Bio Türkiye

International Biotechnology Congress

StartHUB

BIOSphere

100 years

BioTechnology Ecosystem For A Sustainable Life

28 - 30 SEPTEMBER **2023,** Ottoman Archive Complex, Istanbul

ABSTRACT BOOK

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DAVET MEKTUBU

Değerli Akademisyenler, Sayın Meslektaşlarımız, Bürokratlarımız, İlaç Endüstrimizin

Çok Değerli Temsilcileri ve Sevgili Öğrencilerimiz,

Biyoteknoloji ekosistemine katkı sağlamak üzere düzenlediğimiz Uluslararası BIO Türkiye organizasyonumuzun bu yılki teması **'Sürdürülebilir Bir Yaşam İçin Biyoteknoloji Ekosistemi'** olarak gerçekleştirildi. Biyoteknolojinin farklı uygulama alanlarında da artan önemini göz önünde bulundurarak organizasyonumuzda **Sağlık Biyoteknolojisi**'nin yanı sıra **Tarım ve Gıda Biyoteknolojisi**'ne de yer verildi. Sağlık Biyoteknolojinin temel alanları olan **Farmasötik Biyoteknoloji, Hücresel Tedaviler ve Gen Tedavileri, Biyomedikal ve Biyomühendislik Uvgulamaları** ele alınırken, **Gıda Biyoteknolojisi ve Tarım Biyoteknolojindeki** en güncel gelişmeler değerlendirilmiştir. Uluslararası BIO Türkiye Organizasyonu (2023)'nin ülkemizde ve diğer ülkelerdeki paydaşları bir araya getirerek, biyoteknoloji ekosisteminin güçlenmesine ve sürdürülebilir bir yaşam için yenilikler geliştirilmesine katkı sağlamıştır. BIO Türkiye Organizasyonu, biyoteknoloji alanınında üretkenliği destekleyecek bilgilerin paylaşıldığı, zorluk ve sorunları ele alınıp, çözüm önerileri oluşturulacağı, kamu, sivil toplum kuruluşları, üniversite ve endüstri paydaşlarının bir arada sürece katkı sağlayacağı verimli bir platform olması hedeflenmiştir. **Uluslararası BIO Türkiye Organizasyonu (2023)**, 28 – 30 Eylül 2023 tarihlerinde Osmanlı Arşivi Külliyesi, İstanbul'da gerçekleştirilmiştir. Organizasyonumuz kapsamında ana etkinlik düzenlenmiştir.

- BIO Türkiye - Uluslararası Biyoteknoloji Kongresi
- BIO Türkiye - StartHUB
- BIO Türkiye - BIOSphere

BIO Türkiye-Uluslararası Biyoteknoloji Kongresi

BIO Türkiye Organizasyonu (2023) içerisinde yer alan BIO Türkiye-Uluslararası Biyoteknoloji Kongresi, biyoteknolojinin en önemli alanları olan Sağlık, Tarım ve Gıda Biyoteknoloji alanlarındaki en güncel bilimsel araştırmaları ve bilimsel bilgilerin paylaşılacağı, bu alandaki en kapsamlı kongresi olmuştur. Sağlık biyoteknolojisi uygulamalarından Farmasötik Biyoteknoloji, Biyoteknolojik İlaç ve Aşı, Hücresel Tedaviler ve Gen Tedavileri, Biyomedikal ve Biyomühendislik Uygulamaları yanı sıra sürdürülebilir bir yaşamın en temel unsuru olan Gıda ve Tarım Biyoteknoloji alanlarında bilimsel katkıda bulunan tüm paydaşları bir araya getirerek güncel bilgilerin aktarılması ve paylaşılmış oldu. Kongre, biyoteknoloji alanının multidisipliner özelliği nedeniyle ilgili tüm bilimsel disiplinlerden katkı sağlayacak içerik ve kapsamla organize edildi. Uluslararası nitelikte olan BIO Türkiye - Uluslararası Biyoteknoloji Kongresi alanlarında uzman yurtdışı konuşmacı ve katılımcıların katılımıyla güçlenmiştir. En güncel bilimsel gelişmeleri paylaşmaya olanak sağlayacak olan poster, sözlü bildiri ve diğer bilimsel paylaşım platformları yüksek bilimsel standartlara göre hazırladık.

BIO Türkiye-StartHUB

Biyoteknoloji alanında yenilik geliştirmenin lokomotifi olan 'Biyogirişimler'- Start-up firmalarının, akademisyenlerin, üniversitelerin, kamu kurumlarının, sanayici ve yatırımcıların bir araya geleceği StartHUB etkinlikleri, ekosistemde yenilik üretmeye katkı sağlamayı hedeflemektedir. StartHUB organizasyonu verimli bir iş birliği ağı oluşturarak akademik bilginin, ürüne ve hizmete dönüşme sürecinde yer alan tüm paydaşlara bir etkileşim platformu oluşturmuştur. StartHUB organizasyonu öncelikle biyoteknoloji girişimcilerinin temel ihtiyaçlarını tespit ederek, bu ihtiyaçlara yönelik olarak 'startup'a özgü aktiviteler yapmayı planladık. Bu aktivitelerin startupların ihtiyaçlarına göre bir 'Eşleştirme Toplantıları Serisi', 'Eğitim Programları', ve 'Uzmanına Danış' toplantıları olarak çeşitlendirildi. Gençlerin yenilik üretme gayretini destekleyecek üniversite lisans ve yüksek lisans öğrencilerine yönelik etkinliklerle StartHUB (2023)'nin geniş bir etki alanı oluşturmaya çalıştık.

BIO Türkiye-BIOSphere

Biyoteknolojinin ülkemiz için de artan önemine uygun olarak bu alanda yapılan en kapsamlı organizasyonlardan biri olarak planlanan BIO Türkiye Organizasyonu (2023) içerisindeki bilimsel (akademik) bildiri ve sunumlar dışında kalan alanlardaki sunumlar, bildiriler, öneriler, atölye çalışmaları, paneller, sempozyumlar, çalıştaylar, kamu-endüstri toplantıları ve diğer etkinliklerin tamamı BIOSphere etkinliği içinde gerçekleştirilmiştir. BIO Türkiye Organizasyonu (2023) içinde tüm paydaşlar arasında hem iş birliği ağı, hem de güçlü paydaş ilişkileri geliştirilmesi ve bu açıdan kamu destekleri ve organizasyonları gibi tüm destek kuruluş ve organizasyonlarının tanıtımları ve hedef kitlesiyle buluşturulmaları sağlanmıştır. BIOSphere'in her yıl artan paydaş katılım ve katkılarını bu yıl yeni bir boyutta ve verimlilikte gerçekleştirmesini sağladık. Ulusal ve uluslararası iş birliği ve etkileşim ağlarının güçlendirilmesi ile BIOSphere (2023), biyoteknoloji ekosisteminin güçlenmesine katkı sağlamaya çalıştık.

Sevgi ve Saygılarımızla,

Dr. Sevgi SALMAN ÜNVER

BIO Türkiye Organizasyonu (2023) Başkanı

Tayfun GÜMÜŞ

BIO Türkiye Organizasyonu (2023) Genel Sekreteri

KOMİTELER

BIO TÜRKİYE KURULU

KURUL BAŞKANI

DR. MAHMUT TOKAÇ

KURUL ÜYELERİ

UZM. ECZ. MÜCAHİT YİNANÇ

TAYFUN GÜMÜŞ

DR. HAKKI GÜRSÖZ

DR. MUHAMMED ATAK

HIZIR UZUNER

ORGANİZASYON KOMİTESİ

BIO TÜRKİYE ORGANİZASYONU BAŞKANI

DR. SEVGİ SALMAN ÜNVER

BIO TÜRKİYE ORGANİZASYONU GENEL SEKRETERİ

TAYFUN GÜMÜŞ

BIO TÜRKİYE ULUSLARARASI BİYOTEKNOLOJİ KONGRESİ BAŞKANI

PROF. DR. BERRİN ERDAĞ

BIO TÜRKİYE ULUSLARARASI BİYOTEKNOLOJİ KONGRESİ GENEL SEKRETERİ

PROF. DR. HÜLYA AYAR KAYALI

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DR. MUHAMMED ATAK

StartHUB BAŞKANI

PROF. DR. ELİF DAMLA ARISAN

BIO TÜRKİYE – ULUSLARARASI BİYOTEKNOLOJİ KONGRESİ

BİLİMSEL HAZIRLIK KOMİTESİ

BIO TÜRKİYE ULUSLARARASI BİYOTEKNOLOJİ KONGRESİ BAŞKANI

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PROF. DR. HÜLYA AYAR KAYALI

FARMASÖTİK BİYOTEKNOLOJİ ALAN SORUMLUSU

PROF. DR. ALİ DEMİR SEZER

HÜCRE GEN ALAN SORUMLUSU

PROF. DR. HAKAN AKBULUT

BİYOMÜHENDİSLİK VE BİYOMEDİKAL MÜHENDİSLİK ALAN SORUMLUSU

PROF. DR. EMİR BAKİ DENKBAŞ

GIDA BİYOTEKNOLOJİSİ ALAN SORUMLUSU

DOÇ. DR. ENES DERTLİ

TARIMSAL BİYOTEKNOLOJİ ALAN SORUMLUSU

PROF. DR. KAMİL HALİLOĞLU

DANIŞMA KOMİTESİ

PROF. DR. AHMET HACIMÜFTÜOĞLU

ECZ. AYŞE BETÜL ÇINAR

UZM. ECZ. BURCUM UZUNOĞLU

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PROF. DR. GİZEM DİNLER DOĞANAY

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PROF. DR. IŞIL AKSAN KURNAZ

PROF. DR. MELİH BULUT

DOÇ. DR. MUSTAFA GÜZEL

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PROF. DR. OSMAN SAĞDIÇ

RAMAZAN ÖZYURT

ECZ. SELAMET TAŞ

UFUK KARANFİL

UMUT AĞYÜZ

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PROF. DR. AYKUT ÖZDARANDELİ

ECZ. BARIŞ ÖZYURTLU

EBRU ÖZKAN ALTUNTAŞ

PROF. DR. EYÜP İLKER SAYGILI

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ECZ. VOLKAN BÜLENT AKGÜNER

ZEKERİYA AVŞAR

KONUŞMA ÖZETLERİ

CRISPR-CAS SİSTEMİ VE GIDA BİYOTEKNOLOJİSİ

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CRISPR-Cas sistemi, düzenli aralıklarla bölünmüş palindromik tekrar kümeleri ve CRISPR ilişkili protein genlerinden oluşmaktadır. 1987 yılında ilk olarak *Escherichia coli*'de keşfedilen bu tekrar kümelerinin, nükleik asit tabanlı adaptif bağışıklık sisteminin temel bileşenleri olduğu ortaya konulmuştur. Günümüzde bu bağışıklık sisteminin bakteriyofaj enfeksiyonları, istilacı plazmidler ve yabancı nükleik asitlere karşı hücreyi korumayı amaçlayan RNA ve protein tabanlı bir sistem olduğu bilinmektedir. CRISPR sisteminde; Cas proteinleri kısa bir rehber RNA molekülü ile birlikte DNA'ya bağlanarak çift zincir kırıkları oluşturabilmekte, oluşturulan çift zincir DNA kırıkları, homolog olmayan uç birleştirme veya homolog rekombinasyon sistemleri ile farklı biçimlerde tamir edilmekte ve tamir işlemi yeni dizilerin eklenmesi ya da mevcut olanların silinmesi şeklinde sonuçlanabilmektedir. Bu sistemin, hedeflenen bölgeye bağlanarak DNA sekanslarını kolayca ve hassas bir şekilde kesme ve çıkarma yeteneğine sahip olması onu günümüzde önemli bir genom düzenleme yöntemi haline getirmiştir. Rehber RNA'nın tasarım kolaylığı ve uygun maliyeti, DNA kırılmalarının yüksek özgüllüğü ve verimliliği ile hızlı, kolay ve ucuz uygulanabilir olması da bu yönteme, diğer genom düzenleme yöntemlerine kıyasla önemli avantajlar sağlamaktadır. Ayrıca, istenen modifikasyonu içeren ama yabancı DNA ve markör içermeyen genom düzenlemelerinin elde edilebilmesi genoma entegre olabilecek rekombinant bir DNA kullanılmadığı için GDO yönetmeliğinden muaf tutulabilme ihtimalini de artırmaktadır. Özetle değinilen avantajlı yönleri ile önemli bir genom düzenleme aracı haline gelen CRISPR-Cas sistemi, tarım, sağlık ve gıda gibi tüm yaşam bilimleri araştırmalarında büyük bir potansiyel içermektedir. Bu bildiride CRISPR-Cas sisteminin, çalışma prensipleri, gıda alanında kullanım imkanları ve bu teknikler kullanılarak yapılmış bazı çalışmalar hakkında bilgi verilmiştir.

Çip üstü Organ Teknolojileri

Ali Akpek

Kardiyovasküler hastalıklar bütün insan hastalıkları arasında insan ölümlerinin en önde gelen nedenlerinin başındadır. **Thomas vd. (2019)** tarafından yayınlanan Küresel Kardiyovasküler Atlasına göre konu ile ilgili olarak elde edilen bütün başarılara ve yeniliklere rağmen kardiyovasküler hastalıklar hâlâ bütün insan ölümlerinin kadınlarda %20'sini, erkeklerde ise %24'ünü oluşturmaktadır. Dünya çapında her yıl 350 milyondan fazla insanın hayatını kaybetmesine neden olmaktadır. Kardiyovasküler hastalıklar arasında da kalbin kalp kasının yetersiz kalması anlamına gelen iskemik kalp hastalığı yıllık 175 milyon kişi ve kardiyovasküler hastalıkların %49,4'ünü kapsaması sebebiyle insan ölümlerinin en başlıca gelen sebebi konumundadır. **Yadgir vd (2020)** yayınladığı çalışmada ise Kalp kapakçıkları hastalıkları arasında Kalsifik Aortik Kalp Kapakçığı hastalığının ve dejeneratif mitral kalp kapakçığı hastalığının en yoğun görülen hastalıklar olduğunu, bunların sırasıyla 12.6 milyon ve 18.1 milyon hasta olarak görüldüğünü raporlamıştır. Buradan da rahatlıkla anlaşılabilir ki kardiyovasküler hastalıklar daha da özelinde kalp kası ve kalp kapakçığı hastalıkları insan ölümlerinde en başı çeken hastalıklar arasındadır. Bu hastalıkların ölüm oranının yüksekliği bir tarafa, öldürmediği durumlarda da çoğunlukla hastaların hayat kalitesini çok ciddi oranda düşürmektedir. Bu açılardan bu sorunları çözmek için bilim insanları arasında yoğun bir çalışma mevcuttur.

Durum bu şekilde olmasına ve bu konu ile alakalı olarak muazzam yatırımlar yapılmasına rağmen kardiyovasküler hastalıklar karşısında alınabilen mesafe çok sınırlıdır. Bunun en önemli nedeni bu hastalıklara karşı ilaç geliştirme çalışmalarının çok ağır ilerlemesidir. **Abou-Gharbia ve Childers (2014**) gerçekleştirdikleri çalışma da gösterilmiştir ki ilaç başına geliştirme maliyeti ortalama olarak 1 milyar dolardır. İlaç geliştirme süresi de yine ortalama olarak on yıldır. Ayrıca pek çok ülkede etik kaygılar sebebiyle ilaç geliştirilme çalışmalarında hayvan kullanılması çok sıkı denetim altındadır. Yine de bunca masrafa rağmen çalışılan ilaçların önemli bir kısmında gerek klinik çalışmalarda gerekse de pazara çıktıktan sonra ciddi yan etkiler gözlemlenmektedir. **Chu vd. (2013)**, bunun en önemli nedeninin gerek kemirgenlerin gerekse de primatların biyolojilerinin hem genetik hem de metabolik anlamda insanlardan çok farklı olduğu için ilaçlara verdikleri reaksiyonların da insanlardan farklı olması olduğunu ortaya koymuştur. Bu da çok önemli farmokokinetik hatalara sebebiyet vermektedir. **Kola** ve **Landis (2004)** tarafından yapılan araştırmada ise göstermiştir ki misal sadece 2000 yılında ilaç toksisitesi ve ilaç etkinlik gibi en temel klinik güvenlik sebepleri ile bütün ilaç projelerinin %30'u başarısızlıkla sonuçlanmıştır. Kalanların ise büyük çoğunluğu ilerleyen safhalarda başarısızlığa uğramıştır. Yine aynı araştırmaya göre kardiyovasküler hastalıklar özelinde, 1991 ile 2000 yılları arasında bu amaçla geliştirilmiş olan ilaçların sadece %20'si başarılı olabilmiştir. Bundan başka **Menna vd (2008)**, **Piccini vd (2009**) ve **Shah (2006)** gerçekleştirdikleri çalışmalarda son 50 yılda ortaya çıkan kardiyovasküler ilaçlarda ciddi yan etkilerin bulunmasının piyasadan toplatılmalarının en önemli gerekçesi olduğunu ortaya koymuştur. Özetle mevcut ilaç geliştirme yöntemleri bütün zorlu aşamalardan sonra piyasaya çıkmayı başarabilmiş olan ilaçların dahi geri toplatılabilmesine sebep olabilecek ölçüde yetersizdir.

Bütün bu nedenlerden dolayı insan organlarını in vitro ortamda daha iyi ve daha isabetli olarak modelleyebilecek platformlar geliştirilmeye çalışılmaktadır. Bu platformlar Çip üstü Organ platformları olarak adlandırılmaktadır. Bu platformlar sayesinde yeni ilaçlar için daha hızlı ve daha isabetli preklinik testlerin gerçekleştirilmesi hedeflenmektedir.

Çip üstü Kardiyovasküler sistemin önemli olmasının kardiyovasküler hastalıkların en yüksek seviyede insan ölümlerine sebebiyet vermesinin ötesinde neden önemli olduğunu **Pound vd. (2004)** gerçekleştirdiği araştırmada açıklamıştır.

Bunlar şu şekilde özetlenebilir.
1. Kardiyovasküler hastalıklar için hayvan modelleri insanlar için çok zayıf tahminler yürütmemize sebep olabilir **(Bracken 2009)**.
2. Sistemik toksisite ve metabolit oluşumu canlılara özgüdür. Bu sebeple yan etkilerde canlıya özgüdür. Bu nedenlerden dolayı hayvan modellerinde alınacak sonuçların insanlar için işe yaraması zayıf bir olasılıktır (**Barnard ve Kaufman (1997)**).
3. Bazı ilaçlar sadece cinsiyete, yaşa, etnik kökene ya da genotipe göre çok sınırlı bir kitle üzerinde etkili olabilir. Diğer herkes üzerinde etkisiz olabilir. Bunun hayvan denemelerinde ve hatta insan denemelerinde dahi anlaşılması kolay değildir. Misal olarak klinik denemelerde gönüllü olanların büyük ölçüde orta yaşlı kadınlar olduğu raporlanmıştır (**Cato vd. (2012) ve Mattison (2010)**). Bu sebeple ilaçların büyük kitlelerde nasıl reaksiyon vereceğinin anlaşılması çok kolay değildir.
4. Mevcut ilaç geliştirme paradigması son derece pahalı ve zaman alıcıdır (**Institute of Medicine US (2011)**).

Bütün bu veriler aynı sonucu işaret etmektedir. **Chang vd. 2009** ve **Katare vd. 2010** tarafından özetlendiği şekilde ilaç çalışmaları için hayvanlardan elde edilen pozitif ya da negatif sonuçların insanlar için de buna paralel olarak pozitif ya da negatif anlamlar içerme ihtimali çok zayıftır. Arada rasyonel bir bağlantı kurulma ihtimali çok zayıftır ve çoğunlukla olası değildir.

BİYOTEKNOLOJİK İLAÇLARDA KLİNİK ÇALIŞMALAR

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İÜ İlaç Araştırmaları Merkezi

TİTCK kılavuzlarındaki tanıma göre; klinik araştırmalar, beşeri tıbbi ürünlerin klinik araştırması, biyoyararlanım çalışması ve biyoeşdeğerlik çalışmalarıdır. Klinik deneme**,** sağlıkla ilgili bir veya birden fazla müdahalenin, insanda sağlık sonuçları üzerine etkilerini araştırmak amacıyla ileriye dönük olarak insanlar üzerinde yürütülen araştırmalardır. Beşeri tıbbi ürünlerin klinik araştırmalarında Faz I, II, III ve IV (düşük riskli bilimsel çalışma) olmak üzere 4 aşama bulunmaktadır. Faz I-III'ü kapsayan ruhsat öncesi klinik geliştirme süreci 8-10 yıla varabilir ve ilaç geliştirmenin en uzun aşamasıdır.
Biyoteknolojik ürünler, canlı sistemler veya organizmalar tarafından üretilen ürünleri ifade eder. Biyoteknolojik ürünlerin klinik araştırmaları ise küçük moleküllü ilaçlara kıyasla özelleşmiş süreçlerdir ve küçük moleküllü ilaçlardan farklı bir prosedür uygulanabilir.
Günümüzde biyoteknolojik ürünlere giderek artan bir eğilim vardır. İleriye yönelik bu artışın devam edeceğini öngören çalışmalar bulunmaktadır. Onkoloji, immünoloji, romatoloji, nöroloji gibi alanlarda biyoteknolojik ürünlerle daha fazla AR-GE çalışması yapılması bu alanlardaki ürünlerin piyasadaki oranını da arttırmaktadır. Biyoteknolojik ürünlerde özellikle onkoloji alanındaki ilaçlar öne çıkmaktadır. Burada onkoloji alanındaki yeni tedavilere ihtiyaç duyulması ve gelişmekte olan immunoonkolojikler, protein kinaz inhibitörleri, hedefli küçük moleküller, bispesifik antikorlar gibi yeni ürünler olmasının yanında onkoloji klinik çalışmalarına özgü durumlar bulunmaktadır. Onkoloji klinik araştırmalardaki paradigma değişikliğiyle sitotoksik kemoterapilerin yerini molekül hedefli ajanlar, geleneksel klinik araştırma tasarımlarının yerini ise adaptif tasarımlar ve biyobelirteç güdümlü değerlendirmeler almıştır. Onkoloji Faz 1 klinik çalışmaları geçmişten beri klasik ürünlerin sitotoksilk olması sebebiyle sağlıklı gönüllülerde değil hastalarda gerçekleştirilmektedir. Yeni biyoteknolojik ürünler görece daha güvenli ilaçlar olmakla birlikte hastada Faz I çalışması yapılmasının bazı avantajları vardır. Bunlardan bir tanesi eskiden beri yapılan hasta Faz I'lerinden kazanılan deneyimleri bunlarla karşılaştırmalı bilgiler elde edilmesi iken diğeri de biyoteknolojik ilaçlar ile yapılan çalışmalarda biyobelirteçler ve yeni dizaynlar kullanılarak Faz I 'den başlayarak etkililik ile ilgili bilgiler de edinilmesidir.
İstanbul Tıp Fakültesinin T.C. Sağlık Bakanlığı onaylı Faz I ve Klinik Araştırmalar Merkezimizde gündemdeki çalışmalar da ağırlıklı olarak biyoteknolojik, onkoloji ilaçlarını kapsamaktadır.
Onkoloji klinik araştırmalardaki paradigma değişikliğiyle sitotoksik kemoterapilerin yerini molekül hedefli ajanlar, geleneksel klinik araştırma tasarımlarının yerini ise adaptif tasarımlar ve biyobelirteç güdümlü değerlendirmeler almıştır. çalışmaların çoğu da hasta katılımcıların olduğu, biyoteknolojik ürünleri içeren Faz I çalışmalarıdır.
Biyoteknolojik ürünler ile küçük moleküllü ilaçlar arasında immünojenisite, üretim ve saklama koşullarındaki zorluklar, klinik araştırma zorlukları, zaman, maliyet gibi farklar bulunmaktadır. Bu farklar biyoteknolojik ürün geliştirme ve klinik araştırma aşamalarını küçük moleküllü ilaçlardan ayırmaktadır. Klasik çalışma dizaynından farklı olarak geliştirilen çalışma dizaynları zaman, maliyet ve sonuca ulaşma konusunda avantajlar sağlamaktadır. Biyoteknolojik ilaçlarda klinik son nokta çok daha önemli olduğu için biyobelirteçler biyoteknolojik ürün klinik araştırmalarında ön plana çıkmaktadır.
Biyoteknolojik ürünlerin ruhsatlandırılması aşamasında küçük moleküllü ilaçlardan farklıdır. EMA, FDA gibi çeşitli otoritelerin biyoteknolojik ilaçların klinik araştırmalarında birbiriyle örtüşen klasik çalışma

dizaynında bulunmayan bazı istekleri vardır. Bunun yanında preklinik geliştirme sürecinde her otoritenin belirlediği istekler birbirinden farklıdır ve klinik geliştirme kısmına kıyasla daha zorlayıcıdır. Otoritelerin istekleri arasında klinik araştırma sürecinde yeni çalışma dizaynlarının yanında karşılaştırmalı PK/PD çalışmaları ön plana çıkmaktadır.

Biyobenzer ürünler; kalite, güvenlilik ve etkililik konusunda referans ürünle benzer özellikler gösteren biyoteknolojik ürünlerdir. Biyobenzer ürünlerin klinik çalışmalarını biyoeşdeğerlilikten ayıran temel unsur Faz I ve Faz III aşamalarının her ürün için tekrarlanması gerekliliğidir. Biyobenzer ürünlerin piyasa sürülebilmesi için istenilen çalışmaları otoritelerce belirlenmiştir.. Biyobenzer ürünlerin maliyeti bir biyoeşdeğer ürün kadar azalmasa da referans ürüne göre daha düşüktür. Ülkemizde de biyobenzer ürünler ilaç pazarında yer almaktadır.

Biyoteknolojik ürünlerin, farmasötik ilaçlardan farklı olduğu bir konuda da immünojenisitedir. İmmunojeniteye bağlı anaflaksi, hipersensitivite ve infüzyon reaksiyonları gibi olası advers etkiler çalışma güvenliliği açısından tedbir gerektirmektedir. Bu gibi güvenlilik problemlerinin yanı sıra immun yanıtlar ürünün etkililiğini modiye edebilir veya azaltabilir, endojen proteinlerle çapraz reaksiyonlara neden olabilir ya da farmakokinetiğini değiştirebilir.

Biyoteknolojik ürünlerle önemli bir tartışma konusu erişilebilirlik, güvenlilik ve etkililik, gizlilik ve mahremiyet, sosyal değerler ve bilimsel geçerliliğin dengelenmesi gibi etik kaygılardır.

Biyoteknolojik ürünler bazı meydan okumalar ortaya çıkarsa da aynı zamanda yenilikçi yaklaşımlara olanak sağlar. Çevrimsel araştırma, biyoteknolojik ürünlerin gelişmesiyle paralel ilerlemiş bir çalışma yöntemidir. Bu yöntem preklinik-klinik arasındaki sınırları kaldırıp daha hızlı ve insan sağlığını daha fazla gözeten kişiselleştirilmiş çözümlere olanak sağlayacaktır.

Biyoteknolojik İlaçlarda Preklinik Çalışmalar

Prof. Dr. Alper B. İskit

Biyolojik ilaçların gelişim sürecinde, özellikle biyoteknolojik ilaçların ruhsatlandırılması aşamasında, tanımlanmış standart preklinik ilaç çalışmalarının olmaması nedeniyle ilaç otoriteleri arasında çelişkili kararlar çıkmıştır. Özellikle preklinik çalışmalarda uygun hayvan türü seçimi, immunojenisite çalışmaları, rutin preklinik testler (FK/FD, toksisite…) için standardizasyon ciddi bir gereksinim olmuştur. Preklinik çalışmaların standardizasyonu amacıyla öncelikle EMA ilk klavuzu yayınlamıştır (EMEA/CHMP/BMWP/42832/2005). Bu kılavuza göre preklinik çalışmaların amacı klinik çalışma için insanda güvenli, etkin başlangıç dozunu bulmak; toksisiteye maruz kalabilecek organları, varsa toksisitenin geri dönüşümlü olup olmadığını tespit etmek ve klinikte monitorizasyon için güvenlik parametrelerini bulmak olarak tarif edilmiştir. EMA yaklaşımı tüm ürünler için tek bir kuralın bu alanda uygulanamayacağı ve hatta dosya bazında değerlendirme gerekebileceği esasına dayanmaktadır. Preklinik çalışmalara genel yaklaşım:

1. Hücre kültüründe (in vitro) yapılan çalışmalar

Biyoteknolojik ilaç adayının öncelikle biyolojik karakterizasyon/aktivasyon çalışmaları *in vitro* şartlarda gösterilir. İlacın terapötik etkisinin ve etki mekanizmasının anlaşılması amacıyla reseptör bağlanma çalışmaları (afinite), hücre siklusuna etki, hücre içi yolakların inhibisyonu, DNA tamir mekanizmaları ve apoptozise etkisi araştırılır. İlacın terapötik alanına özgü damarlanma üzerine etki, hücre göçü ve tümör hücresi öldürme gibi spesifik araştırmalar da özellikle *in vitro* şartlarda hücre kültüründe çalışılabilir.

2. Deney hayvanı tür seçimi ve farmakoloji çalışmaları

İki farklı tür, "rodent" ve "non-rodent",seçilir. Bu seçim yapılırken ürünün reseptör / epitop dağılımı mutlaka incelenmelidir. Epitop, antijenin özgüllüğünü belirleyen ve antijenin kendi özgül antikorları ile birleşmesini sağlayan kimyasal gruplardır (determinant). Reseptör / epitop dağılımı özellikle *"cevapsız hayvan"* türleri için önemlidir. Reseptör veya epitop ekspresyonu yoksa hayvan türü uygunsuzdur Ekspresyon yapan transjenik hayvanlara gereksinim doğar.

a) Preklinik farmakodinami çalışmaları
b) Preklinik farmakokinetik çalışmalar

FD/FK çalışmaları sonucunda klinik çalışmaya ön bilgi olarak uygulama yolu ve sıklığı, doz seçimi, doz-cevap ilişkisi, maksimum doz- toksik doz ve biyoyararlanım parametreleri hakkında bilgi sahibi olunabilir

3. Farmakolojik Güvenlilik, Toksikoloji ("Safety Pharmacology")
4. İmmunotoksisite değerlendirme çalışmaları
5. Reprodüktif ve gelişimsel toksisite değerlendirme çalışmaları
6. Genotoksisite değerlendirme çalışmaları
7. Karsinojenisite değerlendirme çalışmaları
8. Lokal Tolerans çalışmaları

Biyobenzer ilaçlarda preklinik çalışmalar:

Biyobenzer ilaçlar arasında, preklinik ve klinik çalışmalarda ürün bazında farklılıklar gözlenmekle beraber, genel anlamda orijinal biyolojik ürünlere göre preklinik değerlendirme açısından daha az, daha sınırlı çalışmalar ve veriler beklenir. Kısaltılmış preklinik program istenebilir:

1. Subkronik toksisisite çalışması (4 hafta),
2. Lokal tolerans çalışması,
3. Yeni biyobenzere özgü PK/PD değerleri ve çalışması talep edilir.

PANDEMİLER VE TURKOVAC DENEYİMİ

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Ülkemizde ilk konfirme Covid-19 vakası 11 Mart 2020 tarihinde görülmüştür. İlk konfirme Covid-19 vakanın görülmesinden sonra ülkemizin sayılı Biyogüvenlik seviye-3 (BSL-3) laboratuvarına sahip Erciyes Üniversitesi Aşı Araştırma ve Geliştirme Uygulama ve araştırma Merkezi (ERAGEM)'de Covid-19 hastalığına neden olan SARS-CoV-2 virüsü 2020 Mart ayında SARS-CoV-2 virüsünün hücre kültüründe üretilmesi ve izolasyonu başarılmıştır. Nisan 2020 tarihinde izole edilen SARS-CoV-2 virüsünün ülkemizdeki ilk DNA dizilimini belirlenmiş ve virüsün tam büyüklükteki gen haritası çıkarılmıştır (26).

İnaktif aşı adayının klinik öncesi çalışmaları Erciyes Üniversitesi ERAGEM'de Eylül 2020 tarihinde başarıyla tamamlanmış ve Ekim 2020 tarihinde GMP ve BSL-3 şartlarına sahip Koçak Farma'da BSL-3 ve GMP şartlarında aşının Faz çalışmaları için üretimi ve dolumuna başlanmıştır. 5 Kasım 2020 tarihinde Erciyes Üniversitesi İyi Klinik Uygulama ve Araştırma Merkezi'nde (İKUM) inaktif aşı adayının ilk dozu bir gönüllüye uygulanarak Faz I çalışmaları başlamış ve Şubat 2021 tarihinde Faz 1 çalışmalarıyla ilgili ilk veriler elde edilmiştir (19). Turkovac aşısının Faz II çalışmaları 10 Şubat 2021 tarihinde 250 gönüllüde başlamış ve 9 Nisan 2021 tarihinde 2. doz uygulaması bütün gönüllülerde tamamlanmıştır. Turkovac aşısının 250 gönüllüdeki Faz II çalışmaları, aşının gerek güvenliliği gerekse de immünojenitesi açısından oldukça umut verici sonuçlar elde edilmiştir (19). Faz 3 çalışmalarına 22 Haziran 2021 tarihinde başlanmıştır ve 41 Merkez ve 28 ilde yürütülen iki doz Sinovac aşısı olmuş kişilere bir kol Turkovac diğer kol Sinovac olmak üzere 3. doz uygulanarak (rapel) her iki aşının güvenlik ve etkinlik çalışmaları yapılmıştır. Elde sonuçlar Turkovac ve Sinovac aşılarının benzer etkinlik ve güvenirliğini ortaya koymuştur (44). Bu çalışmalar, Cumhuriyet tarihinin en kapsamlı klinik çalışmaları olarak ifade edilebilir.

Kasım 2021 tarihinde Turkovac aşısının acil kullanım onayı için Sağlık Bakanlığı Türkiye İlaç ve Tıbbi Cihaz Kurumuna (TİTCK) başvuru yapılmıştır. Başvuruda; klinik öncesi çalışmalar, Faz 1, Faz 2 ve Faz 3 çalışmalarının verileri ve rapel doz çalışmalarıyla alınan verileri içeren rapor hazırlanmıştır (19,26,44,45). Ayrıca, üretim süreçlerini içeren kritik 13 basamak ve aşının kalite kontrolleriyle ilgili yaklaşık 400 analizin yer aldığı dosya hazırlanmıştır. Gerek TİTCK gerekse de acil kullanım onayı ile ilgili bilimsel kurulun değerlendirmeleri sonucunda Turkovac aşısı 22 Aralık 2021 tarihinde acil kullanım onayı almıştır. Turkovac aşısının endüstriyel boyutta üretimi Dollvet Biyoteknoloji şirketinde başarıyla yapılmış ve milyonlarca doz aşı üretilmiştir. Ülkemiz 50 yıl sonra ilk defa antijenden seri üretime kadar bir aşının bütün safhalarını kendi imkanlarıyla üretmeyi başarmış ve dünyada, Covid-19'a karşı aşı üreten 9. ülke olmuştur.

Diğer aşı platformlarında olduğu gibi viral inaktif aşı geliştirilmesi de farklı disiplinlerin bir arada çalışmasını gerektiren süreçleri kapsamaktadır. Viral inaktif aşı adaylarının geliştirilme süreçlerinde doktoralarını viroloji biliminde yapmış ehliyetli uzman araştırıcılar büyük öneme sahiptir. Ayrıca, "Aşı Bilimi" alanında uzmanlaşmış immunologlar, moleküler biyologlar, farmakologlar, kimyagerler, hücre kültür sistemleri alanında uzmanlaşmış araştırıcılar ve biyomühendis gibi uzmanların bir araya gelmesi ve multidisipliner çalışmaların yapılması hayati öneme sahiptir. Aşı bilimi konusunda yetişmiş insan gücü yanında aşı Ar-ge çalışmalarının yapılacağı alt yapının sağlanması gerekmektedir. Aşı bilimi Ar-Ge çalışmaları uzun soluklu ve yüksek teknoloji gerektiren laboratuvarlarda yapılan çalışmalardır ve bu bağlamda alt yapı yatırımlarının yapılması önem arz etmektedir. Aşı bilimi konusunda çalışan üniversiteler, enstitüler, kamu kuruluşları ve özel sektör arasındaki iş birliklerinin arttırılması ve aşı

ekosisteminin oluşturulmasıyla gelecekte ortaya çıkabilecek yeni pandemiler ve salgınlara karşı stratejik bir silah olan aşıların kısa sürede geliştirilmesi ve üretilmesi mümkün olacaktır.

Adenoasosiye Virüs Tabanlı Gen Tedavilerinde Güncel Gelişmeler ve Rekombinant AAV'lerde Yeni Ufuklar

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Adenoassoisye virüsler (AAV), güçlü ve uzun süreli *in vivo* transgen ifadesi sağlamaları, toksisite ve immunojenitelerininin düşük olması nedeniyle klinik denemelerde öne çıkmakta ve onaylanmış insan hastalıkların gen tedavilerine temel teşkil etmektedir. Eylül 2023 itibari ile gen tedavisi veri tabanında (*genetherapynet.com*) kayıtlı tüm gen tedavisi klinik denemelerinin % 9.1'i Adenoassosiye virüs (AAV) tabanlı gen tedavi ürünleri içermektedir. 2012 yılında *European Medical Agency* (EMA) tarafından onay verilen ilk gen tedavisi ürünü Glybera, 2017 yılında FDA tarafından onaylanan Luxturna ve takip eden yıllarda onay alan Zolgensma (2019), Hemgenix (2023), Elevidys (2023) ve Roctavian (2023) gen tedavisi ürünlerinde terapötik genin taşıyıcısı AAV tabanlıdır.

Adeno-associated virus küçük, zarfsız tek iplikli DNA genomuna sahip Parvoviridae ailesinden virüslerdir (yaklaşık 25 nm). Adenovirüsler ile ilişkili olmamakla birlikte, 1965 yılında adenovirus üretimi sırasında kontaminasyon olarak ilk kez tanımlandığından dolayı adeno-ilişkili (assosiye) virus ismini almıştır. AAV'ler doğal olarak kendi başlarına replikasyon yapamazlar ve yardımcı bir virüse (Adenovirüs, Herpesvirüs veya Vaccinia virüs) ihtiyaç duyarlar. AAV'ler yardımcı virus yokluğunda tercihen 19q13'de AAVS1 lokusuna entegre olarak latent kalırlar. Yardımcı virus varlığında ise AAV'ler paketlenmiş tam virus formunu alarak litik faza geçer. AAV genomu *rep* ve *cap* olmak üzere iki gen içerir ve bu genler AAV'in yapısını ve yaşam döngüsünü sağlayan proteinlere (Rep78, Rep68, Rep52, ve Rep40, VP1, VP2, VP3, AAP, MAAP) çevrilir. Kapsid, doku tropizmi ve transdüksiyon etkinliğinin en önemli belirleyicisidir. Farklı AAV Kapsid serotipleri özgün reseptörlere ve ko-reseptörlere tutunarak hücre içine girer. Tüm AAV virionları 1:1:10 (VP1:VP2:VP3) oranında 60 VP alt biriminden oluşur. Her altbirim virion yüzeyinde, primer doku tropizmi ve hücre içi trafiğini etkileyen dokuz değişken bölgeden oluşur ve bu bölgeler nötralizan antikorlar ile tanınır. Bu bölgelerin genetik olarak değiştirilmesi AAV'lerin transdüksiyon etkinliğini ve nötralizan antikorların bağlanmasını değiştirir. AAV'ler, hücre yüzeylerindeki primer reseptörleri ve ko-reseptörlerine bağlanarak endositoz ile hücreye girer. Endosozomlar içinde hücreye alınan AAV'ler, endozom içindeki düşük pH nedeniyle, VP1 ve VP2'nin amino uçlarını açığa çıkaran yapısal değişime uğrayarak endozomlardan kurtulur ve hücrenin perinükleer alanında birikir. Çekirdeğe girdikten sonra kapsidini terkeden tek iplikli genom çift iplikli hale gelerek, taşıdığı transgenin transkripsiyonunu gerçekleştirir.

AAV genomunun %96'sı çıkarılarak elde edilen rekombinant AAV'ler (rAAV)'de sadece transgen ekspresyonunu uyaran ve vektör üretimi için esansiyel rol oynayan 145 bazlık uçlardaki AAV ters dönmüş tekrarları (ITR) bulunur. *Rep* ve *cap* genleri çıkarıldığı için rAAV'lerin replikasyonlarını yapabilmeleri ve virus halinde paketlenebilmeleri için rAAV'lerin üretimi esnasında bu genleri ve diğer yardımcı virus genlerini taşıyan plazmidlerin üretim hücre hatlarına transfeksiyonu gereklidir. Genomda boşalan yere ise transgen ekspresyon kaseti klonlanır. Transgen ekspresyon kaseti; promotor, terapötik gen, poliadenilasyon sinyali ve transgenin stabilizasyonu için çeşitli pre- ve post-düzenleyici elemanlarını içerir. rAAV genel paketlenme kapasitesi, 4.1-5.2 kb uzunluğunda olsa da farklı serotiplerde bu kapasitenin üzerine çıkılabildiği görülmektedir. Örneğin AAV-5 kapsidlerinin 8.9 kb'a kadar genomu paketleyebildiği gösterilmiştir. Genoma entegrasyon için *Rep* geni geni gerekli olduğundan, rAAV'ler enfekte ettiği hücreninin genomuna entegre olamaz ve ITR uçları ile konkatemerler yaparak sirkülize olarak hücre içinde epizomal kalır.

Bir genin transferi için hangi tip rAAV kullanılacağının seçiminde; hedeflenecek hücre/doku tipi, aktarılacak genin güvenlik profili, sistemik veya lokal aktarım tercihi ve de dokuya özel gen ifadelenmesi istenip istenmediği göz önünde bulundurulması gerekir. Ayrıca bağışıklık sisteminin uyarılıp uyarılmaması tercihine göre de AAV seçimine gidilebilir. Bazı AAV'ler antijen sunucu hücrelerine etkili bir transdüksiyon yapamamaktadır. Rekombinant AAV'lerde virüse ait gen bulunmadığından diğer vektörlere göre son derece immunojetileri zayıftır. Yine de kapsid proteinlerine ve aktarılan gene karşı immunojenite görülebilir. Ayrıca, tedaviyi alacak bireyin daha önce bazı adeno-asosiye virüslerle karşılaşmış olması ve mevcut immünitesi nedeniyle gen aktarım etkinliğinin zayıflayabileceği göz önünde bulundurulmalıdır.

Günümüzde en az 12 doğal serotip ve 100'den fazla AAV varyantı izole edilmiş durumdadır ve farklı gen tedavisi klinik denemelerinde kullanılmaktadırlar. Bunların bazıları insan dışı primatlardan izole edilmiştir. Örneğin, Duchene Muscular Distrofi için yeni FDA onayı almış Elevidys'de kullanılan ve mikro-distrofin genini taşıyan vektör, maymundan izole edilmiş AAVrh74 serotipidir. Maymundan izole edilen diğer bir serotip AAV8'dir: kemirgenlerin ve insan olmayan primatların karaciğerine AAV8'in etkin gen aktarımı yapabildiği gösterilmesi sonrasında başta hemofili olmak üzere, diğer insan hastalıklarına (göz ve kas) yönelik gen tedavileri için klinik denemeler (*ClinicalTrials.gov* NCT00979238; NCT03066258; NCT03199469) başlatılmıştır ancak henüz onay almamıştır.

Değişik AAV serotiplerinin farklı bağlanma reseptörleri ve dolasıyısı doku tropizmleri mevcuttur. AAV'ler primer olarak hücrenin yüzeyindeki karbonhidratlar ile etkileşerek hücreye girmektedir. Bu karbonhidratlar sialic asid, galaktoz ve heparan sulfat'tan oluşmaktadır. Örneğin birçok dokuyu enfekte edebilmesi ile en geniş tropizme sahip olan AAV9'un, galaktoza ilginliği yüksektir ve kan beyin bariyerini geçerek santral sinir sistemi (SSS) hücrelerini enfekte edebilmektedir. Spinal Muskuler Atrofi için geliştirilen ve 2019 yılında FDA onayı alan Zolgensma gen tedavi ürünü, SMN1 genini taşıyan rAAV9 içermektedir. Primer etkileşim yanı sıra sekonder reseptörler de viral transdüksiyonunda ve hücre ve doku tropizminde rol oynar. Birincil ve ikincil reseptör etkileşimlerindeki ince değişiklikler ile, belirli bir tropizme sahip olan ve tercihen bir hücreyi veya doku tipini diğerlerine göre daha fazla enfekte eden varyantlar elde edilebilir.

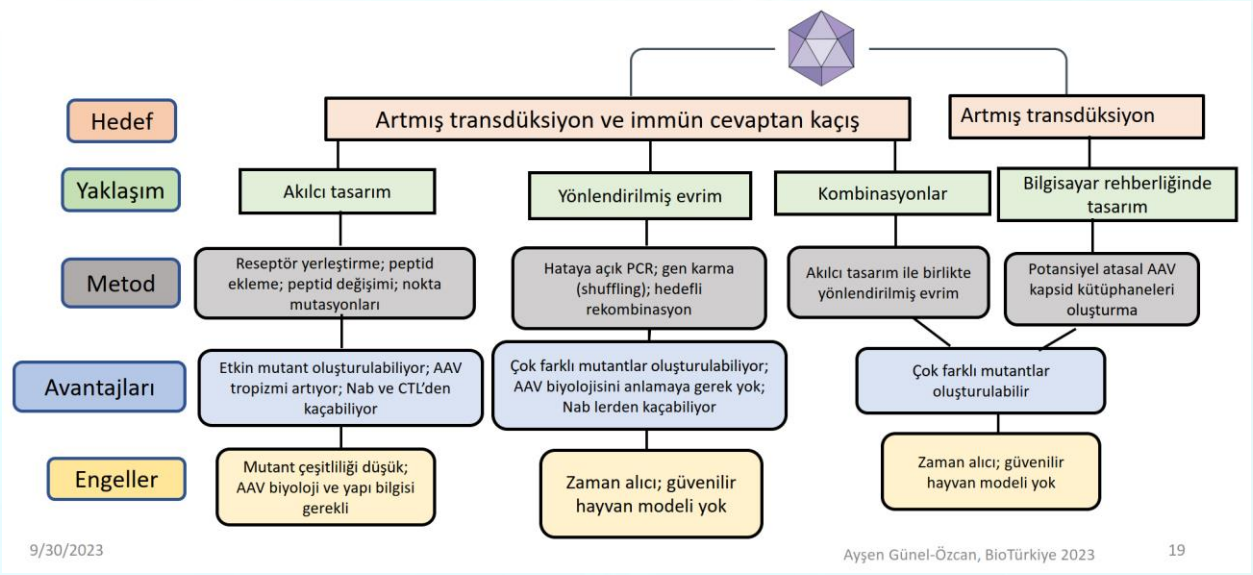
AAV vektörlerinin transdüksiyon etkinliği hücre tipine göre değişmekle birlikte transdüksiyon ünitesinde 25 ile yüzlerce vektör genomu içeren partikülü (VGP) içerebilmektedir. Transdüksiyon etkinliğinde hız sınırlayıcı basamaklardan birisi gen ifadesinden önce tek iplikli DNA olan vektör (ss-AAV) genomunun çift-iplikli DNA'ya (dsDNA) dönüştürülmesidir. Geliştirilen Self-komplementer (scAAV) vektörler dimerik ters dönmüş tekrarlı DNA molekülü nedeniyle ikinci DNA sentezine gerek kalmadan gen ifadelenmesine başlayabilir ancak paketleme kapasitesi 2.7-3.3kb'a düşer ve üretimleri ssAAV'lere göre daha zordur. Bu vektörlerin genomunda yer sınırlı olduğundan dolayı, kodon optimizasyonu yanısıra optimum transkripsiyonel ve post-transkripsiyonel düzenleyici elemanların tasarlanması etkin transgen ifade düzeylerinin elde edilmesi için kritik öneme sahiptir. Diğer hız sınırlayacı basamaklar ise çekirdeğe transport ve/veya kapsid'in açılması (uncoating)'dır. dsDNA oluştuktan sonra kısa süre vektör genom kararsızlığı olması da gen ifadesinde belirgin kayba yol açabilmektedir.

Biyolojik dağılım (*Biodistribution*) düzeyinde veya transkripsiyonel ve post-transkripsiyonel düzeyde yaklaşımlar ile AAV vektörlerin özgüllüğü artırılarak toksisite ve Off-target etkileri önlenebilir. Günümüzde Kapsid mühendisliği ile değişik doku özgünlüğüne sahip ve immün cevaptan kaçabilen yeni AAV vektörleri geliştirilmeye çalışılmaktadır. Dokuya özgü reseptörlere bağlanmayı veya hücre trafiğini değiştirecek modifikasyonlar ile doku spesifik kapsidler geliştirilerek veya en uygun uygulama yolu seçilerek en etkin biyolojik dağılım sağlanabilir. Dokuya özgü promotorlar kullanarak da doku özgünlüğü artırılabilir. Örneğin Hemofili B tedavisi için geliştirilmiş hFIX variant R338L genini taşıyan rAAV5

vektörünü içeren Hemgenix gen tedavisi ürününde ve Hemofili A tedavisi için geliştirilmiş hFVIII-SQ genini taşıyan rAAV5 vektörünü içeren Roctavian gen tedavisi ürününde karaciğere özel promotor dizileri kullanılmaktadır (https://www.ema.europa.eu/en/medicines/human). Off-target etkileri önlemek için diğer bir yaklaşım ise, transgen ifadelenmesinin istenmediği dokuda yüksek ifadelenen bir miRNA'nın hedef dizilerini transgenin 3'-UTR ucuna eklemektir. Böylelikle o miRNA'nın yüksek ifade olduğu dokular, rAAV'nin taşıdığı transgenin susturulması sonucu, gen tedavi ürününden korunur.

Genetik hastalıklar yanı sıra kanser gen tedavilerinde de AAV vektörler umut vaat etmektedir. Çeşitli kanserler için onay almış (Metastatik melanoma, renal kanser ve hairy cell lösemi) yüksek toksisite gösteren rekombinant sitokinlerin yerine transgeni taşıyan AAV vektörler daha düşük dozlarda daha güvenli antikanser etki gösterebilir. AAV vektörleri transgene karşı hem humoral hem hücresel immün cevabı uyarabilir. rAAV'lerin hücreye etkin transdüksiyonu ve antijenin uzun süreli ifadesi birçok tümör antijene karşı güçlü ve sürekli sitotoksik T –lenfosit cevabın oluşmasını da sağlamaktadır. İmmunojenik cevabın şiddeti serotipe, doza veya uygulama yoluna bağlı değişebilir. İmmunojenik cevap, transdükte edilen hücrelerden vektörün eliminasyonuna sebep olarak transgenin ifadelenmesini kısıtlanmasına sebep olabilir. İmmunojenite gen aktarımını sınırlayabilse de vektörün bu özelliği genetik immünizasyon sistemleri geliştirmek için avantaj da olabilir. Bu bağlamda literatürde HPV16, COVID gibi immunizasyonları geliştirmek için AAV vektörleri ile ilgili araştırmalara rastlanmaktadır. Örneğin, AAV-tabanında spike antijen genini taşıyan COVID-19 aşısı AAVCOVID maymunlarda SARS-COV2 enfeksiyonundan koruduğu ve nötralizan antikor düzeylerinin bir yıl süre ile saptanabildiği gösterilmiştir. AAVCOVID'in yüksek miktarda endüstriyel üretilebildiği ve bir (1) ay oda ısısında kalabildiği 2021 yılında Cell Host & Microbe dergisinde Zabaleta ve arkadaşları tarafından bildirilmiştir. Bunların da ötesinde, son yıllarda genom düzenleme aracı olarak AAV vektörlerin kullanılması da dikkat çekmektedir.

Sunum esnasında, yukarıda açıklanan AAV biyolojisinden gelen bilgiler ile AAV mühendisliğinde kullanılan transdüksiyon etkinliğini artırma ve immün cevaptan kaçınmaya yönelik stratejiler (*Bkz Şekil*), küçük laboratuvar ölçeğinde AAV vektör geliştirme ve üretim süreçleri yanı sıra, endüstriyel ölçekte üretim ve saflaştırma aşamalarını içeren 'upstream' ve 'downstream' süreçler ve bu süreçlere özel sorunlar (örneğin son üründe görülebilecek paketlenmiş boş plasmidler) ve olası çözüm yolları (doğru kromatografi yöntemi seçimi gibi), klinik denemelerde rekombinant rAAAV ile görülen toksisite ve bunlara bağlı mortalite olguları da bu sunum kapsamında tartışılmıştır.



Şekil: Kapsid mühendisliği ile değişik doku özgünlüğüne sahip ve immün cevaptan kaçabilen yeni AAV vektörleri geliştirme stratejileri (*Li C ve Samulski RJ, Engineering adeno-associated virus vectors for gene therapy, Nature Reviews Genetics, Volume 21, 2020* makalesinden adapte edilmiştir*).*

Fermente gıda ürünlerinde küfler

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Dünyada küf kullanılarak olgunlaştırılan çeşitli fermente gıda ürünleri bulunmaktadır. Bu ürünler arasında Uzak Doğu kaynaklı olmakla birlikte artık tüm dünyada tüketilmeye başlayan tempeh ve kojiyi, Avrupa'da ise küflü peynirleri sayabiliriz. Koji, pirinç ve soya gibi tahıl ürünlerinin küf yardımıyla şekerlendirilmesine dayanan bir ara üründür. Tahıllarda şekerlendirme, dünyada büyük oranda çimlendirmeye dayanan malt üretimi ile yapılmakta iken, Uzak Doğu'da *Aspergillus* türleri kullanılmaktadır. *Aspergillus* sporları haşlanmış tahıl ürünlerine eklenmekte ve fermantasyon ardından koji elde edilmektedir. Koji bir ara ürün olup, başka fermentasyonlar ile soya sosu, miso adı verilen fermente tahıl ezmesi, sake denilen Japon pirinç şarabı ve bu şarabın distilasyonu ile shochu içkisi veya şarabın oksidasyonu ile pirinç sirkesi elde edilebilmektedir. Üretilecek ürüne göre starter olarak değişik *Aspergillus* türleri kullanılabilmektedir. Örneğin, *A. oryzae* her tür koji üretiminde kullanılabilirken, *A. sojae* çoğunlukla soya sosu üretiminde, *A. luchuensis* ise çoğunlukla shochu üretiminde kullanılmaktadır. Küf kullanılarak üretilen başka bir fermente ürün olan Tempeh ise Endonezya kaynaklı bir fermente tahıl ürünüdür. Genellikle soya kullanılarak üretilen tempeh üretiminde *Rhizopus* türleri kullanılmaktadır. 1980 öncesi üretilen tempeh örneklerinden izole edilen küfleri *R. delemar* ve *R. arrhizus* (*R. oryzae*) türleri oluştururken, starter kültür kullanımının yaygınlaşması ile son yıllarda *R. oligosporus* türlerinin öne çıktığı gözlenmiştir. Dünya üzerinde küfle olgunlaştırılan ürünlere Avrupa kaynaklı bir örnek de küflü peynirlerdir. En iyi bilinen küflü peynir türlerinden biri, üretiminde *Penicillium roqueforti* türünün kullanıldığı mavi peynirlerdir. Bu peynirler arasında Roquefort ve Gorgonzola gibi peynirler verilebildiği gibi, ülkemizde üretilen küflü peynirler de bu gruba girmektedir. Avrupa tipi küflü peynirlerde genellikle starter kültür kullanılırken, ülkemizde kendiliğinden küflendirme yöntemi tercih edilmektedir. Ülkemizde üretilen küflü peynirlerden elde edilen küflerin çoğunluğunu *Penicillium* türleri ve *P. roqueforti* oluşturmaktadır. Avrupa küflü peynirlerinden ve başka substratlardan elde edilen *P. roqueforti* populasyonunun dört alt gruba ayrıldığı izlenmiştir. Türkiye *P. roqueforti* izolatlarının bu popülasyonlara benzer üyeler barındırdığı gibi, farklı özelliklere sahip kendine özgü izolatlara da sahip olduğu görülmektedir. Sonuç olarak, küfler dünyada çeşitli karakteristik fermente ürünlerin üretiminde starter kültür olarak kullanılmaktadır. Bu ürünler arasında fermente tahıl ürünlerinde *Aspergillus* ve *Rhizopus* türleri, peynirlerde ise *Penicillium* türleri öne çıkmaktadır. Küfler, gerçekleştirdikleri biyokimyasal reaksiyonlarla bu ürünlerin karakteristik renk, lezzet ve tekstürlerin ortaya çıkmasında önemli rol oynamaktadırlar.

Anahtar kelimeler: *Aspergillus*, koji; *Penicillium roqueforti*; *Rhizopus*, tempeh

Tarımda Yapay Zeka Uygulamaları

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Yapay Zeka, günlük hayatı kolaylaştırmak ve işleri maksimum doğrulukla yeniden tanımlamak amacıyla Bilgisayar Bilimleri Mühendisliği'nin öncü alanlarından biridir. Yapay Zeka, birçok farklı alanda kullanılmakta olup, tarım sektöründe, arazinin işlenmesinden bitki ekimine, hasattan sonraki işlemlere kadar birçok alanda yoğun bir şekilde kullanılmaktadır. Yapay zekanın tarım alanında uygulanmasının en büyük avantajı, tarımsal faaliyetlerin verimliliğini artırmaya yardımcı olabilmesidir.

Bu sunumda tarımsal yapılar ve sulama alanında yapay zeka konuları ile ilgili yapmış olduğum yaklaşık 20' ye yakın SCI ve SCIE dergilerde yayınlanmış çalışmalarımı anlatacağım. Tarımsal yapılar ve sulama alanında yapay zeka çalışmaları dört farklı alanda gerçekleştirilmiştir. Bu çalışmalar sulama ve hidroloji, tarımsal yapılar, sulama suyu kalitesi ve toprak bilimi ile ilgili yapay zeka çalışmalarından oluşmaktadır. Ayrıca TÜBİTAK tarafından desteklenen "Patlıcan Bitkisinin Sulama Programlamasının Belirlenmesinde Yapay Zekâ Uygulamalarının Kullanılması" ve "Tarım Alanlarının izleme ve değerlendirilmesine yönelik, Uzaktan algılama ve yapay sinir ağlarıyla yüksek çözünürlüklü evapotranspirasyon, verim ve su kullanım etkinliği haritalarının oluşturulması" isimli projeler hakkında bilgi verilmiştir. Birinci projede patlıcan bitkisinin sulama programlamasında yapay sinir ağları ve bulanık mantık uygulamaları kullanılmıştır. İkinci projede ise termal banda sahip Landsat 8 uydu görüntüleri ile geliştirilen yapay sinir ağları modeli ile termal bandı olmayan Kompsat 3 ve Rapid Eye uydu görüntüleri için yüksek çözünürlüklü termal görüntüler elde edilmiştir. Yer ölçümleri ile oluşturulan yapay zeka modelleri ile termal bandı olmayan Kompsat 3 ve Rapid Eye uydu görüntüleri için yüksek çözünürlüklü termal görüntüler elde edilmiştir. Yüksek çözünürlüklü termal görüntülerle yüksek çözünürlüklü evapotranspirasyon haritaları elde edilmiştir. Yapay zekanın tarım sektöründeki diğer uygulama alanları, hastalık tespiti, yabancı ot kontrolü, bitki sınıflandırma ve daha birçok alan bulunmaktadır. Geleneksel yöntemlerle bitki hastalığı ve yabancı ot tespiti genellikle görsel inceleme ile yapılırken, yapay zeka ile hastalıklı bitkilerin ve yabancı otların fotoğrafları ve spektral görüntüleri analiz edilerek daha başarılı tespitler yapılabilmektedir.

Tarım sektöründe yapay zeka uygulamalarının karşılaştığı bazı kısıtlamalar da vardır. Bunlar arasında, çiftçilerin yapay zeka çözümlerini araştırmak için yeterli zaman ve dijital becerilere sahip olmamaları, mevcut altyapının ve sistemlerin entegrasyon zorlukları, modellerin eğitilmesi ve doğru tahminler yapabilmesi için büyük miktarda veri gerekliliği, mekansal verilerin kolayca elde edilmesine karşın zamansal verilerin elde edilmesinin zorluğu, bitkilere özgü verilerin sıklıkla yılda bir kez elde edilmesi ve veri altyapısının olgunlaşma sürecinin zaman alması gibi faktörler yer almaktadır.

Gelecekte yapay zeka, tarım sektöründe daha fazla kullanılabilirliğe sahip olabilir. Yapay zeka şirketleri, çiftliklerde görevlerini yerine getirebilecek robotlar geliştirebilir ve bu robotlar bitkileri insanlardan daha hızlı ve ayrıntılı bir şekilde toplayabilirken aynı zamanda bitki kalitesini ve yabancı otların kontrolünü sağlayabilirler. Yapay zeka algoritmaları, uydu görüntüleri ve geçmiş verileri kullanarak böcek türlerini tespit edebilir ve çiftçilere hastalık-zararlı yönetimi konusunda yardımcı olmak için cep telefonlarına bildirimler gönderebilirler. Gelecekteki teknolojik gelişmeler, tarıma yönelik eğitim verileri, dronlar ve otomatik makine imalatı gibi yapay zeka temelli ürün ve hizmetler geliştirmek isteyen işletmelere destek sağlayabilir. Yapay zeka destekli teknolojiler, sıcaklık, yağış, rüzgar hızı ve güneş radyasyonu gibi verileri kullanarak bitkilerin su ihtiyacını, besin gereksinimlerini ve verimini analiz edebilir. Tarımsal uygulamalarda yapay zeka, tüm tarım sürecini geliştirmek için kullanılacaktır.

Geleneksel Ürünlerden Starter Kültür Geliştirme Stratejileri

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Fermantasyon 8000 yıl öncesine dayanan tarihi ile en eski gıda işleme ve muhafaza metotlarından biridir. Birçok bilim insanının katkıları ve Louis Pasteur'ün 19. y.y. sonlarında kanıtladığı bilimsel veriler ile fermantasyon alanındaki çalışmaların sayısı ve bilimsel kaliteleri artmıştır. 20 yy boyunca dünyanın farklı yerlerinde üretilen fermente gıdalar üzerine yapılan çalışmalar artmış bunun sonucu olarak bir çok ürün yöresel üretimden ulusal veya uluslararası üretimi gerçekleştirilen ticari ürünlere dönüşmüştür. Ülkemizde bilinen yoğurt, ayran, kefir, turşu, sucuk, boza ,alkollü içecekler yanında uluslararası anlamda kimchi, sauerkaraut, kumis, amasi, soya sosu gibi gıdalar ilk akla gelenlerden bazılarıdır. 2022 yılı itibari ile fermente gıdaların dünya ekonomisinde 575,8 milyar dolarlık bir hacme ulaştığı bilinmektedir.

Fermente gıdaların sahip olduğu ekonomik hacim ve insan sağlığına olan katkıları düşünüldüğünde insan beslenmesine katkısının her geçen gün artacağı açıktır. Ancak fermente gıdalardan starter kültür geliştirme süreci boyunca karşılaşılan bazı sorunların olduğu görülmektedir. İzolasyon için yeterli sayıda fermente gıdaya ulaşılamaması, uygun izolasyon ve identifikasyon metodlarının uygulanmaması, suş karakterizasyonun yeterli yapılmaması/yapılamama, kültürlerin depolama işlemlerinin sağlıklı yapılamaması ve uygun tuşların ticari boyutta üretilemi boyunca karşılaşılan güçlükler bunlardan bazılarıdır

Fermantasyonun anlaşılmasında mikrobiyal metabolizmanın keşfi ve mikroorganizmaların sağlıklı bir şekilde izolasyon ve tanı işlemlerinin yapılabilmesi en önemli iki gelişmedir. Günümüzde özellikle spesifik besiyerlerinin sayısındaki artış ile istenen mikroorganizma çeşitliliğinin belirlenmesi izolasyon için önemli imkanlar oluşturmuştur. İzolatların farklı moleküler yapılarını temel alarak geliştirilen SDS-PAGE, MALDI-TOF MS, PFGE, MLST, RAPD, Rep-PCR, DGGE, RT-PCR gibi tanı ve tespit metodlarının kullanılabiliyor olması ise mikroorganizmaların özelliklerinin belirlenmesinde ve tanısında her zamankinden daha iyi sonuçların alınmasını sağlamaktadır. Ayrıca herhangi bir nedenle kültüre edilemeyen türlerin belirlenmesi ve fermente gıdaların mikrobiyotalarının doğru bir şekilde belirlenebilmeleri için NGS (Next Generation Sequencing) kullanımı önemli imkânlar sunmaktadır. Ancak tüm bu imkânların kullanılabilmesi yetişmiş insan gücü ve alt yapı ile mümkün olabilecektir.

Quality of Biotechnological Medicinal Products

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Biotechnological medicinal products are produced in living organisms by using recombinant DNA technology. They vary in terms of manufacturing process, size, stability and immunogenicity from small chemical molecules. Biotechnological drugs, also called biopharmaceuticals, can be grouped as cytokines, enzymes, hormones, blood or plasma products, immunological products, monoclonal antibodies, gene and cell therapy products and peptide therapeutics (1). To accomplish the quality target product profile (QTPP), the manufacturing process needs to be well designed. The expression system, should be carefully selected. Appropriateness of the suggested formulation in terms of integrity, activity, strength of the active ingredient, stability, and compatibility (i.e., interaction with excipients, diluents, and packaging materials) should be evaluated The stability of the biotechnological product should be determined according to ICH Q5C guideline. The product's claimed shelf life should be supported by comprehensive stability data (2). Extensive state-of-the-art characterisation studies are required to assess the quality of biotechnological products. It is important to properly characterize the existence and degree of post-translational modifications, such as glycosylation, oxidation, deamidation, and truncation. Ligand or receptor binding assays, enzymatic assays, cell-based assays and functional assays can be used for determination of biological activity. The process-related or product-related impurity profiles of the biotechnological medicinal product should be determined by a combination of analytical procedures (3).

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Non-viral Delivery Systems for Gene Therapy

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Gene therapy aims treatment of genetic diseases by transferring functional genes into a human cell or removing the faulty gene (1). Gene therapy can be done in two ways either ex-vivo or in-vivo. Ex-vivo gene therapy is the removal of cells from the patient, transfer of the desired gene outside the body, and the returning the modified cells to the patient by transplantation or transfusion (2). In-vivo gene therapy is the replacement of missing genes or the administration of therapeutic genes to the patient in a drug delivery system, providing treatment without removing the patient gene from the cell. In order for a gene therapy to be successful, genes must be given to patients in a way that they can be expressed, they must be transferred stably to target cells and protected by serum nuclease enzymes (3).

Gene therapy medicinal products consist of a viral vector or non-viral delivery system containing a therapeutic gene. Nucleic acids such as DNA, RNA, siRNA, mRNA, CRISPR/Cas9 system, ZFNs and TALENs can be transferred into the cells by viral or non-viral delivery systems to accomplish nucleotide modifications, gene silencing, exon skipping and gene editing (4). Non-viral delivery systems have more advantages than viral vectors. They have low toxicity and they can be manufactured on a large scale.

The most commonly used viral vectors are retroviruses, adenoviruses, adeno-associated viral vectors, lentiviruses and herpes simplex virus (5). Non-viral gene transfer is done by physical methods, chemical methods or drug delivery systems. Physical methods such as electroporation, microinjection, particle bombardment and sonoporation deliver naked DNA molecules directly to the cytoplasm, while protecting them from enzymatic degradation by skipping the endosomal and lysosomal stages. These methods use a physical force to overcome the membrane barrier of cells. Polymeric or lipidic, particular or vesicular drug delivery systems can be prepared on nano size and their surfaces can be funtionalized and they can be targeted to tissues or cells (6). Non-viral gene delivery systems consisting of lipids and polymers generally include many systems such as polymeric and metallic nanoparticles, liposomes, niosomes, polymeric micelles, nanogels, nanocapsules, dendrimers, carbon nanotubes, nanocrystals and solid lipid nanoparticles in the size range of 10-1000 nm (6, 7).

The gene delivery system that can efficiently transport therapeutic genes to the target cell must be able to carry a large gene, ensure the expression of the gene it carries at the desired level and duration, be reproducible and validated, be specific, have low immunogenicity and remain stable under specified storage conditions (8).

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Mikrobiyal polimerlerin üretiminde biyoproses süreçlerinin geliştirilmesi ve optimizasyonu

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İnsan nüfüsundaki hızlı artış, buna bağlı olarak ortaya çıkan artan enerji gereksinimleri, doğal kaynakların aşırı tüketimi, ve sanayi alanındaki gelişmeler, kontrol edilemeyen çevre kirliliğine sebep olmuştur. Çevresel sorunlar ve bu konudaki tartışmalar yüzyıllar öncesine dayansada, ondokuzuncu yüzyıla gelindiğinde, çevresel sorunların artması ve buna bağlı olarak iklim değişikliği, ciddi önlemlerin alınması konusunu çok daha fazla tartışılır hale getirmiştir. Fakat çevreye verdiğimiz olumsuz etkiler ve sera gazlarındaki artış yıllar boyunca artarak devam etmiştir. Yirminci yüzyılın ortalarına gelindiğinde 'insanların ihtiyaçlarını, gelecek nesillerin de aynısını hatta daha iyisini yapmasına olanak tanıyacak şekilde yönetmeye çalışması' anlamına gelen sürdürülebilirlik kavramı bugünkü anlamı ile hayatımıza girmiştir. Bu doğrultuda Birleşmiş Milletlere üye ülkeler, Eylül 2015'de, gezegenimizi korumak amacı ile 17 başlık altında, 2030 yılında tamamlanacak, yol haritasını belirlemişler ve 'Sürdürülebilir Kalkınma Amaçları' üye ülkeler tarafından kabul edilmiştir. Bunun yanı sıra, Avrupa Birliği, Aralık 2019 da, 2050 yılında Avrupa kıtasını karbon-nötr kıta haline getirmek için, 'Avrupa Yeşil Mutabakatı' açıklamıştır. Eğer radikal önlemler alınmaz ise gelecek, daha fazla sera gazı, buna bağlı olarak da, daha fazla iklim değişikliği ve doğal afetler demektir.

Sera gazı oluşumuna sebep olan çok farklı etmekler bulunmaktadır. Bu etkenlerden biri de fosil bazlı plastiklerdir. Literatüre göre 2019 yılında, fosil bazlı plastiklerin yaşam döngüleri boyunca yol açtığı toplam sera gazı emisyonları 1,8 gigaton karbondioksitdir. Her ne kadar plastiklerin geri dönüştürülmesine yönelik büyük çabalar harcansa da, plastiklerin sadece %10-15 kadarı geri dönüştürülmekte, geri kalan kısmı ise doğada birikmektedir. Plastiklerin doğada bozunmasının onbinlerde yıl sürmesi, biyobazlı, biyolojik olarak parçalanabilir ya da her iki özelliği de taşıyan plastik arayışını ortaya çıkarmış ve günümüzde bu konuda bir çok ülkede zorlayıcı tedbirler alınmaya başlanmıştır.

Önümüzdeki yıllarda yıllık 100,000 tonun üzerindeki hacimlere ulaşması beklenen ticari bir pazara sahip olan polihidroksialkalonatlar (PHA), tamamen bozunabilen biyopolimer olarak son yıllarda oldukça ilgi çeken bir biyopolimer ailesi olarak karşımıza çıkmaktadır.

Mikrobiyal polimerlein üretiminde en önemli parametre hücre başına üretilen polimer miktarında artış sağlanmasıdır. Bu doğrultuda, öncelikle üretici mikroorganizmanın geliştirilmesi, sonrasında besi yeri ve çevre koşullarının optimizasyonu, ve üretim modunun belirlenmesi gerekmektedir.

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Plant-Based Metabolite Production Systems

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Plant primary metabolites are essential compounds produced by plants as part of their normal growth, development and basic metabolic processes. These metabolites are involved in fundamental biological functions and are crucial for the plant's survival and growth. Plant secondary metabolites are organic compounds that are not *directly* involved in the essential growth and development of plants, but they play crucial roles in the plant's interactions with its environment. These compounds often serve as defence mechanisms against herbivores, pathogens, and environmental stressors. Plant biotechnology tools are the robust one which can be exploited to induce primary or secondary metabolites in diverse plant species. The optimum level of the metabolites could be attained by fine tuning the in vitro culture of plants via phytohormones, mineral nutrients or nanoparticles. The targeted metabolites with high level of content can be achieved by genetic transformation of the genes into plastid or nucleus of the plant cells.

The advantages of plant-based metabolite productions are (1) they are economically more convenient due to the fact that there is no need for cold chain transportation, (2) there is no need to worry about being contaminated by toxins and pathogens that usually occur in bacterial vaccine production, (3) there is no reverse virulence event, (4) it is easier to edit (increase and decrease) the production scale, and (5) it is extremely easy and practical to store.

Plant-based metabolites can be produced with two systems; (1) *nuclear transformation*: It is a system with a relatively low production capacity, and (2) *chloroplast (transplastomic) transformation*: It is a system with a much higher production capacity. The chloroplast transformation system, in which the desired gene is transferred to a large number of chloroplast organelles present in the cytoplasm instead of the nucleus only (i.e., nuclear transformation) of the plant cell.

Patates Islahında Biyoteknolojik Yaklaşımlar

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Patates dünyada mısır, çeltik ve buğdaydan sonra en fazla üretimi yapılan dördüncü bitki olup tahıllar dışında ise en fazla üretilen bitkidir. Geniş adaptasyon yeteneği nedeniyle halen dünyada insan yerleşiminin olduğu tüm kıtalarda 160'tan fazla ülkede üretilmektedir. Kısa yetişme süresi, birim alandan yüksek verim potansiyeli ve zengin besin içeriği ile gıda güvenliği açısından stratejik öneme sahip bitkilerden birisidir. Bu nedenle birçok ülkede uzun zamandır patates ıslah çalışmaları yürütülmektedir. Patateste ıslah edilen yeni bir çeşidin üretici ve tüketiciler tarafından kabul edilmesi ve pazar payı bulabilmesi için yüksek yumru verimi ve kalitesine sahip olması yanında önemli hastalıklara ve depolamaya dayanıklı, tüketici tecihlerine uygun olması gerekmektedir. Son yıllarda kuraklık, yüksek sıcaklık, tuzluluk gibi abiyotik stres faktörlerine tolerans da ıslah amaçları arasına girmiştir. Bu nedenle patates ıslah programlarında çok yönlü seleksiyonlar yapılması gerekmektedir. Ancak patatesin yumruları ile vejatatif çoğalan bir bitki olması, tetrasomik kalıtıma sahip olması, yüksek heterozigotluk nedeniyle istenmeyen özelliklerin de döllere aktarılması nedeniyle çeşit ıslahı zor bir bitkidir.

Klasik ıslah yöntemleri uygulanarak istenilen özellikte çeşit geliştirmek hala günümüzde kullanılan en etkin yöntemlerden biri olmakla birlikte ıslah programının süresi, seleksiyonun etkinliği ve maliyeti göz önünde bulundurulduğunda ıslah programlarının güncel biyoteknolojik gelişmelerle desteklenmesine ve daha etkin ve pratik yöntemlerin geliştirilmesine ihtiyaç vardır. Patates genomunun dizilenmesi ve NGS teknolojilerinin gelişmesiyle patateste biyoteknolojik alanda önemli gelişmeler olmuştur. QTL haritalama ve genom boyu ilişkilendirme çalışmalarının (GWAS) yanı sıra artık patateste alternatif olarak genomik seleksiyon yöntemi de kullanılmaya başlanmıştır. Bu sistemin uygulanmasında hala bazı zorluklar olsa da ıslah sürecinde kolaylık sağlaması heyecan verici bir gelişmedir. Multi-omiks teknolojileriyle birlikte artık sadece transkriptom seviyesinde değil bir deneme uygulandığında metabolomik ve proteomik verileriyle daha detaylı bir ağ oluşturulup özellikle kompleks özelliklerin aydınlatılması sağlanabilecektir ve alel madenciliği çalışmalarına çok önemli katkılar sağlayacaktır. Patates, transgenik yöntemin uygulandığı ilk bitkilerden biri olsa da artık günümüzde yemeklik ve hayvan yemi olarak ticari olarak üretilen transgenik patates çeşitleri mevcuttur. Patates, genom düzenleme yöntemleri için kullanılan model organizmalardan biridir ve çok önemli başarılar elde edilmiştir. İleride özellikle diploid patates ıslahı için çok önemli katkıları olacağı düşünülmektedir.

Bu bildiride, moleküler ıslah yöntemleri, markör destekli seleksiyon, haritalama çalışmaları, genomik seleksiyon, omiks ve multi-omiks teknolojileri, genom düzenleme ve gen aktarım yöntemleri ile bugüne kadar yapılmış olan çalışmalar ele alınmış olup bu yöntemlerin ilerleyen dönemlerde mevcut ıslah çalışmalarına nasıl katkıda bulunabileceği hakkında detaylı bir tartışma yapılmıştır.

BAHÇE BİTKİLERİNDE DOKU KÜLTÜRÜ UYGULAMALARI

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Tarımın meyve, sebze, asma ve süs bitkileri yetiştiriciliği üzerine çalışılan dalı olan bahçe bitkilerinde nüfus artışı, göç artışı, değişen iklim-toprak koşulları gibi nedenlerle ürün miktarına ve çeşitliliğine olan talep her geçen gün artmaktadır. Tohumla veya çeşitli vejetatif yöntemlerle üretilen bahçe bitkilerinde hem birim alandan elde edilecek veriminin arttırılması, hem de ürün çeşitliliğinin sağlanması yönünden ıslah çalışmaları ile yeni çeşitlerin geliştirilmesi bir zorunluluktur. Tohumla üretime dayalı sebzecilik sektöründe hem açıkta hem de örtüaltı yetiştiriciliğinde verim, kalite, hastalıklara dayanıklılık, abiyotik stres faktörlerine tolerans yönünden F_1 hibrit çeşitlerin kullanımı yaygınlaşmaktadır. Genelde vejetatif üretime dayalı meyvecilik sektöründe ise hastalıklardan arındırılmış sertifikalı fidan ihtiyacı ile farklı ticari çeşitlere olan talep sürekli artmaktadır. Tüm bunlar tohuma ve özellikle vejetatif üretime dayalı bahçe bitkilerindeki uzun süren çeşit ıslahı sürecinin kısaltılmasını, bunun için de başta bitki doku kültürü (BDK) olmak üzere biyoteknolojiden faydalanılmasını zorunlu kılmaktadır.

Temeli totipotensi (bütünü verme yeteneği) ve kompetens (farklılaşma yeteneği) kavramlarına dayanan BDK nde çeşitli bitki eksplantları (tohum, embriyo, organ, doku, hücre veya protoplastları) steril koşullarda ve yapay besin ortamlarında kültüre alınarak yeni bitki, bitki parçacıkları veya bitkisel ürünler üretilir. 1902'deki ilk aseptik kültür denemesi ile başlayan BDK; başta iyileştirilmiş ticari çeşitlere artan talep, sürdürülebilir tarım uygulamaları, bitki genetik kaynaklarının korunması ihtiyacı, genetik mühendisliği ve teknolojideki gelişmeler gibi nedenlerle günümüzde önemi gittikçe artan ve hızlı büyüyen bir sektör olup, 2030 yılına kadar pazar değerinin 900 milyon dolara ulaşması beklenmektedir. Klasik kültür yöntemlerine göre çok daha küçük bir alanda bitkisel üretime imkan tanıyan BDK ile zamandan, yerden, emekten, işçilikten ve ekonomiden tasarruf sağlanabilirken, bitki hücre ve protoplastlarının kültür edilebilmesi ve manipulasyonlarına olanak sağlanması gibi avantajlarla bitkinin normal gelişim seyri değiştirilerek farklı tekniklerle rejenerasyon sağlanabilmektedir. BDK nün en fazla uygulandığı bitki grubu bahçe bitkileri grubu olup, bu grupta uygulanan BDK teknikleri; mikroçoğaltım, meristem kültürü, embriyo kültürü veya embriyo kurtarma, dihaploid (doubled haploid = DH) bitki üretimi (ovül, ovaryum, anter, mikrospor, shed-mikrospor kültürleri), somatik embriyogenesis, sentetik tohum üretimi, somaklonal varyasyon, kimerik bitki üretimi, *in vitro* sekonder metabolit üretimi, *in vitro* mutasyon - *in vitro* seleksiyon, germplasm muhafazası (kriyoprezervasyon), gen transferi ve bitki rejenerasyonu, protoplast kültürü ve füzyonu (somatik hücre melezlemesi) olarak sıralanabilir.

Sebzecilik sektöründe BDK tekniklerinden; tohum yoluyla çoğaltılması zor olan bitkilerin üretimi, genetik olarak özdeş (klon) bitkilerin kitlesel üretimi, gen kaynaklarının muhafazası, virüssüz bitki materyallerinin üretimi, ürün iyileştirme ve ıslah sürecinin kısaltılması amacıyla faydalanılmaktadır. BDK teknikleri meyvecilikte; anaçların-germplazmların çoğaltılması-muhafazası, talebi yüksek veya yeni ıslah edilen bitki çeşitlerinin kitlesel üretimi, virüssüz, hastalıksız-patojensiz bitkilerin üretimi ve ıslah programları için haploid bitki üretimi amacıyla kullanılırken, süs bitkileri sektöründe ise ticari çeşitlerin hızlı kitlesel üretimi, yeni çeşit veya genotiplerin klonal üretimi, hastalıksız bitki üretimi, somaklonal varyasyon ve ıslah amacıyla yararlanılmaktadır.

Çok çeşitli teknikleri içinde barındıran ve çok geniş kullanım alanlarına sahip BDK sektörü yeni araştırma alanlarıyla büyümeye devam etmekte ve etki alanını genişletmektedir. En güncel konular ve yeniliklere; bitki kök hücrelerinin kozmetik sektöründe kullanımı, nanoteknolojinin BDK ne entegrasyonu ve alacalı kimerik süs bitkilerinin üretimi örnek gösterilebilir. Sonuç olarak BDK nün en kapsamlı uygulama alanı

tüm bitki grupları arasında bahçe bitkileridir. Hızla artan nüfusun ihtiyaçlarını karşılamak için verimliliği ve çeşitliliği artırmak amacıyla bahçe bitkilerinin kitlesel olarak çoğaltılması, ürünlerin kalitesi ve beslenme değerlerinin arttırılması ve hızlı ıslah teknikleri ile iyileştirilmiş yeni çeşitlerin geliştirilmesinde BDK teknikleri son derece etkili ve önemli bir role sahiptir.

Fermantasyon ve diyet polifenollerinin dönüşümü

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Fermantasyon geleneksel ürün üretimi için çok eski yıllardan beri kullanılmakta olup son yıllarda atıkların değerlendirilmesi ve biyoaktiviteyi artırma gibi farklı amaçlarla kullanımı da araştırılmaktadır. Fermantasyon gıdalardaki fenolik bileşikleri doğrudan ya da dolaylı olarak etkileyebilmektedir. Başlıca etkisi fenolik bileşiklerin matristen salımını artırması olup fermantasyon süresince mikroorganizmaların sahip oldukları enzimler tarafından fenolik bileşikler parçalanmakta ve böylelikle fenolik bileşikler serbest ve erişilebilir hale geçmektedir. Fermantasyonun gıda ürünlerindeki bir diğer etkisi ise fenolik bileşiklerin daha basit yapıya biyotransformasyonudur. Fermantasyonda görev alan mikroorganizmalar, fenolik bileşiklerin biyotransformasyonuna neden olan enzimlere sahiptir. Örneğin brokoli püresinin fermantasyonunda klorojenik asit, *Lactiplantibacillus plantarum, Limosilactobacillus fermentum, Lm. reuteri, Levilactobacillus spicheri* mikroorganizmalarının sahip olduğu klorojenik asit esteraz, hidroksisinnamik asit redüktaz enzimleri sayesinde kafeik asit, kinik asit, dihidrokafeik asit gibi metabolitlere dönüşebilmektedir. Böylelikle fermantasyon ile fenolik bileşik kompozisyonunu değiştirerek daha yüksek biyoaktiviteye sahip gıda üretimi mümkündür. Ancak fermantasyonun farklı fenolikler ve gıda matrisi üzerindeki etkisi değişken olabilmektedir. Bu nedenle farklı gıda ürünleri için farklı mikroorganizma türü ve fermantasyon koşullarına göre işlem istenilen doğrultuda optimize edilmelidir. Benzer şekilde, gıda ürünlerinde fermantasyon sonrası fenolik madde miktarı çoğunlukla artış göstermesine rağmen, *in vitro* sindirim sonrasında gıdaların biyoaktif özellikleri değişkenlik gösterebilmektedir. Fermantasyon, gıdalardaki fenoliklerin biyoyararlılığını artırma potansiyeline sahip gibi görünse de kullanılan spesifik mikroorganizmaların ve gıda matrisinin etkisini analiz etmek için daha fazla çalışmaya ihtiyaç vardır.

BİOTÜRKİYE: SÜRDÜRÜLEBİLİRLİK VE BİYODÖNÜŞÜM

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Ülkemizde; ekonomik katma değer ve ileri teknolojik ürün ihracatının, endüstriyel simbiyoz ve döngüsel ekonomi çalışmalarının artmasıyla mümkün olabileceği döneme giriyoruz.

Sürdürülebilirlik temelinde bir kalkınma, doğanın küçük bir parçası olan insanın bu gerçekle yola çıkarak ekosistemlerin ihtiyaçlarının karşılanması, gelecek nesillerin ihtiyaçlarının gözetilmesi için biyoçeşitliliğin etkin planlanması ve çevresel faktörlerin, ekonomik ve ekolojik sürecin birlikte planlanması ile mümkün olacaktır. Sürdürülebilir kalkınmanın temelinde en önemli değer ekolojik sürdürülebilirliktir. Gelecek nesillerin ihtiyaçlarının karşılanabilmesi için endüstride tanımlanacak ekonomik süreçlerin ekolojik tabanda planlanması önemlidir. Ekonomik süreçte bizler için en değerli faktörlerin başında; materyal verimliliği, enerji verimliliği, Ar-Ge insan kaynağı ve Ar-Ge bütçeleri gelmektedir. Bu süreçte en verimli olduğumuz noktaların; hammadde, materyal ve enerji verimliliği olduğu belirtilmektedir. Bu noktalarda coğrafi konum itibariyle bulunduğumuz avantajlı konum ve insan kaynağı bu süreci daha da hızlandıracak potansiyele sahiptir. Ar-Ge ve Ür-Ge çalışmalarına verimli ve nitelikli genç nüfusumuzu entegre ederek teknolojide daha yüksek yüzde ile katma değer üretebilmemizin mümkün olduğunu düşünüyorum. Sürdürülebilirliğin etkin planlamasında inovasyon sürecindeki değişimi de gözlememiz oldukça değerli olacaktır. Günümüzde yenileşme mekanik yeniliklerden daha çok biyolojik yeniliklere aittir. Bu doğrultuda; biyolojik yeniliklerin kavram ve çalışmaların bu tabanda planlanabilmesi için farklı terimlerin günlük hayatımıza yerleşmesi önemlidir. Eğitim ve öğretimde "Bio-eğitim" ile "Bio-ekonomik" hammadde dönüşümünün farkındalığının sağlanması gerekmektedir. "Bio" tabanında başlayacak çalışmaların toplumsal faydayı sağlaması için "Bio-etik" çerçevenin korunması ve "Bio-çeşitliliğin" sürdürülebilmesi geleceğimiz için önemli olacaktır. Gelecekte küreseldeki nüfusun artacak olması hammadde ve besin ihtiyaçlarının karşılanması için bio-çeşitliliği önemli kılmaktadır. İklim krizi ve hammadde sürdürülebilirliğinin önümüzdeki süreçte tehlike altında olması nedeniyle özellikle "Bio-ekonomi" ülkemiz için oldukça önemli bir fırsat alanı olacaktır. Bio-ekonomi strateji olarak oldukça önemli olup ülkelerin bio-politikaları içerisinde etkin yer alması dikkat çekmektedir

BioTürkiye'nin bu strateji kapsamında oluşturacağı planlama ve verebileceği yönün çok değerli olacağına inanıyorum. Önümüzdeki yıllarda Biyoteknolojide ilk sıralarda TÜRKİYE olması bu stratejiye ve planlamaya yaklaşımla çok daha hızlı gerçekleşecektir.

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Biotechnology: Past, present and future of Agri-Food Biotechnology

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Biotechnology is the use of biological processes, organisms or biosystems to develop new and different products that are expected to improve human life. The history of biotechnology began with agricultural practices to meet the food needs that emerged as a result of human beings starting to live in communities around 12,000 BC, and progressed with fermentation technologies in bioreactors in 1940s. In prehistoric times, a primitive form of biotechnology called ancient biotechnology was practiced by agriculturalists who created higher quality plant and animal species through cross-pollination or hybridization of crops, breeding of animals, and the use of microorganisms to produce foods such as cheese and yogurt, bread, beer and wine. In 1980s, advances in molecular biology and recombinant DNA technology developed modern biotechnology which primarily uses genetic engineering to produce new products and/or substances in the field of agriculture, food, health and defense industries. Modern biotechnology covers a wide range of disciplines, including bio-molecular sciences, genetics, developmental biology, cell biology, biochemistry, microbiology, animal sciences, plant sciences, neurobiology and psychology, human diet and health, biomaterials science, soil science and related areas of engineering. Biotechnology has applications in four major subfields, called red, white, green, and blue. Red biotechnology is applied to medical processes to produce medicine and drugs such as pharmaceutical drug discovery and production, pharmacogenomics, and genetic testing. Green Biotechnology is applied to agricultural processes involving the use of scientific tools and techniques such as genetic engineering, molecular markers, molecular diagnostics, vaccines, and tissue culture, to modify living organisms including plants, animals, and microorganisms for improving the nutritional quality, quantity and production economics. White biotechnology involves industrial processes used for sustainable manufacturing new chemicals, biomaterials and alternative energy sources. Blue biotechnology covers the marine and aquatic applications of biotechnology which used to improve the health, reproduction, development and growth of aquatic organisms, to control proliferation of noxious water-borne organisms, and develop new drugs. In the future, the development and use of modern biotechnology could help not only meet the rapidly growing need and demand for energy, food, healthcare and appropriate environmental management, but also increase productivity and create new jobs. However, the advancement in this fields has also the potential for misuses that lead to some concerns and controversies about the ethical, legal, and social implications of biotechnology.

Keywords: Agri-Food biotechnology, genetic engineering, recombinant DNA , bioprocess, fermentation

Next Generation Biological/Biotechnological Medicinal Products

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Second and next generation biological/biotechnological drugs are developed to provide some advantages in terms of quality, efficacy and safety compared to first generation products and to find solutions to rare diseases that are impossible or very difficult to treat. Although it took many years for the first generation products to reach patients, the next generations were introduced to human health quickly and in increasing numbers.

First generation biotechnological medicinal products were recombinant proteins, monoclonal antibodies (mAbs) and vaccines, that led to revolutionary changes in the pharmaceutical industry from 80s to 2000s. Since the early 2000s new versions of first-generation products such as polymer-protein conjugates, fusion proteins, mAb fractions, bispecific mAbs and antibody-drug conjugates have been introduced to human healthcare (1,2)

Nucleic acids or closely related chemical compounds have been on the rise in recent years as an emerging new class of pharmaceuticals. They modulate gene expression by adding, replacing, inhibiting, or editing at the DNA or RNA level. DNA therapeutics, antisense oligonucleotides (ASOs), micro RNAs, short interfering RNAs(siRNA), and mRNAs appear to be the most promising nucleic acid modalities as new generation biologics. In the last two years, the Covid-19 pandemic accelerated the rapid introduction of DNA and RNA products to clinic in the form of vaccines.

This presentation will review and present new generation biologic/biotechnological medicines.

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BIOTECHNOLOGICAL APPROACHES IN MAIZE BREEDING

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Maize is the most productive grain in terms of volume produced and also the most traded cereal. The population of the world is predicted by the UN to reach 9.7 billion people by the year 2050. Food security will become very important subject for the near future. It is necessary to increase corn production to overcome future food security problems. Maize production can be increased by the effective breeding methodologies using biotechnological approaches. This presentation provides a brief overview of genomics tools and genome editing implications in maize breeding. Genomic tools have revolutionized maize breeding by enabling more efficient and precise selection of desirable traits. Especially, marker assisted selection (MAS) have been used in maize breeding program for selecting diseases resistance or high yield individuals or other desirable traits. Another approaches of genomic tools is genomic selection which predicts a plant's breeding value by utilizing extensive genetic data. Breeders can predict which plants will perform best overall with greater accuracy by examining a wide variety of genetic markers and phenotypic data. Next-generation sequencing technologies allow researchers to sequence the entire maize genome quickly and cost-effectively. This contributes to trait discovery and marker development by offering a thorough understanding of maize's genetic composition. Genome-Wide Association Studies (GWAS) identifies associations between specific genetic markers and traits by analyzing genetic variation across a broad population of maize plants. This helps identify candidate genes responsible for important traits. The use of genome editing technology in maize breeding has recently emerged as a groundbreaking advancement. This revolutionary technology allows breeders to directly modify specific genes in maize plants to introduce or remove desired traits. It has the potential to rapidly accelerate the development of new maize varieties.

Transgenik Hayvan Modelleri

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Uzun yıllardan beri transgenik hayvan çalışmalarında birçok klasik yöntem kullanılmıştır. Ancak son yıllarda Transgenik hayvan üretiminde genom düzenleme teknikleri kullanılmaya başlandı. CRISPR/Cas9 genom düzenleme teknolojisi son yıllarda hızla gelişmektedir. Dokuya özgü nakavt farelerin (KO) üretilmesine yönelik geleneksel strateji, *in vivo* gen fonksiyonunun hızlı işleyişini kısıtlayan, zaman alıcı ve emek yoğun bir süreçtir. CRISPR/Cas9 sistemi basit ve etkili bir gen düzenleme tekniğidir; Bu yöntem, CRISPR/Cas9'un doğrudan zigotlara enjekte edilmesiyle nakavt farelerin kısa sürede hızlı bir şekilde elde edilebilmesini sağlar.

CRISPR/Cas9 sistemi önemli genlerin düzeltilmesi amacıyla hayvanlar, insanlar, bitkiler, bakteriler gibi birçok organizmada genler arzu edilen değişikliklere uğramıştır. Bu teknikte maya, Drosophila, maymunlar, tavşanlar, domuzlar, sıçanlar ve fareler de kullanıldı. 2015 yılında Çinli bilim insanları, 3 çekirdekli insan zigotlarındaki hastalıklı insan beta globin genini düzeltmek için CRISPR/Cas9 teknolojisini kullandı.

Şu anda hassas genom mühendisliği için mevcut olan tasarımcı nükleaz sistemlerinden CRISPR/Cas9 sistemi en mükemmeli gibi görünmektedir. Son yıllarda CRISPR/Cas9 sistemi kullanılarak yüzlerce transgenik hayvan kolay, ucuz ve hızlı bir şekilde üretildi.

CRISPR/Cas9 tekniğinin gen ve hücresel tedavilerde, knock-out ve knock-in hayvan üretiminde rutin olarak kullanımdadır. Ayrıca klinikte, özellikle genetik kökenli hastalıkların tedavisinde faz denemeleri devam etmektedir.

Ayrıca bu teknoloji 2022 yılında Nobel ödülünü aldı. CRISPR/Cas9 ile bilim insanlarının görüşü ise; bu teknolojinin 21. yüzyılın en büyük teknolojisi olduğu yönündedir.

Anahtar Kelimler: CRISPR/Cas9**,** Transgenik Fare, Knock-out, Konock-in

Transgenic Animal Models

Many classical methods have been used in transgenic animal studies for many years. However, in recent years, genome editing techniques have been used in transgenic animal production. CRISPR/Cas9 genome editing technology has been developing rapidly in recent years. The traditional strategy for generating tissue-specific knockout mice (KO) is a time-consuming and labor-intensive process that constrains the rapid functioning of *in vivo* gene function. The CRISPR/Cas9 system is a simple and effective gene editing technique; This method enables a rapid retrieval of knockout mice in a short time by injecting CRISPR/Cas9 directly into zygotes.

The CRISPR/Cas9 system has undergone desired changes in many organisms such as animals, humans, plants, bacteria, in order to correct important genes. Yeast, Drosophila, monkeys, rabbits, pigs, rats and mice were also used in this technique. In 2015, Chinese scientists used CRISPR/Cas9 technology to fix the diseased human beta globin gene in 3-nucleated human zygotes.

Of the designer nuclease systems currently available for precision genome engineering, the CRISPR/Cas9 system appears to be the most perfect. In recent years, hundreds of transgenic animals have been produced easily, cheaply and quickly using the CRISPR/Cas9 system.

The CRISPR/Cas9 technique is routinely used in gene and cellular therapies, knock-out and knock-in animal production. In addition, phase trials continue in the clinic, especially in the treatment of genetic diseases.
In addition, this technology received the Nobel Prize in 2022. The opinion of scientists with CRISPR/Cas9; that this technology is the greatest technology of the 21st century.

Keywords: CRISPR/Cas9**,** Transgenic Mice, Knock-out, Knock-in

Haploid Bitkiler *in vitro* Uygulamalar

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Biyoteknoloji, ıslah programlarının etkinliğini artırmak ve ıslah sürecini kısaltarak belirlenen hedeflere daha hızlı ulaşmak için önemli bir teknoloji alanıdır. Biyoteknolojik yöntemler arasında haploid teknolojisi, bitki ıslahına yardımcı olmak için uzun zamandır etkin bir şekilde kullanılmaktadır. Haploidler ve dihaploidler (DH), bitki ıslahında çok önemlidir ve homozigot hatların üretilmesi için gereken sürenin geleneksel ıslaha kıyasla büyük ölçüde kısaltılmasını sağlar. Hibrit ıslahının temel kuralı, melezlemede kullanılacak olan ebeveyn hatların homozigot olmasıdır. Bitkilerde homozigot hatlar geleneksel olarak kendileme ıslahı yöntemiyle elde edilmektedir. Özellikle yabancı döllenen bitkilerde kendileme ile homozigot hatların elde edilmesi 6-8 generasyon sürmektedir. Bu nedenle son yıllarda hibrit ıslahında, *in vitro* haploid ve DH teknolojisi yaygın olarak kullanılmaktadır. Bu teknolojide öncelikle haploid bitkiler elde edilmekte ve sonrasında haploid bitkilerde kromozom katlaması yaparak DH yani saf (%100) homozigot bitkiler elde edilmektedir. *In vitro* koşullarda haploid bitki üretimi için en çok kullanılan iki yöntem, androgenez (anter ve mikrospor kültürü) ve ginogenez (yumurta ve yumurtalık kültürü) yöntemleridir. Son yıllarda özellikle Cucurbitaceae familyasında, ışınlamış polen tekniği ile partenogenetik embriyo oluşumu yaygın olarak kullanılmaya başlanmıştır. Günümüzde androgenez, haploid bitki üretimi için en çok tercih edilen yöntemdir. Haploid bitki rejenerasyonu, donör bitkinin genotipi, alındığı mevsim, fizyolojik ve büyüme durumu gibi çeşitli faktörlerden etkilenir. Özellikle bitkinin genotipi başarıyı doğrudan etkilemektedir. Genel olarak çift çenekli bitkiler tek çenekli bitkilere göre daha başarılı sonuçlar vermektedir. Haploidler, saf homozigot hatların geliştirilmesine, ıslah döngüsünün veya süresinin kısaltılmasına, gelişmiş genetik saflığa, QTL haritalaması için etkin popülasyona, markör geliştirmeye ve markör destekli ıslah programını hızlandırmaya yardımcı olmaktadır. Bu nedenle, DH teknolojisinin birçok üründe ıslah programının hızlandırılmasında önemli katkıları bulunmaktadır.

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Biobenzer İlaçlar

Münis Dündar

Biyolojik tedaviler, doğal kaynaklardan izole edilen ve/veya biyoteknolojik yöntemlerle üretilen ürünlerdir. Biyolojik tedaviler, aşılar, kan ve kan bileşenleri, alerjenikler, somatik hücreler, gen terapisi, dokular ve rekombinant terapötik proteinler gibi geniş bir ürün yelpazesini içerir. Biyolojik tedaviler karbonhidratlar, proteinler, nükleik asitler ve bu elementlerin kombinasyonlarından oluşabilir ve/veya hücreler ve dokular gibi canlı varlıklar olabilir. Biyolojik tedaviler canlı hücrelerde yapıldığından, süreçlerde ve sistemlerde küçük değişiklikler bile biyolojik üründe farklılıklara neden olabilir. Karşılaştırılabilirlik genellikle yalnızca analitik verilere dayalı olarak gösterilebilir (yani fizikokimyasal özellikler, biyolojik aktivite, immünokimyasal özellikler, saflık ve stabilite), ancak bazen klinik veya klinik olmayan çalışmalardan farmakokinetik ve/veya farmakodinamik, klinik etkinlik, spesifik güvenlik, immünojenisite ve farmakovijilans çalışmaları gibi ek kanıtlar istenebilir. Pazarlanmadan önce biyolojik tedavileri de içeren tüm yeni ilaçlar düzenleyici bir makamın onayından geçmelidir. ABD'de biobenzer ürün, klinik olarak inaktif bileşenlerdeki küçük farklılıklara rağmen, bir referans ürüne oldukça benzer bir biyolojik ürün olarak tanımlanır. Çoğu biyolojik ürün, düzenleyici onay başvurusunun bir parçası olarak devam eden pazarlama sonrası güvenlik izleme (farmakovijilans) programlarına sahiptir. Bunun nedeni, klinik çalışmaların genellikle tüm potansiyel güvenlik sorunlarını, özellikle de nadir olanları belirlemek için çok sınırlı olmasıdır.

Keywords: Biobenzer, İlaç, Farmakodinamik, Farmakokinetik, biyolojik tedavi

RNA Müdahalesi (RNAi) ve Tarımda Kullanım Potansiyeli

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RNA müdahalesi (RNAi) bitkilerde spesifik genleri susturmanın çok özel bir yoludur. Bitkinin kendisine ürettirilen (transformatif) veya harici olarak verilen (transformatif olmayan) çift sarmal mRNA bitkinin kendisinde doğal olarak bulunan doğrayıcı adı verilen bir enzim kompleksi ile siRNA adı verilen 20-24 baz uzunlukta küçük RNA parçalarına ayrılır. Çeşitli proteinlerden ve RNA'dan oluşan RISC kompleksi adı verilen ikinci bir diğer doğal enzim kompleksi siRNA'ları rehber olarak kullanarak onlarla dizi benzerliğine sahip tek iplikçikli doğal mRNA'ları parçalar ve böylece ilgili genin protein sentezi susturulur. Transformatif olan ve olmayan RNAi farklı uygulama alanlarına sahiptir. Transformatif RNAi bitkilerde kalıcı uygulama ile yağ asiti sentez yolaklarının değiştirilerek yağ asidi kompozisyonunun değiştirilerek yağ kalitesinin artırılması, olgunlaşma hormonları üreten genlerin susturulması ile uzatılmış raf ömrüne sahip ürünlerin geliştirilmesi, pamuk çiğidindeki gossipol gibi zehirli maddelerin veya çay ve kahve gibi bitkilerde kafein içeriğinin düşürülmesi ve çekirdeksiz meyveler üretilmesi gibi uygulama alanlarına sahiptir. Transformatif olmayan RNAi, bitkiye dışarıdan çift sarmal RNA veya siRNA verilmesini ilgilendirmektedir. Harici RNA bitkiye yaprak spreyleri, sulama suyu, turunçgillerde ve asmada gövdeye enjeksiyon, transgenik mikroorganizmalarla bitkide çift sarmal RNA üretimi şeklinde verilebilmektedir. Transformatif olmayan RNAi uygulaması, tarlada veya bahçede halihazırda yetiştirilmekte olan ürüne genetik müdahale yapılmasını sağlamak gibi oldukça yenilikçi bir yaklaşım sunmaktadır. Tarımda zararlı böceklerin gelişiminde önemli olan bazı proteinlere ait mRNA'ların hedeflenmesi durumunda RNAi böceklerle mücadele için pestisitlere alternatif olan ekonomik, etkili, sağlık ve çevre açısından güvenli bir yöntem oluşturmaktadır. Bugüne kadar yapılan çalışmalarda, RNAi'nin patates böceği, mısır kurdu, afitler ve beyazsinek gibi zararlılara karşı etkili mücadele sağladığı ortaya konmuştur. Benzer şekilde, farklı yaklaşımlarla da olsa RNAi'nin mantari, bakteriyel ve viral hastalık etmenlerine karşı da etkili mücadele yöntemleri sağladığı anlaşılmıştır. Yine RNAi'nin yabancı otlara karşı koruma sağlayabildiği belirlenmiştir. RNAi tekniğinin apomiksis ve geçici erkek kısırlık oluşturulması gibi yollarla yakın gelecekte bitki ıslahı süreçlerinde de faydalı olması beklenmektedir. Tekniğin çok fazla mRNA kullanımı gerektirmesi ve bitkiler tarafından RNA alım yollarının iyileştirilmesi alanlarında gelişmelere ihtiyaç bulunmaktadır. RNAi kullanılan bitkiler ABD'de 2017 yılında, Çin'de ise 2021 yılında onaylanmış ve böylece tarımsal üretimde kullanımının önü açılmıştır. Tarımda devrim niteliğinde uygulama alanlarına sahip olan tekniğin gelecekte daha farklı yaklaşımlarla farklı bitkisel özellikler için kullanılması beklenmektedir.

Bitki ıslahında hızlı ıslah teknolojileri

Nevzat Aydın

Küresel iklim değişikliği ve nüfus artışının beklendiği dünyada en stratejik başlık gıda güvenliği ve teminidir. Başka bir ifadeyle sağlığı bozulan bir dünyada daha fazla insanı beslemek zorundayız. Bu kapsamda bitki ıslah gıda güvenliği açısından en çevreci ve en etkin çözüm olarak karşımıza çıkmaktadır. Gelişen teknolojiyle birlikte birçok alanda olduğu gibi bitki ıslahı alanında da genotipik ve fenotipik bilgi üretimi hızla artmaktadır. Hızlı ıslah teknolojisiyle birlikte bitki ıslahında açılan generasyon dediğimiz ıslah aşmasında zamandan büyük kazanç sağlanmıştır ve çeşit ıslahına önemli bir yenilik kazandırılmıştır. Hızlı ıslahın yenilikleri üç başlık altında toplanabilir: ışıklanma süresinin uzatılması, erken hasat ve tohumlarda dormansinin kırılması. Buğday örneğinde bitkiler 22 saat ışıklı, 2 saat karanlık periyotta yetiştirilmiş, çiçeklenmeden 15-20 gün sonra hasat edilmiş ve kurutulan tohumların dormansisi 4 derecede tutularak kırılmıştır. Son birkaç yılda yapılan çalışmalarla hızlı ıslah teknolojisi uzun gün bitkileri yanında kısa gün bitkileri hatta meyvelerde kullanılmaya başlanmıştır. Hızlı ıslah teknolojisi zaman avantajı yanında moleküler yöntemler ve fenotipleme çalışmalarının kombine edilmesiyle bitki ıslahında önemli bir ekosistem haline gelmiştir. Özellikle haritalama popülasyonlarının geliştirilmesi ve markör destekli seleksiyon programların uygulanmasında hızlı ıslah yöntemi büyük avantajlar sağlamaktadır. Tahıllarda yaptığımız çalışmalar sonucunda hızlı ıslah teknolojisi kullanılarak dünyada en hızlı geliştirilen çeşitlere özel sektör işbirliği ile üretim izni alınmış ve tescil denemeleri devam etmektedir. Hızlı ıslah teknolojisi ıslah programlarına değerli allellerin kazandırılması, hızlı çeşit geliştirme ve araştırma materyalinin hızla hazırlanması için ümitvar bir teknolojidir.
Anahtar Kelimeler: Markör destekli seleksiyon, Tahıl Islahı, Haritalama Popülasyonu

Reverse translational R&D in biotechnology and its contribution to cruelty-free products

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Conventionally, following the development of a candidate drug product to reach the clinic is not so easy. A lot of time, money and effort is necessary for pre-clinical and clinical trials to take place. When the case is translating biotechnological processes into clinical implementation, it takes no less than 10-15 years and over $2-3 billion at the luckiest. Even though the investigational new drug (IND) passes the pre-clinical phases without a problem, clinical phases of development may not respond that effectively due to the first-in-human efficiency and safety monitoring which does not always fully extrapolate from pre-clinical animal models. During the four phases of translational R&D, there might, therefore be a need to repurpose the drug to a new clinical indication or even go back to developing more efficient doses or routes of administration of the same active ingredient. As stem cell research has provided scientists the chance to reprogram somatic cells harvested from patients and differentiate them into cellular-based 2D, 3D and 4D disease models, there is a chance the revert bench-to-bedside practices into bedside-to-bench reverse translational R&D applications. In other words, it is now possible to use such cruelty-free disease models of human origin as pre-clinical models yet with the chance to test first-in-humans before reaching the actual patients. In this session, the pros, cons and future prospects of how biotechnological reverse translational R&D serves fort he benefit of cruelty-free products is discussed in depth.

Reflections on AI and ethics Are we heading in the right direction?

Perihan Elif Ekmekci M.D., Ph.D.

The recent advancements in artificial intelligence (AI) underscore ethical dilemmas that prompt contemplation on our trajectory. The rapid development of AI, compounded by the emergence of generative AI, introduces fresh ethical controversies. These debates were brought to the forefront of attention within the spheres engaged in AI design, utilization, or implementation, followed by the Future of Life Institute's call to temporarily halt large-scale AI experiments for six months.

Efforts have been made to regulate AI ethics, such as the Montréal Declaration for Responsible Development of Artificial Intelligence, the European Commission's Ethics Guidelines for Trustworthy AI, and UNESCO's Recommendations on AI. While these represent significant documents that establish fundamental values and principles in the field, they fall short in addressing two critical problems: the absence of a designated interlocutor and the lack of guidance for ethical quandaries.

AI and digitalization pose immense potential for radical innovation and widespread adoption, fundamentally altering operations across various domains of life. This transformative capacity establishes AI and digitalization as a long-wave phenomenon capable of reshaping the entire societal framework. This transformation is notably evident in the moral domain, where social moral change brought about by the long-wave phenomenon of AI manifests across multiple levels. The primary impact is pragmatic mediation, which signifies technology's influence in either adding or subtracting morally charged decisions from our lives. Hermeneutic moral mediation elucidates how societies begin to interpret aspects of the world differently post-digitalization and AI. Additionally, secondary and tertiary effects transfer the influence of the primary effects onto areas such as the economy, business, and service provision. These secondary and tertiary effects reciprocally impact the primary effects, determining the ongoing and future shifts in established values.

Presently, there exists no unified approach to address the ethical predicaments of AI. This lack of consensus can be attributed to several factors:

- Epistemic Complexity of AI
- Uncertainties in identifying the "accountable/responsible" party
- The normative approach is inadequately swift to keep pace with technological progress
- Inability to establish effective regulation/oversight
- Opacity in legislative power
- Variations in the depth and efficacy of social moral change
- Disparities in sectoral risk/benefit balance
- Multiplicity of stakeholders

A comprehensive AI framework should encompass the following considerations:

1. Enabling the intended benefits for users and society without the necessity of storing or selling data, such as through Data Visitation.
2. Maintaining data integrity throughout its life cycle.
3. Seeking consent for data usage, excluding cases specifically meant for research purposes.
4. Establishing Ethical Committees across various domains including research, public sectors, NGOs, the private sector, and regulatory agencies to oversee and guide ethical practices.

Gıda Biyoteknolojisi ve Güncel Mevzuat Uygulamaları

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Gıda hukuku, gıdanın tarladan çatala üretim, işleme ve dağıtımının her aşamasında yönetimi için kanunlar, yönetmelikler ve idari hükümleri içermektedir. Hukuki düzenlemeler, sağlıklı ve besleyici gıdaların mevcudiyeti, insan yaşamının ve sağlığının korunması, tüketicilerin çıkarlarının korunması, yurtiçi gıda ticaretinde adil uygulamalar ve uluslararası kurallara göre sağlıklı gıdanın küresel ticaretinin kolaylaştırılması açısından önemlidir.

Gıda daima temel bir insan hakkı olarak tanımlanır. Bu nedenle en eski tarihi yazılardan tüketicileri gıda satışında dürüst olmayan uygulamalardan korumak için kuralların düzenlenmesine ilişkin kanıtlar bulabiliriz. Örneğin Asur tabletleri gıda tahılları için doğru ağırlık ve ölçüler tanımlar; eski Mısır parşömenleri belirli gıdalara uygulanacak etiketlemeyi açıklar; antik Atina üretilen bira ve şaraplar saflık ve sağlamlık açısından denetleniyordu. Romalılar ise tüketicileri sahtekârlığa ve kötü ürünlere karşı korumak için iyi organize edilmiş gıda kontrol sistemi açıkladılar ve Orta Çağ'da Avrupa'da tek tek ülkeler yumurta, sosis, peynir, bira, şarap ve ekmeğin kalitesi ve güvenliğine ilişkin yasalar çıkarılmıştır ve bu eski yasaların bazıları hâlâ mevcuttur. Yusuf Has Hacib tarafından XI. yüzyılda (M. 1069) Kaşgar'da yazılan Kutadgu Bilig doğru beslenmenin önemi ve insan sağlığına etkileri, besinlerin hijyen açısından nasıl olması gerektiği konusunda beyitler yer almaktadır.

Bugün değişen çevre ve ihtiyaçlarla birlikte evrimleşen gıda hukuku yaklaşımında 1940'lardan itibaren uluslararası aktörlerin katılımı; İnsan Hakları Evrensel Beyannamesi (1948) ve Dünya Gıda Zirvesi (1996) ile kendine sağlıklı bir yaşam ve refah sağlayacak yeterli gıdaya erişim her insanın hakkıdır ve herkesin güvenli ve besleyici gıdalara erişim hakkı tanımlandı. Bu kapsamda gıda güvencesi (Food Security) ve gıda güvenliği (Food Safety) kavramları ortaya çıktı. Gıda güvencesi tüm insanların her zaman, aktif ve sağlıklı bir yaşam için beslenme ihtiyaçlarını ve gıda tercihlerini karşılayacak yeterli, güvenli ve besleyici gıdaya fiziksel ve ekonomik erişime sahip olduğu zaman var olur. Gıda Güvenliği ise gıdada tüketicilerin sağlığına zarar verebilecek tehlikelerin (mikrobiyolojik, kimyasal veya fiziksel) olmaması veya güvenli, kabul edilebilir düzeylerde olmasıdır. Üretimden hasada, işlemeye, depolamaya, dağıtıma, hazırlama ve tüketime kadar gıda zincirinin her aşamasında gıdanın güvenli kalmasını sağlamaktır.

Gıda yaşamın vazgeçilmezi ise ve iyi yönetilmezse zarar verebiliyorsa "Gıda Güvenliği Kanunu"na ihtiyaç vardır. Türkiye'de gıda kanunu 2010 yılında yayınlanan 5996 Sayılı Veteriner Hizmetleri, Bitki Sağlığı, Gıda ve Yem Kanunu Çerçeve Kanunu ve 5977 Sayılı Biyogüvenlik Kanunu ile 2011 yılında yayınlanan Resmi Kontroller ve Hijyen Yönetmeliği ile Kodeks Yönetmelik ve Tebliğleri ve 1995'ten bu yana AB ile Gümrük Birliği gereği düzenlemelere dayanmaktadır. Ayrıca 10 Temmuz 2018 Tarım ve Orman Bakanlığının Görevlerine İlişkin Cumhurbaşkanlığı Kararnamesi ile gıda kanunun uygulaması desteklenmiştir.

Türkiye'de 5977 sayılı Biyogüvelik Kanunu'nun uluslararası dayanakları ve temel ulusal dayanakları aşağıda sıralnmıştır: 1) Biyolojik Çeşitlilik Sözleşmesi 14 Mayıs 1997'de yürürlüğe girdi. 2) Kartagena Biyogüvenlik Protokolü 24 Ocak 2004'te yürürlüğe girdi. 3) İkinci Ulusal Rapor hazırlandı (2011) 4) Üçüncü Ulusal Rapor hazırlandı (2015) 5) Dördüncü Ulusal Rapor hazırlandı (2019) 6) Ulusal Biyoçeşitlilik Stratejisi ve Eylem Planı (2007). Bu kanunun amacı bilimsel ve teknolojik gelişmeler dikkate alınarak modern biyoteknoloji kullanılarak üretilen GDO'lardan ve ürünlerden kaynaklanabilecek risklerin önlenmesini; çevrenin, biyolojik çeşitliliğin ve insan, hayvan ve bitki sağlığının korunmasını ve

sürdürülebilirliğini sağlamak amacıyla biyogüvenlik sistemini kurmak ve uygulamak; kanun kapsamındaki faaliyetleri denetlemek, düzenlemek ve izlemektir. Bu amaçla risk değerlendirme hem bilimsel hem de sosyo-ekonomik açıdan gerçekleştirilmektedir. Türkiye'de Tarım ve Orman Bakanlığı tarafından Onaylı GDO listesi yayınlanmaktadır. Buna göre 36 adet mısır ve soya çeşitleri olmak üzere yem amaçlı onaylanmış GD bitki bulunmaktadır. Ayrıca 9 adet GD mikroorganizma endüstriyel uygulama amaçlı onaylanmıştır.

Revolutionizing Health: Gene and Cell Therapy Unveiled

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In the realm of medical innovation, the fields of gene and cell therapy have emerged as transformative forces, offering novel approaches to treating a myriad of diseases. This abstract provides a comprehensive overview of the current progress in these dynamic fields, highlighting key advancements, challenges, and future prospects.

Gene therapy, the direct modification of an individual's genes to treat or prevent disease, has witnessed unprecedented progress in recent years. Central to this advancement is the revolutionary CRISPR-Cas9 technology, which has bestowed scientists with an unparalleled ability to precisely edit genetic sequences. This tool, likened to molecular scissors, allows for the targeted removal, addition, or alteration of specific DNA segments, opening avenues for the correction of genetic mutations underlying various disorders.

The clinical application of CRISPR-Cas9 extends across a spectrum of genetic diseases, from monogenic disorders like sickle cell anemia to complex conditions such as cystic fibrosis. Trials and studies have demonstrated promising outcomes, showcasing the potential to alleviate disease burden at its genetic roots. Moreover, the CRISPR technology's versatility has spurred investigations into its application beyond the correction of genetic defects, including the modulation of gene expression for therapeutic purposes.

In tandem with gene therapy, cell therapy has emerged as a potent strategy for tackling diseases, particularly in the realm of cancer treatment. Chimeric Antigen Receptor T-cell therapy (CAR-T) stands out as a prominent example, exemplifying the marriage of genetic and cellular interventions. CAR-T therapies involve the extraction and modification of a patient's own immune cells to express chimeric receptors that target and eliminate cancer cells with remarkable precision. Clinical successes, notably in hematological malignancies, underscore the potential of this personalized immunotherapy approach.

The application of gene and cell therapies is expanding beyond oncology, with researchers exploring avenues for treating inherited genetic disorders and autoimmune diseases. Preliminary studies indicate progress in addressing conditions like muscular dystrophy and hemophilia through gene therapy, hinting at a future where inherited diseases may be mitigated or even eradicated. In autoimmune diseases, efforts are underway to modulate immune responses through cell-based therapies, offering a potential breakthrough in diseases where the immune system turns against the body.

While the progress in gene and cell therapy is undeniably promising, challenges abound on the road to widespread clinical implementation. Concerns regarding off-target effects and unintended genetic consequences of CRISPR-Cas9 interventions necessitate rigorous safety assessments. Ethical considerations surrounding germline editing, which involves altering the genes passed onto future generations, add layers of complexity to the discourse. Moreover, the long-term safety and durability of gene and cell therapies remain subjects of ongoing investigation, emphasizing the need for comprehensive follow-up studies.

In conclusion, the current progress in gene and cell therapy represents a paradigm shift in medical therapeutics. The convergence of advanced gene-editing tools, such as CRISPR-Cas9, with innovative cell-based approaches like CAR-T therapy, has ushered in an era of precision medicine with transformative potential. While challenges persist, the strides made in understanding and harnessing

the power of genetic and cellular interventions offer a glimpse into a future where previously incurable diseases may be effectively treated or even eradicated. The journey from laboratory breakthroughs to widespread clinical application is ongoing, promising a future where the principles of gene and cell therapy redefine the boundaries of medical possibility.

Hayvan Biyoteknolojisi

Sema Birler

Hayvanlarda üreme biyoteknolojisi, dişi ve erkek gamet hücrelerinin elde edilmesi ve saklanmasından, ileri teknikler olan klonlama ve transgenezise kadar geniş bir yelpazeyi kapsar. Bu teknolojilerden bir tanesi olan suni tohumlama, ülkemizde 1926 yılında ilk kez atlarda uygulanmış ve Türkiye dünyada bu teknolojiyi uygulayan ikinci ülke olmuştur. Ülkemizdeki büyükbaş ve küçükbaş hayvan varlığında çok büyük bir değişim olmamasına karşın (2022 yılı, sığır: 16.851.956; koyun: 44.687.888), bu hayvanlarda üreme biyoteknolojisi yöntemlerinin yeterince uygulanamaması, mevcut hayvanlardaki verim düzeylerinin istenen seviye gelememesinin en önemli nedenlerinden biridir.

Üreme biyoteknolojisi yöntemlerinden bir tanesi olan in vitro fertilizasyon (IVF) ve intrasitoplazmik sperm enjeksiyonu (ICSI), günümüzde insanlarda da infertilite durumunda uygulanan ilk seçeneklerdendir. ICSI metodu, hayvanlarda spermanın liyofilizasyon ile dondurularak kullanılması yanında infertilite sebeplerinden biri olan anormal sperm morfolojisi durumlarında da yavru elde edilebilmesini sağlayan yöntemlerdendir.

Dünyada ilk kez somatik klonlama ile yavru (Dolly) elde edilen 1996 yılından günümüze kadar birçok hayvan türünde bu teknoloji başarı ile kullanılmıştır. Genetik olarak çok üstün bireyler ile aynı genetik yapıya sahip canlılar üretilmesini sağlayan klonlama teknolojisi ile, soyu tükenme tehlikesi altında olan, hatta soyu tükenmiş canlılar üretilebileceği gibi, birçok türde transgenik yavruların üretilmesi, transgenik canlıların sayısının artırılması da mümkündür. Ayrıca terapötik klonlama, birçok hastalığın tedavisi için bir ümit ışığıdır.

Transgenik teknoloji üreme biyoteknolojisi alanında en önemli teknolojilerden biridir. Özellikle 2020 yılında kimya alanında Nobel ödülü alan transgenik metod ile gündemde üst sıralarda olan bu teknoloji sayesinde; hastalık modelleri oluşturmaktan gıda üretimine, ilaç üretiminden ksenotransplantasyona kadar çok sayıda alanda ilerme elde edilmiştir.

Ülkemizde, İstanbul Üniversitesi (İstanbul Üniversitesi-Cerrahpaşa) Veteriner Fakültesi, Dölerme ve Suni Tohumlama Anabilim Dalı bünyesinde, hayvanlarda üreme biyoteknolojisi alanında ilk çalışmalar başarıyla gerçekleştirilmiştir. İneklerde ilk embriyo transfer çalışması (İleri ve ark, 1985), IVF ile ilk kuzu (Birler ve ark, 2000), ilk klonlama (Birler ve ark, 2007), çiftlik hayvanlarında ilk ICSI (Birler ve ark, 2010), ilk transgenik tavşan (Birler ve ark, 2013), ilk transgenik kuzu (Birler ve ark, 2013) bu alanda ülkemizde gerçekleştirilen ilk çalışmalardır.

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Biotechnological Approaches in Sugar Beet Breeding

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Sugar is produced economically in the world from sugar cane and sugar beets. Although sugar beet was cultivated approximately 200 years ago, its yield and quality were significantly improved in a short time with classical breeding methods. However, in recent years, advanced in vitro culture and genetic transformation technologies and selection with molecular markers have been included in classical breeding programs. High-yielding varieties resistant to herbicides, diseases and pests have been developed. In vitro culture techniques such as clonal propagation, haploid plant production, somaclonal variation and in vitro cell selection are used most in sugar beet breeding. Various gene transfer techniques such as protoplast culture and somatic hybridization, transformation via Agrobacterium, particle bombardment and somatic hybridization are also used in sugar beet variety development studies. Herbicide-resistant transgenic sugar beet varieties have been developed by using Agrobacterium and it has been commercialized and used widely for cultivation since 2008. However, efforts to obtain transgenic plants resistant to viruses, fungi and insects are ongoing and have not been commercialized yet. In addition, studies about converting sucrose into fructan in sugar beet by gene transfer techniques have not been commercialized. Herbicide-resistant smart varieties obtained using somaclonal variation are widely used in production. There are important problems such as genotypic variation, low regeneration and transformation frequency in vitro studies on sugar beet. Molecular applications are widely used in sugar beet breeding and shorten the long breeding period. O-type plants are very important in sugar beet hybrid breeding and they are selected by using molecular markers in large quantities and short periods thus shortening long breeding periods when compare to the classical breeding methods. Different virus types and resistant genes related to Rhizomania disease were identified with the help of marker-assisted selection. The sugar beet varieties used widely in cultivation carry one Rhizomania-resistant gene. In recent years, breakage in the resistance provided by this gene is determined by molecular markers, and additional resistance genes are transferred to plants through hybridization to provide resistance to the Rhizomania virus. Molecular markers are widely used to obtain plants with resistance/tolerance to Cercospora leaf spot, root rot and nematode. Gene editing techniques (TALEN, ZFN, Crisper) have also begun to be used in plant breeding programs in recent years.

Keywords: Sugar beet, biotechnology, breeding, in vitro culture, transformation, molecular markers

Tarımda Dijital Teknolojilerin Geliştirilmesi

Prof. Dr. Ünal Kızıl
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Türkiye'de tarımsal amaçlı faaliyet gösteren işletmeler büyüklük olarak gelişmiş ülkelerle kıyaslandığında oldukça küçük kalmaktadır. İşletmelerin %80'den fazlasının ortalama arazi büyüklüğü 100 da'dan daha azdır. Ayrıca, sağmal süt sığırı sayıları açısından değerlendirilecek olursa işletmelerin %99'dan fazlasında 50 baş veya daha az sayıda hayvan beslenmektedir. İşletmelerin büyüklük veya kapasitelerinin küçük olması dijital teknolojilere geçişlerinde önemli bir engel olmaktadır. Bu teknolojilerin büyük çoğunluğunun ithal olması hem maliyetlerini artırmakta hem kullanımını küçük işletmeler için zorlaştırmaktadır. Dolayısıyla, ülkemiz koşullarında faaliyet gösteren küçük aile işletmelerinin daha düşük maliyetlerde satın alabileceği, kullanımı kolay, yazılımı Türkçe olan yerli teknolojilerin geliştirilmesi oldukça önem taşımaktadır. Bu bağlamda Çanakkale Onsekiz Mart Üniversitesi, Ziraat Fakültesi bünyesinde kurulmuş olan Dijital Tarım Laboratuvarında çeşitlik AR-GE çalışmaları yapılmaktadır.

Küçük aile işletmelerince kullanılabilecek barınak içi ses takip sistemiyle üreticinin uzakta olması durumunda bile hayvanlarda meydana gelebilecek huzursuzlukları takip edebileceği, barınak içinde her noktayı uzaktan cep telefonuyla dinleyebileceği bir sistem geliştirilmiştir. Başka bir çalışmada da yine süt sığırı işletmelerinde kullanılabilecek Sıcaklık-Nem İndeksine dayalı bir iklimlendirme otomasyon cihazı geliştirilmiştir. Seralarda rüzgar ve kar yükünden dolayı taşıyıcı sistemde meydana gelebilecek zorlamaları önceden haber verebilecek bir erken uyarı sistemi de yine bu amaçlarla geliştirilmiştir.

Önemli bir başka çalışma alanımızı ise elektronik burun tasarımı oluşturmaktadır. Hayvan gübresinde bulunabilecek patojenlerin koklanarak bulunabilmesini sağlamak amacıyla geliştirilmiş cihaz bu konudaki önemli çalışmalarımızdandır. Bu teknolojinin kokuya dayalı bazı hastalıkların insan ter ve idrar kokusundan tespitine yönelik çalışmalar devam etmektedir. Bu amaçla gelişen teknolojiye paralel olarak sürekli yeni versiyonların üretimi devam etmektedir. Multidisipliner çalışmalar ile geliştirilen teknolojilerin farklı alanlarda kullanımına yönelik projeler de devam etmektedir. Çevre mühendisliği, gıda mühendisliği, biyoloji, inşaat mühendisliği gibi alanlar dijital tarım laboratuvarının potansiyel işbirliği alanlarını oluşturmaktadır.

Nanoparçaçık Ortamlı Histotripsi ve Kavitasyon Çekirdeği Olarak Çok Fonksiyonlu Nanoparçacıklar

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Histotripsi, kısa, yüksek yoğunluklu ultrason (US) sinyallerini kullanarak, akustik kavitasyon mekanizmasını ile mekanik olarak doku parçalama tekniğidir [1]. Uzaklaştırılmak istenilen dokuda halihazırda çözünmüş olan gaz öbeklerini veya US enerjisi ile sıvının buharlaşması ile oluşan gaz öbeklerini çekirdek olarak kullanır ve bu çekirdekleri daha büyük gaz baloncuklarına dönüştürür (bubble clouds). Belirli bir negatif basınç değeri geçildiğinde bu baloncuk öbekleri patlar (kavitasyon) ve hücre üzerinde stres oluşturarak hücre zarının zarar görmesine neden olurlar. Böylece çevre dokulara zarar vermeden ve ısı çıkısına neden olmadan istenmeyen dokular sıvılaştırılarak uzaklaştırılır. Zamanla bu dokular vücut tarafından sağlıklı doku ile yenilenir.
Kavitasyon oluşturmak için gerekli yüksek eşik basınç değeri histotripsi için bir sınırlamadır ve histotripsiyi ikinci bir görüntüleme sistemine mecbur bırakır. Bu yüksek kavitasyon eşik basınç değerinde uzaklaştırılmak istenen noktanın doğru belirlenmesi önemlidir, aksi halde odaklanılan herhangi bir sağlıklı doku zarar görebilir. Bu sınırlamaya çözüm olarak nanoparçacık ortamlı histotripsi (NMH) geliştirilmiş ve ilk örnek olarak nanodamlacıklar (ND) kavitasyon çekirdeği olarak kullanılmıştır [2,3]. NMH'in eşik basınç değeri 1/3'üne kadar düşürülmüş olmasına rağmen üretim zorluğu, raf ömrü ve perflorokarbon (PFC) miktarının belirlenememesi gibi sınırlamalarından ötürü yeni nesil NMH ajanı olarak nanokap kümelenmeleri (NCC)'leri geliştirilmiştir [4]. Basitçe siklodektrin (CD) ve PFC bileşiklerinin suda karıştırılması sonucu çökerek ayrılan bu ajanlar 100 nm'den küçük boyutta ve PFC miktarı belirlenerek hazırlanabilmişler ve kavitasyon eşik basıncını ND'lar kadar etkin bir şekilde düşürmeyi başarmışlardır. Detaylı incelemeler NCC'lerin serbest PFC molekülleri etrafında organize olmuş CD-PFC konuk-konak komplekslerinden oluşan kümelenmeler olduğunu göstermiştir [5]. Ana bileşenlerinden biri beta-siklodektrin (β-CD) olan bu nanokap kümelenmelerini β-CD'ni birincil yüzeyinde gerçekleştirilen tekli modifikasyonlar ile fonksiyonel NCC'ler elde etmek mümkündür. Bu yolla sıklıkla kullanılan ve histotripsiyi gerektiğinde başka tedavilerle bir araya getirebilecek biyokonjugasyon örnekleri gösterilmiştir [6].
Son olarak, histotripsi başarılı tümör tedavi örnekleri ortaya koysa tedavi sonrasıtümör kalıntılarına rastlanabilmektedir. Histotripsi sonrası bölgesel olarak tümör dokusunun ulaştırılan antikanser ajanları bu duruma çözüm olabilir. Bu nedenle NCC'lerin ilaç içerecek şekilde optimize edilerek kavitasyon çekirdeği olarak kullanılması ve hapsedilmiş antikanser ajanın kavitasyon gerçekleştiğinde tümör mikro çevresine ultrason etkisi ile yayılması amaçlanmıştır. İlaç yüklü NCC'ler seçici olarak kanser dokusuna biriktirilip, histotripsi aracılığıyla hem doku parçalanması (NMH ajanı) hem de bölgesel ilaç salım sistemi olarak kullanılacak sistemlerin geliştirilmesine odaklanılmıştır.

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Bakteriyofajlar ve Gıda Biyoteknolojisi Uygulamaları

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Tek Sağlık yaklaşımı dünyadaki insanların, hayvanların, bitkilerin ve çevrenin sağlığının birbirine bağlı olduğunu ve birindeki değişimin diğerini de etkilediğini savunmaktadır. Artan nüfus ve iklim değişikliği ile bu etkileşim daha hızlı ve Tek Sağlık sorunları, zoonotik hastalıklar, antimikrobiyal direnç ve gıda güvenliği, vektör kaynaklı hastalıklar, çevresel kontaminasyon ve insanlar, hayvanlar ve çevre tarafından paylaşılan diğer sağlık tehditlerini içerir. Zoonotik hastalıkların önlenmesinde ve tedavisinde antibiyotiklere alternatif olacak bakteri yiyen virüsler olan bakteriyofajların kullanımı önem kazanmaktadır.

SÖZLÜ BİLDİRİLER

SS-01

Pharmacoeconomic Evaluation of Nusinersen for Spinal Muscular Atrophy (SMA) in Türkiye

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Introduction: Spinal muscular atrophy (SMA) leads to progressive muscle weakening, categorized based on onset and severity [Table 1]. With 4-10 cases per 100,000 live births, SMA is the leading monogenic infant mortality cause [1, 2]. While supportive care dominated, novel drugs like nusinersen have become available, offering varied benefits based on cost, availability, and side effects [3].

Keywords: Spinal Muscular Atrophy, Antisense Oligonucleotide, Pharmacoeconomics

Table 1: Clinical classification of SMA

Type	Age of onset	spiratory sup at birth	Able to sit	Able to stand	Able to walk	Life expectancy	% of Cas
0	Prenatal	Yes	No	No	No	<6 months	45%
1	<6 months	No	No	No	No	<2 years	
2	6 to 18 month	No	Yes	No	No	10 to 40 years	20%
3	>18 months	No	Yes	Yes	Assisted	Adult	30%
4	>5 years	No	Yes	Yes	Yes	Adult	< 5%

Nusinersen, an antisense oligonucleotide, alters the *SMN2* gene's splicing, promoting essential full-length SMN synthesis. After the four initial loading doses, it's intrathecally administered every four months. Clinical trials demonstrated its therapeutic benefits, with SMA Type 1 patients showing a 25.05-month survival rate at a 33-month follow-up [4-6]. However, a study noted that survival rates could only be confidently assessed up to 36.3% at the 13th month due to limited trial completion [7]. Although unregistered in Türkiye, roughly 1,300 patients receive Nusinersen under foreign medicine provisions. Considering Türkiye's annual birth rate (14.28 births/1,000 population) and a life expectancy of 76.21 years, the potential patient pool remains vast [8].
The reference countries for pricing in Türkiye are France, Spain, Italy, Portugal, and Greece. The lowest reference price for a vial is around 73,000 Euros. For biosimilars, it can be 100% of the reference price. For biosimilar products produced domestically, prices can also be determined according to their cost. The requested prices' ratio from the cost should be 15% of the reference, if not they are evaluated by the Commission [9].

Materials and Methods: Data on prices, products, and regulations were sourced from TEBRP, CIA Factbook, and TITCK [8], with Excel 2209 employed for analysis.

Results: Figure 1 depicts anticipated annual SMA cases in Türkiye, revealing a noticeable discrepancy when juxtaposed with the current 1,300 Nusinersen recipients, implying potential access barriers.

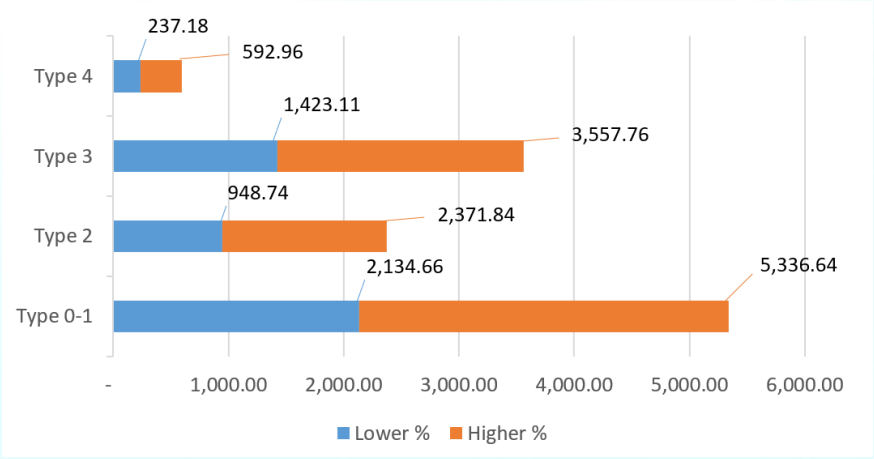

Figure 2: Estimation of annual new SMA cases in Türkiye

Conclusion: The biotherapeutics sector's growth seems inevitable, driven by impending patent expirations of numerous biosimilars. Biosimilar development, costing 100-200 million USD and lasting 8-10 years, emerges as a feasible solution. Nevertheless, the diverse SMA types and limited long-term treatment data impede definitive survival rate assessments. Retrospective studies might offer more clarity, as highlighted in Figure 2. Restrictive Nusinersen policies could be mitigated with a domestic biosimilar, facilitating broader access. This approach, combined with MCDA evaluations, could yield both patient and economic benefits, urging manufacturers to consider biosimilar development.

SS-02

Domestic Production of Monoclonal Antibodies Used in Clinical Pathology

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INTRODUCTION: The process of producing monoclonal antibodies (mAbs) by hybridomas consist of immunizing a host animal, followed by obtaining mAb-producing hybridomas by fusion of spleen B cells with immortal myeloma cell lines. Monoclonal antibodies have a wide range of uses, including the diagnosis and treatment of diseases like cancer, immune system disorders, viral infections and so on. The monoclonal antibody industry has grown exponentially and it is estimated that in 2022, global monoclonal antibody market value is around 205 billion dollars, and it will reach 534 billion by 2030. Türkiye imports the majority of its mAbs used for diagnostics in medicine and related sectors. TUBİTAK has initiated 1003 programs for domestic production of such mAbs. Using funds provided by TÜBİTAK, TÜBA and host institutions, we produced a few antibodies with potential applications in clinical pathology including p53, p40, Napsin A and cytokeratin 5.

MATERIALS-METHOD: 5-6 weeks old BALB/c mice were immunized three times intraperitoneally at two-week intervals with the corresponding antigen. Serum antibody levels of mice were subjected to serial dilution and determined by indirect ELISA method. Fusion of SP2/F0 myeloma and spleen cells was performed in the presence of polyethylene glycol, then cells were seeded in 96-well plates in selective hypoxanthine-aminopterin-thymidine

medium. Screening was performed by ELISA using supernatants of hybridoma colonies that had grown in an average of 2 weeks. Colonies that gave positive signals for the antigens we targeted were subjected to single cell cloning and hybridoma clones were obtained. Monoclonal antibodies were purified by protein A affinity chromatography and their binding to the selected antigen was additionally confirmed by western blotting or flow cytometry. Selected antibodies were tested in immunohistochemistry (IHC) using the Roche Ventana device.

RESULTS: Among monoclonal antibodies tested in IHC, we obtained satisfactory results with several mAbs. We will present data on hybridoma technology and characteristics of domestically produced monoclonal antibodies against p53, p40, Napsin A and cytokeratin 5.

CONCLUSION: Our studies and results show that Türkiye has the capacity to produce mAbs currently used in medical diagnostics, immunohistochemistry in particular.

Keywords: Monoclonal antibody, hybridoma, immunohistochemistry, clinical pathology

SS-03
Synthesis and In Vitro Characterization of Antibody Drug Conjugates

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Introduction : Cancer is among the leading causes of death worldwide. Current treatment approach include surgery and chemotherapy. However, the disease may still relapse and related deaths may occur. In addition, considering the side effects of chemotherapeutics and developing drug resistance against these drugs, there is a need to find new treatment approaches. Biotherapeutics in cancer treatment began to come to the fore. Among these, target-specific monoclonal antibodies (mAbs) are one of the most studied and used in clinical. However, a new field, Antibody Drug Conjugates (ADCs), has emerged, as cancer cells develop resistance to therapeutic mAbs over time, and most importantly, the power that can be obtained from chemotherapeutic drugs cannot be met by mAbs. In this way, ADCs, which minimize the side effects and multi-drug resistance of cytotoxic drugs, and combine the potent effect of cytotoxic drugs with the target-specific therapeutic power of mAbs alone, have begun to be a hope in cancer treatment. ADCs are structures in which potent cytotoxic drugs are attached to mAbs via a linker. The monoclonal antibodies contained in the ADCs are specific to the target cancer antigen and the cytotoxic drug used in the ADC is activated after it is taken into the cancer cells. Therefore, ADCs are more effective and safer than chemical drugs [1-3].

Materials and Methods: The disulfide bonds of a mAb were reduced with tris(2-carboxyethyl)phosphine hydrochloride (TCEP) at 37 ◦C. Then, a cytotoxic drug conjugated to a linker was added to the reduced mAb and incubated at +4 ◦C to construct ADC. After the ADC was synthesized, it was characterized by SDS-PAGE, UPLC-SEC, LC-MS/MS, UPLC-HIC. The binding affinity of ADC was evaluated by cell-based ELISA and efficacy of ADC in ovarian cell lines (OSE-SV40, A2780, A2780cis and OVCAR–3) was demonstrated by cell viability assay.

Results : than 5% and the average drug to antibody ratio (DAR) was 4. The binding affinity of the synthesized ADC did not change significantly compared to the naked antibody. Additionally, its IC50 value was approximately 6 nM and it affected cancerous cells without affecting healthy cells.

Conclusions: The reductive conjugation method used enabled selective delivery of the cytotoxic drug to cancer cells while maintaining the affinity of the mAb. Therefore, the synthesized ADC is a more potent and targeted drug candidate on ovarian cancer cells. It is envisaged that the studies of the drug candidate with proven in vitro efficacy will continue with in vivo studies.

Keywords
Antibody-Drug Conjugates, Ovarian Cancer, Physicochemical Characterization, Biological Characterization

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SS-04
Exploring Growth Media Impact on Recombinant Monoclonal Antibody Production: A Fed-Batch Study for Quality and Quantity Assessment

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INTRODUCTION: Although chemotherapeutics used in cancer treatment have strong cytotoxic effects, they are not specific targeted molecules. This leads to increased side effects and drug resistance. In order to increase the therapeutic efficacy, it is of great importance to produce targeted monoclonal antibodies (mAbs) with high selectivity and to use them for therapeutic purposes.
In mAb development, the most important parameters that need to be optimized for protein quality are the media and its components [1, 2, 3]. It is necessary to optimize the medium in order to increase both cell growth and mAb quantity and quality. By optimizing the composition of the medium, increasing the density of viable cells, prolonging the culture period, and revealing the true potential of a cell line, high titer of high quality produced mAb can be achieved [4].
Here, it was aimed to determine the optimum yield and quality growth medium by comparing the activities of recombinant mammalian cell lines carrying the gene to express the desired protein in different growth mediums.

MATERIALS-METHODS: The mammalian host cells were transfected with the vector carrying the desired gene region to be expressed, and pools of cells that produce the recombinant protein were formed. In order to determine the medium and conditions in which cell pools produce the best, fed batch studies were carried out with cells seeded in two different growth media. In this context, the cell pool with the high production capacity was determined by controlling data such as viable cell numbers, % viability, glucose amounts and pH of the

medium. The production amounts of these pools with high production were analyzed in protein A column, BCA kit, SDS Page and 280nm absorbance.

RESULTS: The titers of mAbs were found to be in the range of 0.5 – 1,5 mg/ml. As a parallel method, the BCA kit was used and the consistency of production quantities was demonstrated. The molecular weights of these mAbs were found to be in the expected range.

Keywords: CHO cells, mAb production, fed batch optimization.

SS-05
Biotechnological Production of Antitumoral Phytochemicals in the Plant Endemic Centaurea kilaea

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Over 60% of cancer drugs are currently derived from natural compounds, emphasizing the importance of plant secondary metabolites in cancer treatment. Chemical synthesis of these complex compounds is often challenging. Natural plants have low levels of these metabolites, requiring significant plant material. Biotechnological production offers advantages like consistent, scalable, year-round sustainable output, as well as simplified extraction and purification processes. Plant cell culture and tissue culture show promise as alternatives for secondary metabolite production. While large-scale commercialization is pending, ongoing research suggests in vitro plant systems, like cell suspension cultures and transgenic hairy roots, hold potential as bioactive compound sources.
Centaurea kilaea, an endemic plant in our country, holds promising phytochemicals with potential medicinal applications. Notably, compounds like 3'-O-methyleupatorin and jaceosidin have shown potent cytotoxicity against various cancer cell lines. Despite this, there is no prior research on biotechnological production of these secondary metabolites. Our study aims to cultivate these anti-tumoral compounds through plant tissue culture, optimizing their production in Centaurea kilaea. We will also assess their anti-tumoral properties, striving to establish cost-effective, large-scale production methods without harming this endemic species.
To select the optimal growth regulator, calli obtained from Centaurea kilaea plants were cultured in various media compositions, including MS + 1mg/L BAP, MS + 0.5mg/L BAP, MS + 1mg/L 2,4D, MS + 0.5mg/L 2,4D, and transferred to MS + 0 media. The culture in MS + 1mg/L 2,4D medium exhibited the highest effectiveness in terms of supporting callus production and increasing biomass. Cell suspension cultures were subsequently established from calli with active division and subcultured every 6 weeks. It was found that 1 mg/l concentration of synthetic 2,4-dichlorophenoxyacetic acid (2,4-D), an auxin group plant growth regulator, significantly promoted cell proliferation in the cultures. Detailed analysis of secondary metabolites was conducted using an LC-MS/MS device, with callus and cell suspension culture cells divided into 6 weeks (young) and 12 weeks (old) groups.

This study enhances our understanding of endemic Centaurea kilaea, paving the way for detailed metabolite analysis and production enhancement. Sustainable, controlled production of these bioactive compounds offers new avenues for drug development, showcasing biotechnology's potential to meet rising demands for effective cancer treatments. Further research into scaling up and optimizing these biotechnological methods is crucial for successful integration into the pharmaceutical industry and harnessing the full potential of plant cell culture technology in anticancer agent production.

Keywords: Secondary metabolites, Centaurea kilaea, callus,Cell suspension culture, -O-methyleupatorin, jaceosidin

SS-06

High IgG4 Monoclonal Antibody Production Using Suspension CHO Cell Line

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BACKGROUND: The cancer incidents and death-rates are increasing significantly each year. The chemotherapeutic agents used in cancer treatment have strong cytotoxic effects but this cytotoxicity isn't restricted to cancer cells. Therefore, it is of great importance to produce targeted biological molecules against cancer such as mAbs with high selectivity to reduce off-target effects and increase the efficacy of the treatment. CHO cells are mammalian cells that can make complex post-translational modifications on proteins that are close to the post-translational modifications in humans. This directly affects protein activity and immunogenicity, therefore CHO cells are widely used in monoclonal antibody production (Dumont, Euwart, Mei, Estes & Kshirsagar, 2015). In this study, we aimed to develop a biosimilar monoclonal antibody producing CHO cell line and a fed-batch process using ArtiaBio HyPerX technology.

METHODS-RESULTS: The amino acid sequence of the selected antibody was obtained and confirmed for accuracy. A cDNA construct was then developed using codon optimization and with the selection of a leader sequence. The resulting cDNA was cloned to ArtiaBio proprietary expression vector which was used to transfect the ArtiaBio parental cell line. Different conditions of electroporation were investigated, and the best condition was used to electroporate CHO cells to produce pools. The transfectant pools were evaluated and ranked based on productivity. Highest producing and well-growing 2 pools were selected for single cell cloning. Based on productivity, 11 top clones were selected. Selected clonal cells were fed using ArtiaBio feeds daily starting from day 3. In a 14 day fed-batch culture, cell densities of 25 million/ml was achieved and high titers were obtained.

CONCLUSIONS: To sum up, we were able to generate a stable cell line and develop a fed-batch process resulting in high cell densities and titers using ArtiaBio HyPerX technology.

REFERENCES: Handlogten, M., Lee-O'Brien, A., Roy, G., Levitskaya, S., Venkat, R., Singh, S., & Ahuja, S. (2017). Intracellular response to process optimization and impact on productivity and product aggregates for a high-titer CHO cell process. Biotechnology And Bioengineering, 115(1), 126-138. doi: 10.1002/bit.26460

Keywords: monoclonal antibodies, production, CHO cells, suspension

SS-07
Valorization of Natural Polysaccharides in Biomedical Applications

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INTRODUCTION: Polysaccharides have remarkable physicochemical and physiological properties such as biocompatibility, biodegradability, and low immunogenicity, which are important for biomedical applications. Natural polysaccharides have a considerable impact due to their structural diversity, wide availability, low cost, and ease of modification chemically and biochemically. Xylan is a hemicellulosic polysaccharide, which is found widely in nature. It can be obtained from forest and agricultural industry debris. Similar some other polysaccharides, xylan can find application in drug delivery and tissue engineering due to its availability, structural diversity, biocompatibility, biodegradability, and low cost.

METHODS: In this study, xylan-based nanoparticles and biofoams was developed for drug delivery and tissue engineering applications. The xylan-based drug carrier was combined with a polymeric micelles system to increase the bioavailability of hydrophobic bioactive molecules by combining the thin-film hydration and ionic gelation method. The drug loaded xylan-based biofoams were synthesized by the oil in water emulsion templated method. They were fully characterized by SEM, STEM, zeta potential analysis, DLS, BET, and FTIR. The in vitro drug release profile and the cytocompatibility was examined.

RESULTS: The synthesized uniform spherical nanoparticles have high loading capacity and maintained their structure and the drug was released to the target site. They did not exhibit significant cytotoxicity. Besides, drug-loaded xylan-based biofoams exhibited promising properties such as stability, swelling ability, and mechanical strength. The adhesion and differentiation of fibroblast cells on biofoams clearly supported that the physicochemical properties provided a satisfactory framework and can assist new tissue formation.

CONCLUSION: This study showed that xylan is a promising feedstock for the synthesis of stable and biocompatible materials in biomedical applications, which reveals its potential capability in drug carriers and scaffolds.

Keywords: Xylan, Polysaccharides, Targeted drug delivery, Scaffold

SS-08
In-vitro evaluation cytotoxic potential of novel isoindole derivatives on various cancer cell lines

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Cantharidine and its analogs are natural anhydrides with an inhibitory effect on protein phosphatases. However, they are not included in cancer therapies due to their toxicity. In recent studies, it has been found that derivatives of cantharidin as isoindole- 1,3-dione and its derivatives have anticancer effects.
The main purpose of this study to investigate the effects of four different drugs, which are newly synthesized isoindole derivatives for use in cancer treatment, on different cancer cells.

The study was investigated the effect of the synthesized compounds on five different cancer cell lines (A549, HeLa, PC3, MCF-7, and Caco-2) over different time periods (24, 48, and 72 hours) and various drug concentrations. The results showed that as the drug dose and incubation time increased, the cell viability decreased. This suggests that the compounds have cytotoxic effects on the cancer cells. The IC50 values, which indicate the concentration of a drug needed to inhibit the growth of cells by 50%, were calculated. The compound with the highest cytotoxic effect was identified as OG4, and it had the greatest effect on the Caco-2 cell line. Then, the IC50 concentrations of the drugs were applied at 48 hours, which is the optimum incubation period, and apoptotic stages and cell cycle stages were compared using flow cytometry to understand whether the drugs have a suppressive function in cancer development.

Cells were exposed to IC50 concentrations of the compounds for 48 hours, and the extent of apoptosis (programmed cell death) was measured. Different compounds showed varying rates of apoptosis induction in different cell lines. OG3 had the highest apoptosis rate in A549 and HeLa cells, OG1 in PC3 and MCF-7 cells, and OG4 in Caco-2 cells. This demonstrated that the compounds have the potential to trigger cell death pathways in cancer cells.

The study also investigated the effect of the compounds on cell cycle progression of different cell lines. OG4 caused G2 phase arrest in A549 and HeLa cells, while OG2 induced G2 phase arrest in PC3, MCF-7, and Caco-2 cells. G2 phase arrest indicates that cells are prevented from entering the mitosis phase, which is an essential step in cell division. This finding suggested that the compounds could disrupt cell division and inhibit cancer cell proliferation.

The compounds were tested for their ability to inhibit cell migration, which is a critical process in cancer metastasis. Different compounds showed varying inhibitory effects on cell migration in different cell lines. OG2 had the highest inhibition rate in A549 and MCF-7 cells, OG3 in HeLa cells, and OG4 in PC3 and Caco-2 cells. This suggests that the compounds could potentially impede the spread of cancer cells.

The study demonstrates the potential of the synthesized isoindole derivatives as effective biological agents against various cancer cell lines. The compounds show promising cytotoxic effects, apoptosis induction, cell cycle disruption, and migration inhibition, which are key features in developing cancer treatments.

Keywords: anticancer activity, biochemistry, chemistry, isoindole derivatives

SS-09

An Insight Into The Biotechnology Literacy Attitudes Of The Students Of Health Related Fields: An Observational Study

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INTRODUCTION: Biotechnology is one of the leading fields in the health sector in the world. It continues to exist in many different fields as well as pharmaceutical, diagnostic and treatment systems. Countries that want to keep

up with advancing technology should increase biotechnology literacy (BL) and awareness among society. The aim of this is to measure attitudes and behaviors of students health related science.

METHOD: An observational online survey was administered to health students and graduates in Turkey. Ethical approval was given by Bezmiâlem Vakıf University ethics committee with decision number 2023/244. The questionnaire was disseminated between August and September 2023. The questionnaire consists of two sections: demographics and biotechnology literacy questionnaire using the Theoretical Domain Framework scale with 14 domains containing 44 items. The domains measured based on the TDF scale are 14; knowledge (3 items), skills (2 items), social/professional role and identity (2 items), beliefs about abilities (5 items), optimism (2 items), beliefs about outcomes (5 items), reinforcement (4 items), intentions (3 items), goals (4 items), memory, attention and decision processes (1 item), environmental context and resources (6 items), social influences (3 items), emotion (1 item), behavioral regulation (3 items). To assess the attitudes of BL comparison made between students who had biotechnology-oriented education and who did not. Reliability and T-test were used in data analysis. Statistical significance was set to $p<0.05$

RESULT: 202 respondents participated in the survey, leaving 173 results after excluding data with insufficient information. Of the respondents, 41.62% (72) were pharmacists and 25.43% (44) were medical doctors; 72.83% (126) were female and 27.17% (47) were male; of those with a biotechnology-based bachelor's degree, 24.28% (42) are female and 6.94% (12) are male. The mean age of the participants was 28.7 years and the standard deviation was 7.95. The questionnaire reliability was high with Cronbach's Alpha value of 0.972. The Cronbach's alpha values of questionnaire sub-domains is between 0.623 - 0.983. A significant ($p<0.05$) difference was observed in the subdomains of knowledge, skills, social/professional role and identity, beliefs about abilities, reinforcement, intentions, goals; memory, attention and decision processes, environmental context and resources, social influences, emotion, behavioral regulation when those who received biotechnology-based undergraduate education were compared with those who did not. Non-significant ($p>0.05$) difference was observed in the subdomains of optimism and beliefs about outcomes when those who received biotechnology-based undergraduate education were compared with those who did not.

CONCLUSIONS: Although our study was conducted with participants who studied in the field of health, significant differences in BL attitudes were observed. The opinions of the individuals about the benefits of biotechnology are positive regardless of their BL level. Our results pointed out that increasing the BL levels of individuals would increase the contributions to biotechnology.

Keywords: biotechnological literacy, education, attitude and behavior, biotechnology, theoretical domain framework

SS-10
Purification Optimization Of A Recombinant Single-Chain Variable Fragment (SCFV) Antibody

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Introduction: Monoclonal antibodies that specifically recognize tumor-associated antigens are a promising targeted cancer therapy class that suppresses and destroys cancer cells by activating natural immune system

functions. However, recombinant antibody fragments with improved tumor penetration and more advantageous production procedures have been developed because they have several therapeutic disadvantages, such as difficulty penetrating solid tumors. The single-chain variable fragment (scFv), which forms most antibody fragments in late-stage clinical studies, can be produced by phage display technology. Antibody purification from adherent cells is one of the most challenging steps in antibody research. Efficient purification of scFv antibodies is complex due to the competitive binding of albumin in fetal bovine serum (FBS) with the poly-histidine tag attached to the scFv antibody to the nickel column. In this study, the effects of the FBS ratio used in the cells on the scFv antibody production and purification efficiency were investigated during the expression of the scFv antibody produced by the phage display method in HEK293 cells.

Materials and Method: The scFv antibodies, including a poly-histidine tag produced by the phage display method, were expressed in HEK293 cells by stable transfection of an expression plasmid. To investigate the effect of FBS ratio in cell culture medium on scFv antibody production in HEK293 cells, we tested different FBS ratios of cell culture medium changing between 0 and 10%. We used nickel resin (Ni-NTA resin) for purification. Cibacron Blue 3GA columns were used to remove albumin from cell culture supernatants. SDS-PAGE and Western blot techniques evaluated the effects of different FBS ratios in cell culture media at the transfection stage on antibody expression and albumin elimination efficiency.

Results: We found that HEK293 cells fed with DMEM containing 10% FBS produced much more antibodies than with 5% and 2.5% FBS, and no antibody production was detected in the cell culture medium containing 0% FBS. Cell culture supernatants containing 10% and 5% FBS were passed through a Cibacron Blue 3GA column, which allows separation and purification by binding to albumin with high affinity before adding to nickel columns. Multiple passings of cell culture supernatants containing 10% FBS through Cibacron Blue 3GA columns resulted in efficient recovery of the scFv antibody from nickel columns.

Conclusions: The single-chain antibody production in adherent HEK-293 cells in DMEM medium containing 10%FBS yields the best results, and multiple passing of culture supernatant through the Cibacron blue 3GA columns is an efficient way of getting rid of albumin during the purification of single-chain antibodies using IMAC method.

Keywords: IMAC, Ni-NTA, purification, single-chain antibody

SS-11

A Bibliometric Analysis of Pfizer-BioNTech (BNT162) Vaccine Side Effect and Safety Profile in Turkey

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INTRODUCTION: COVID-19, known as coronavirus disease 2019, is a globally widespread and highly contagious pandemic. Vaccination stands as one of the crucial strategies for preventing the spread of infectious diseases as well as COVID-19. The first vaccine to have emergency use authorization for COVID-19 was Pfizer/BioNTech's BNT162 in January 2021. Despite having approval for emergency use validation, the side effect and safety profiles of these vaccines are still an important research topic. The aim of this study is to conduct a bibliometric analysis

of the publications on the side effect profiles of the Pfizer-BioNTech (BNT162B2) vaccine and to examine the side effects in Turkey.

METHODS: Initially, an exploration for scientific articles was conducted employing an advanced query within the Web of Science™ database core collection, on the 30th of August 2023. The search was conducted using the term "BioNTech OR "BNT162b2" AND ("safety" OR "side effect" OR "adverse effect" OR "death" OR "mortality") AND "Article" (Document Type) along with the inclusion of "TURKEY" or "TURKIYE" (Countries/Regions) as search parameters.

RESULTS: Data extracted from 71 articles were subjected to analysis employing the Vosviewer® package program. The articles under scrutiny primarily appeared in the year 2022 (39, 54.93%). Notably, the scientific journals "Vaccines" and the "International Journal of Rheumatic Diseases" emerged as prominent publishers with 5 and 4 publications, respectively, regarding Pfizer-BioNTech (BNT162B2) in the context of Turkey. Among the institutions, the University of Hacettepe and Istanbul demonstrated the highest productivity within the Turkish context. Turkish scientists were mostly collaborated with scientist from England and Germany. Vaccination, Vaccine, BNT162b2 were the most preferred keywords following COVID-19 SARS-COV-2. In comparison of number of citations per papers, which published with contribution of scientist from Hacettepe, Marmara, Istanbul and Istanbul-Cerrahpaşa universities were the highest among Turkish peers. Significant adverse events reported with BNT162 vaccines include hypersensitivity reactions, local reactions, myocarditis and pericarditis, systemic reactions (excluding hypersensitivity). It should be noted that gastrointestinal, nervous system and musculoskeletal side effects have also been reported.

CONCLUSION: According to the results of our bibliometric analysis, it is seen that different institutions and researchers in Turkey are contributing significant knowledge to the literature regarding the use of COVID-19 vaccines. Vaccines have an essential role in the protection against COVID-19, the effects of which are still heavily influenced all over the world. However, it is necessary to keep in mind that these vaccines are not entirely safe and the risk of adverse effects due to their use, especially in fragile populations, should not be neglected.

Keywords: COVID-19, Vaccine, Pfizer-BioNTech (BNT162B2), Side Effect, Bibliometric Analysis, Türkiye

SS-12

Chemometrics Assisted Spectrophotometric Method Development and Validation for Simultaneous Analysis of Clopidogrel and Acetylsalicylic Acid in Commercial

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Clopidogrel and acetylsalicylic acid, which are used in atherothrombotic diseases, myocardial infarction and stroke, are produced as both single and combined drugs. [1] Various analytical methods are employed for quality control analysis of these combined medications. Among these methods, spectrophotometric techniques are preferred due to their ease of use and cost-effectiveness. However, the overlapping spectra of the active ingredients in these combined drugs can restrict the applicability of spectroscopic methods in quality control analysis. To overcome this limitation, chemometric-assisted analytical methods are employed. [2] Chemometrics is a chemistry discipline involving the processing of chemical data using statistics, mathematics, and computers to

extract meaningful information from chemical analyses or uncover hidden knowledge. [3] In this study, it is aimed to develop and validate the chemometric approach UV-Vis (Ultraviolet-Visible) Spectrophotometer method for the simultaneous determination of clopidogrel and acetylsalicylic acid in a commercial formulation called Klogel-A®, which contains the active ingredients of clopidogrel and acetylsalicylic acid together. Standard solutions of acetylsalicylic acid and clopidogrel were prepared, and their spectra were recorded using UV-Vis Spectrophotometry to determine the wavelengths of maximum absorbance. Calibration sets were prepared, and absorbance values were determined under specified conditions. Calibration curves were derived and the developed method was validated. The method was then applied to analyze acetylsalicylic acid and clopidogrel in the Klogel-A® commercial formulation.

Keywords: Acetylsalicylic acid, Chemometry, Clopidogrel, UV-Visible Spectrophotometer

Introduction: In this study, it is aimed to develop and validate the chemometric approach UV-Visible Spectrophotometry method for the simultaneous quantification of acetylsalicylic acid and clopidogrel active substances found together in commercial formulations and to show that it is applicable in real samples.

Materials and Methods: For spectrophotometric measurements, Termo Brand double beam UV - Visible Spectrophotometer was used. First, standard working solutions of acetylsalicylic acid and clopidogrel were prepared. By scanning in the wavelength range of 200-400 nm, it was determined that acetylsalicylic acid gave maximum absorbance at 225 nm and clopidogrel at 220 nm wavelength, and then the method conditions were optimized. Calibration sets containing different concentrations of both active substances were prepared and absorbance values were determined at predetermined wavelengths. The obtained data were evaluated using chemometric approaches (PLS, Partial Least-Squares and PCA, Principal Component Analysis). The method, which was developed and validated by quantifying acetylsalicylic acid and clopidogrel in the combined drug Klogel-A® commercial formulation, was shown to be applicable in real samples.

Results: It was determined that the UV-Visible Spectrophotometer method for acetylsalicylic acid and clopidogrel active ingredients was linear in the concentration range of 0-50 µg/mL. Synthetic mixture solutions of acetylsalicylic acid and clopidogrel were prepared. Absorbance values were determined and PLS calibration sets were created. The equations of the linear regression line of the actual and estimated concentrations for acetylsalicylic acid and clopidogrel are A= 1.0102x + 0.1472 (A: absorbance, x: acetylsalicylic acid concentration) and A=0.9882x+0.511 (A: absorbance, x: clopidogrel concentration), respectively. In addition, the calibration curve correlation coefficients were determined as 0.9999 (acetylsalicylic acid) and 0.9998 (clopidogrel).

Conclusion: For the simultaneous determination of acetylsalicylic acid and clopidogrel in the combined drug Klogel-A® commercial formulation, a chemometric approach UV-Visible Spectrophotometry method was developed and validated. Since the developed spectrophotometric method is fast, non-pre-processed and cost-effective, it is thought that it can be used successfully in the simultaneous determination of acetylsalicylic acid and clopidogrel in combined drugs in quality control laboratories.

SS-13

Development Novel and Efficient Peptides for Gene Delivery

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INTRODUCTION: The most important problem in the gene therapy is gene delivery. Cell-penetrating peptides (CPPs) have emerged as versatile tools for delivering various cargoes into cells. However, the exact mechanism of cellular uptake by CPPs remains elusive. This study aimed to explore the efficacy of new peptides, a specific class of CPP-nucleic acid binding peptide hybrids as gene delivery agents. A novel, highly efficient, cost-effective method was developed using these peptides. Eight peptides (named TM1 to TM8), composed of a nucleic acid binding part and a hydrophobic part of different cell-penetrating peptides, were synthesized, and tested for their ability to facilitate intracellular delivery of plasmid DNA. The results demonstrated that TM3 peptide exhibited the best performance among all TM peptides, in delivering the gene of interest to the nucleus. This study highlights the potential of TM peptides, specifically TM3, as a novel gene delivery vector in the field of gene therapy.

Cell-penetrating peptides (CPPs) have garnered significant attention due to their ability to traverse cellular membranes and deliver diverse cargoes, including drugs, nucleic acids, and proteins, into cells. This unique property of CPPs has positioned them as promising tools for drug delivery and various biomedical applications. While CPPs are known to penetrate cells through mechanisms such as endocytosis, direct translocation, or a combination thereof, the precise mechanism of cellular uptake by CPPs remains to be clarified (1). Currently liposomes, vesicles composed of phospholipids capable of encapsulating hydrophilic and hydrophobic molecules, are commonly used for gene delivery (2). This study presents a novel method characterized by high efficacy, low toxicity, and cost-effectiveness agent compared to liposomes, as a gene delivery tool.

TOOLS AND METHOD: Eight TM peptides were designed and synthesized using a peptide synthesizer. These peptides incorporated a short positively charged segment derived from nucleic acid binding peptide and a hydrophobic portion from different cell-penetrating peptides. The synthesis of TM peptides was followed by incubation with different dilutions of pcDNA-GFP (which expresses green fluorescent protein in the cell), at room temperature. The resulting hybrid peptide-pcDNA-GFP complex was then delivered into HeLa cells. RESULTS: The investigation revealed that the utilization of TM peptides facilitated fast and efficient expression of GFP which indicated successful delivery of pcDNA-GFP into HeLa cells, achieving cellular entry efficiency as high as 70-80%. Among the tested TM peptides, TM3 exhibited superior performance compared to other TM variants, as confirmed by fluorescence microscopy assays measuring total cellular uptake and cytosolic delivery. DISCUSSION: In conclusion, this study highlights the potential of TM3 peptide as a novel gene delivery agent in gene therapy. The efficient intracellular delivery and nuclear targeting capabilities of TM3 make it an attractive candidate for targeted gene delivery applications. The development of TM peptides as gene delivery vectors offers promising opportunities for advancing gene therapy and treating various diseases. Further optimization and investigations are warranted to fully exploit the potential of TM peptides in gene delivery applications. TM peptides may be an efficient tool of gene therapy.

Keywords: Cell-penetrating peptides (CPPs), TM peptides, Gene delivery, Gene therapy, Plasmid DNA delivery

SS-14

Unleashing the Potential of "Smart" Nanoparticles in Chordoma Therapy: Conquering Treatment Resistance via Nanomedicine

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In 1990, Professor Aufderheide examined the well-preserved remains of the 16th-century Kiribaya tribe and discovered an unusual pathology: an "onion-like mass" in the skeletal remains of a woman in her thirties. Further analysis revealed that this mass was a malignant bone tumor that was surprisingly preserved in the mummy for over a thousand years. Chordoma, one of these disease types with a history dating back to 4000 years ago, is a rare bone tumor with an exceptionally low incidence of one in a million cases, but its resistance to contemporary therapeutic interventions and its high susceptibility to relapse continue to pose significant clinical challenges. Therefore, there is an urgent demand for the application of advanced technological therapeutic strategies. In recent years, the field of nanomedicine has made significant contributions to the diagnosis and treatment of various ailments, including cancer. Continuing advances in nanotechnology have led to the design of innovative treatment strategies involving a wide variety of nanomaterials that facilitate the targeted delivery of therapeutic agents such as genes and drugs to diseased cells. In the context of this work, the aim is to create an innovative therapeutic approach for chordoma that uses smart polymer nanoparticles as carriers for genes. Our experimental findings demonstrated the remarkable non-toxicity of the designed smart gene carrier nanoparticles on the chordoma cell line CH22. Additionally, gel electrophoresis experiments confirmed the successful formation of a complex between the NLS+β-CD structure and CRISPR/Cas9 pDNA. DLS has been utilized in measuring the particle sizes and zeta potentials of "smart" NPs at different N/P ratios. All "smart" NPs with varying N/P ratios had particle sizes of less than 200 nm. The charge of "smart" NP and NLS peptide is positive, whereas the charge of CRISPR/cas9 plasmid is negative, while all complexes had a positive surface charge. Importantly, fluorescence imaging revealed efficient nuclear entry of the NLS peptide-containing pDNA+β-CD complex group within 6 hours, whereas the group lacking the NLS peptide showed no nuclear entry. Notably, the delivery of the plasmid to the nucleus and subsequent activation were confirmed by GFP transfection 48 hours after the application of the NLS+β-CD+pDNA complex to the chordoma cell line. Collectively, our study underscores the crucial role of polymer nanoparticles in facilitating gene therapy against the rare chordoma cancer, highlighting their potential in improving treatment outcomes for this challenging disease.

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Keywords: chordoma cancer, CRISPR/cas9, gene delivery, nanomedicine, smart nanoparticles

SS-15

Low-Dose Chemotherapy Inhibits Proangiogenic Factors in Endothelial and Cancer Cells

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INTRODUCTION: Despite the progress in developing chemotherapy and anti-angiogenic treatment agents, the severe side effects of those drugs have brought the search for alternative treatment plans. Metronomic chemotherapy, the so-called use of low doses of conventional chemotherapy drugs, has been used frequently in recent years due to its anti-angiogenic effects. 5-fluorouracil (5-FU), irinotecan (IR), and oxaliplatin (OX) are frequently used in terms of their anti-angiogenic activity potential. The rationale for using metronomic chemotherapy as an anti-angiogenic treatment modality must be clarified. The effects of this application on cancer cells and tumor endothelial cells are not well known. In the current study, we aimed to investigate the cytotoxic effects of chemotherapy at metronomic doses on tumor and endothelial cells and the effects on the functions of these cells and pro-angiogenic and anti-angiogenic gene and protein expressions.

MATERIALS-METHOD: We determined the cytotoxic effects of 5-fluorouracil, irinotecan, and oxaliplatin on the human lung cancer cell line (A549) and human umbilical vein endothelial cell line (HUVEC) by MTT (3-(4,5-dimethylthiazol-2- IL)-2-5-diphenyltetrazolium bromide) test. -1logIC50, -2logIC50, and -3logIC50 doses of the drugs were considered metronomic doses. The effects of metronomic doses of the drugs on VEGF-A, PDGF-BB, bFGF, Endothelin-1, VEGFR1, VEGFR2, iNOS, eNOS, Angiopoietin-2, and Thrombospondin-1 gene expressions were assayed by real-time polymerase chain reaction (PCR) and protein levels were determined by Western blot method in A549 and HUVEC cells. In addition, VEGF-A, PDGF-BB, ANG-2, END-1, and TSP-1 cytokine and NO levels secreted by HUVEC cells were determined. The effects of the metronomic drug doses on endothelial migration and microvasculature formation functions in endothelial cells were investigated with a commercial kit.

RESULTS: While 5-fluorouracil completely suppressed pro-angiogenic factors in A549 cells, an increase was observed in the level of ANG-2, which acts as an anti-angiogenic factor, but not in TSP-1. The angiogenic factors such as VEGF-A, PDGF-BB, bFGF, END-1, VEGFR1, eNOS gene, and protein levels decreased. In contrast, ANG-2 and TSP-1 levels were increased in HUVEC cells treated with 5-FU, while pro-angiogenic factors were suppressed. While the 5-FU application significantly reduced the migration ability of HUVEC at metronomic doses, no significant difference was observed in microvessel formation. While the irinotecan and oxaliplatin decreased the expression levels of pro-angiogenic genes and proteins in A549 cells and increased ANG-2, the pro-angiogenic factors were suppressed in HUVEC cells, an increase in anti-angiogenic factor expression and a significant decrease in microvessel formation and migration abilities were detected.

CONCLUSIONS: Our findings show that 5-fluorouracil, irinotecan, and oxaliplatin administered at doses much lower than those used clinically metronomically can stimulate the production of anti-angiogenic factors while reducing angiogenic factors, especially in tumor endothelial cells. In addition, it has been determined that cancer

cells can be eliminated at metronomic doses. In conclusion, the metronomic doses of 5-FU, irinotecan, and oxaliplatin should be tested in further animal models and clinical trials as anti-angiogenic treatment modalities.

Keywords: Angiogenesis, Cancer, Metronomic Chemotherapy, Tumor Endothelium

SS-16

The Importance of Innovative Approaches (Machine Learning, Artificial Intelligence and Deep Learning) on the Pharmacovigilance of Gene Therapy Products: Prediction and Monitoring

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Introduction: Understanding the impact of genetic background on diseases is crucial for diagnosing and treating patients effectively. The concept of "precision medicine" is gaining prominence, emphasizing individualized approaches, including gene therapy, to optimize treatment outcomes [1]. However, it's equally important to consider the pharmacovigilance aspects of treatment to ensure safety and sustainability. Adverse drug reactions (ADRs) are influenced by factors like medication errors, polypharmacy, and misuse of medicines, and poor management of ADRs has led to unnecessary hospitalizations and economic burdens, in addition to harming patients [2,3]. Some gene therapy products, like Fomivirsen, have been withdrawn from the market due to uncontrollable adverse events [4].

The pharmacovigilance studies can be examined under different subgroups such as preclinical, clinical, and post marketing phases. Various innovative options such as Deep Learning (DL), Machine Learning (ML), and Natural Language Processes (NLP) are the cornerstones of pharmacovigilance systems, especially for conventional medicines nowadays [5,6]. Thanks to ML techniques, it is possible to analyze structured data which includes imaging and genetic source. The unstructured and free-text form is detected by NLP which can understand and interpret human language [7].

The FDA currently recognizes the existence of 25 FDA-approved cellular and gene therapy products, a number expected to grow substantially due to rising demand. However, these therapies pose challenges in terms of discovery and manufacturing, often making them costly for patients and healthcare authorities. Furthermore, safety concerns that frequently emerge during clinical trials can prevent many products from advancing to the marketing phase. In light of these considerations, the primary objective of this study is to assess diverse approaches for identifying and categorizing the side-effect profiles and benefit-to-harm ratios of gene therapy products during their preclinical stages. A novel hybrid deep learning (DL) model to give a descriptive prediction of drug side effects was reported. The model consists of two main components: a graph convolutional neural network (GCNN) with inception modules to allow more effective learning of drug molecular features and bidirectional long short-term memory (BiLSTM) recurrent neural networks associate drug structure with its associated side effects [9,10].

Material and Methods: In this study, via using of the Embace database, a search was made in the following criteria: (safety:ti OR pharmacovigilance:ti) AND ('deep learning':ti,ab OR 'machine learning':ti,abOR 'artificial intelligence':ti,ab) AND (predict*:ti). 40 related article was found in this search.

Also the search was repeated with ('gene therapy':ti OR 'gene treatment':ti OR biotech*:ti) AND (safety:ti OR pharmacovigilance:ti) AND ('deep learning':ti,ab OR 'machinelearning':ti,ab OR 'artificial intelligence':ti,ab) AND predict*:ti. No related studies were found.

Results: It is seen that the number of publications related to the use of AI in pharmacovigilance has hardly increased in the last 4 years consistent with previous literature data [11]. Pharmacovigilance management is shaped day by day in line with innovative options. Artificial intelligence applications are gaining importance in terms of confirming drug safety. The use of these tools by providing various modifications for gene therapy products can be quite effective.

Conclusions: By examining the relevant articles in Embace, innovative approaches were examined and their applicability to gene products was considered. Adapting various artificial intelligence applications, especially used in the discovery of small molecules, by considering various parameters in terms of applicability in gene therapy products, may help develop more cost- effective R&D.

Keywords: Gene therapy, Precision Medicine, Pharmacovigilance, Safety, Artificial Intelligence

SS-17

Assessing the Impact of Imazalil Treatment after Harvest on the Quality Characteristics of Moroccan Pomegranate Variety during cold Preservation

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INTRODUCTION: Pomegranate (Punica granatum L.) has received special attention from fruit growers and consumers around the world due to its diverse functionality and famous nutritional benefit in the human diet. Harvested pomegranate fruit is highly susceptible to high weight loss and deterioration in technological quality and nutritional components during postharvest handling and storage. Cold storage is one of the best common methods of conservation technologies performed to extend its availability in the market. This leads to myriad problems of this method is that low temperature causes deterioration of quality and nutritional values in pomegranate fruit. The ultimate aim is to find a new way to effectively maintain fruit quality during cold storage.

TOOLS AND METHOD: Thus, the effects of treatment based on Imazalil on their technological quality (Weight loss, color attributes (L*, a*, b*, C and h°), pH, titratable acidity and total soluble solids), and nutritional components (total anthocyanins contents (TAC), and total phenolics contents (TPC)) in Pomegranate fruits of the variety 'Sefri Ouled Abdellah' collected from the Béni Mellal region and immediately stored at 4°C for 120 days.

RESULTS: Fresh untreated Pomegranates showed high general quality deterioration (weight loss, color changes, acidity and total soluble solids) during cold storage. The Treatment based on Imazalil was more effective in delaying the changes and losses in bioactive components when compared with those in control.

CONCLUSION: This experiment adds to a growing corpus of research showing treatment based on Imazalil is effective in prolonging the technological quality and nutritional components of pomegranate in postharvest during cold storage. Our data suggest that we still have a long way to find the best treatments and storage conditions for pomegranate fruit.

Keywords: Pomegranate, Cold storage, technological quality, nutritional components, Morocco.

SS-18
Production of Aroma Compounds by Biotechnological Methods

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Background: Ecological concerns such as climate change and ocean pollution force many industries to implement more sustainable and environmentally friendly processes and develop products. This circumstance necessitates the inclusion of biological elements in production, and today, aroma substances are produced by biotechnological methods.

Scope and approach: The concentration of aroma substances in food material may decrease by some factors such as temperature changes or long storage times during food processing. For this reason, some aroma compounds can be produced as food additives to strengthen the aroma of food products. Aroma substances can be produced by chemical synthesis, natural extraction, and biotechnological methods. However, each technique, not the biotechnological process, has specific advantages and disadvantages.

Key findings and conclusion: Chemical synthesis or natural extraction procedures are commonly performed in the manufacturing of aroma substances. Natural extraction is dependent on the climate, on the other hand. In addition, the aroma production process is expensive, and the process has a low yield. These raise the necessity of producing aroma substances by biotechnological techniques.

Keywords: Aroma, Biotechnology, Flavoring Agents

SS-19
Lipoprotein-Mediated Disruption of the Cell Membrane and ROS Production as Mechanisms of Antifungal Activity of Lactic Acid Bacteria

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LAB are the main microbiota in the production of fermented foods and beverages, and they are known for their health-promoting properties, including their antifungal activity. [1] Fungal formation in food and feed, along with the subsequent release of mycotoxins, pose a significant threat to human health, with acute cases potentially leading to death. [5] Therefore, the control and prevention of foodborne poisoning have become crucial tasks in the field of public health. In response to this demand, biopreservation has emerged as one of the safest and most reliable methods for inhibiting fungal growth in foods. Lactic acid bacteria (LAB) have garnered considerable interest as biological additives in food due to their GRAS classification and probiotic properties. [2]

Among the microorganisms widely used for biological protection, LAB stand out for their capacity to produce a range of antimicrobial compounds. These compounds include organic acids, hydrogen peroxide (H2O2), proteinaceous compounds, reuterin, and certain volatiles, such as diacetyl fatty acids, volatile acids, bacteriocins, cyclic peptides, and low molecular weight substances. [2, 3, 6] These compounds impede fungal growth by disrupting the cell membranes of fungi. [3] The antifungal activity of LAB can be attributed to multiple factors. LAB produce antimicrobial compounds, acidify the environment, compete for nutrients, and generate lipoproteins. Antifungal fatty acids break down the lipid bilayers of fungal membranes, leading to the disruption of membrane integrity, cell lysis, and the release of intracellular proteins and electrolytes. [2] Proteinaceous compounds produced by LAB include ribosomal (RSPs) and non-ribosomal (NRPs) peptides, as well as peptides derived from the enzymatic hydrolysis of proteins. For instance, the Lf (1-11) peptide produced by LAB exhibits antifungal activity against Candida albicans and Aspergillus fumigatus. [4]
LAB primarily generate energy through lactic acid fermentation, resulting in the production of various byproducts, including peptidoglycan, polysaccharides, enzymes, and lipoproteins. Lipoproteins, composed of lipids and proteins, play a crucial role in the antifungal effect of LAB. They bind to fungal cell membranes, disrupting membrane integrity, inhibiting fungal cell survival, and impairing the activity of enzymes in fungal cells, thus affecting metabolic functions. Consequently, the lipoproteins produced by LAB effectively inhibit the growth and reproduction of fungi.
The antifungal mechanism of action of lipoproteins involves increasing the permeability of the fungal cell membrane by altering the lipid composition. This disruption of the internal balance within fungal cells ultimately leads to their death. Additionally, lipoproteins induce oxidative stress in fungal cells, resulting in the accumulation of intracellular reactive oxygen species (ROS), which cause oxidative damage and death of fungal cells. Under normal physiological conditions, an appropriate level of intracellular ROS can stimulate cell proliferation and facilitate adaptation to oxidation. However, when the concentration of ROS exceeds the antioxidant capacity of the cells, oxidative stress occurs, leading to damage to DNA, proteins, or lipids, and ultimately cell death. [5]
In conculation, fungal contamination in food and feed poses a significant threat to human health. To address this issue, biopreservation using lactic acid bacteria (LAB) has emerged as a safe and effective method. LAB metabolit lipoproteins, are promising biological additives in food.

Keywords: Lactic Acid Bacteria, Antifungal Activity, Lipoprotein, Cell Membrane, Reactive Oxygen Species

SS-20
Microbial Diversity and Health Benefits of Water Kefir Grains

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Fermented beverages are produced through the process of fermentation, wherein microorganisms (bacteria and yeasts) convert sugars and starches. Microbial diversity plays a crucial role in determining the product's aroma, taste, and nutritional value, while contributing to the overall ecosystem health. Water kefir, one such beverage, is produced by fermenting a mixture of water and sugar with water kefir grains. Water kefir grains form a symbiotic colony consisting of various lactic acid bacteria, acetic acid bacteria, and yeasts. Among these microorganisms are lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Pediococcus*, and *Bifidobacterium*; acetic acid bacteria like *Acetobacter* and *Gluconobacter*; and yeasts including *Saccharomyces*, *Candida*, and *Zygosaccharomyces*. These microorganisms actively participate in the fermentation process, imparting the beverage's characteristic flavor, aroma, and health benefits. Throughout the

fermentation process, they catalyze the transformation of water and sugar, resulting in the production of several health-promoting components, including prebiotics and vitamins. The microbiota of water kefir can influence nutrient absorption and metabolism. Probiotics can aid in the breakdown of specific nutrients, which may enhance nutrient absorption and metabolism. For instance, some probiotics can assist in the biosynthesis of certain vitamins such as Vitamin B and Vitamin K. However, the microflora of water kefir grains may vary depending on geographical region and fermentation duration. Factors such as the type of grains, fermentation time, and the type and quantity of fruits used in the production influence the microflora of this beverage, ultimately affecting its properties. Water kefir also exhibits probiotic properties that support the human gut microbiota, strengthen the digestive and immune systems, and improve nutrient absorption and metabolism. Consequently, the microbial diversity of water kefir grains plays a pivotal role in determining the quality of the final product and its associated health benefits.

Keywords: Fermented beverages, water kefir, microbial diversity, probiotics, health benefits, fermentation process.

SS-21
Characterization and potential applications of specific glucansucrases isolated from *Weissella* spp

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Introduction: Glucansucrases are members of the glycoside hydrolase 70 (GH70) enzyme family, which synthesizes polysaccharides and oligosaccharides using sucrose as a substrate. These enzymes are generally responsible for the formation of different glucans, and this difference is due to the α-(1→6) bonds in the main chain and the varying amounts of α-(1→2), α-(1→3) and α-(1→4) bonds in the chain. Weissella confusa can be isolated from different environments such as humans, animals, fermented foods and beverages, grains and vegetables. In previous studies, it has been stated that Weissella confusa strains have high glucansucrase activity and the enzymes responsible for production were determined. Even if Weissella strains have similar glucansucrases, different polysaccharides and oligosaccharides produced may have different functional and technological properties. In this study a novel glucansucrase from sourdough isolate Weissella confusa SDE was identified, heterologously expressed and the structure of glucan SDE was determined.

Materials and methods: Weissella confusa SDE was isolated and cultivated. Whole genome sequencing of Weissella confusa SDE was performed and the similarities between glucansucrase SDE and other glucansucrases available in the NCBI database were identified using NCBI BLASTp searches. Multiple amino acid sequence alignments were conducted with Clustal Omega Tool. The aLICator Ligation Independent Cloning and Expression Kit (Thermo Scientific, USA) was used to clone the glucansucrase gene from Weissella confusa SDE. This gene was cloned into the IPTG-inducible vector pLATE31 and expressed in the host E. coli BL21 (DE3). For the production of glucan with glucansucrase SDE enzyme, the reaction medium was established with sucrose and the resulting glucan was lyophilized. Structural, molecular and thermal characterization of this glucan conducted with the 1H NMR analysis, Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimeter (DSC) analysis, respectively.

Results: Under in vitro conditions, a glucan SDE was produced by glucansucrase SDE using sucrose as the substrate. FTIR analysis of glucan SDE revealed the presence of functional groups within the structure, suggesting

that this polymer is a polysaccharide. According to the results of DSC analysis, the degradation of glucan SDE started around 200°C and the melting point of glucan was determined to be approximately 233 °C. Overall, 1H NMR analysis results showed the production of glucans with α-(1→3) and α-(1→6) glycosidic linkages via the reaction of glucansucrase SDE.

Conclusions: Weissella spp., which are very widely found in nature and has been limited studied so far, it is important to isolate its new glucansucrases, expression of recombinant enzymes, determination of their characteristic properties and investigation of their potentials. In this study, these findings showed the production of glucan with α-(1→3) and α-(1→6) glycosidic linkages. The FTIR analysis showed the presence of the functional groups within the glucan structure. The thermal properties of glucan SDE may enable its use in many food matrices, including bakery products. Consequently, the physicochemical properties of glucan SDE would make it a potential candidate for applications in the food industry.

Keywords: Glucansucrase, glucan, Weissella

SS-22

Effect of in vitro Digestion and Ultrafiltration on the Bioactive Properties of Unfermented and Fermented Spirulina Products

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Proteins are an essential part of a healthy diet, and today animal-based protein sources (meat, egg, milk etc.) are the main source for the protein intake. Therefore, alternative proteins such as plant proteins, edible insects, single-cell proteins and microalgal biomass are attracting researchers to use in the diet. Among these microalgae are one of the most promising candidates. Despite their high nutritional value, there are several factors that limit the use of microalgae in food products such as taste, odor and limited information related to their bioavailability. In this case, fermentation can be a good approach to overcome these obstacles and promote the development of microalgae-based products as microorganisms and fermentation conditions can diversify the properties of the final product. Previously, we have screened several lactic acid bacteria, Bacillus and yeast species and optimized the fermentation conditions at the flask level, followed by the optimization of the conditions (e.g., aeration, agitation and pH) in a laboratory scale (3L) bioreactor in the batch mode and evaluated the effect of these production conditions on some bioactive properties (total phenolic content, antioxidant activity, antimicrobial activity and ACE-inhibitory activity). The present work aimed to focus on the most promising production mode, cascade, in terms of the effect of in vitro digestion and ultrafiltration of unfermented Spirulina (unFS) and fermented Spirulina (FS) on the bioactive properties such as total phenolic content (TPC), DPPH inhibitory activity and Trolox Equivalent Antioxidant Capacity (TEAC) and ACE-inhibitory activity (ACE-I). UnFS and cascade FS products were subjected to the in vitro gastrointestinal digestion. The results showed that there was a slight increase in the TPC of unFS from 10.88 mg GAE/g to 12.42 mg GAE/g sample followed by a decrease (8.79 mg GAE/g) after intestinal digestion. On the other hand, the TPC of FS decreased from 49.02 mg GAE/g to 27.71 mg GAE/g after gastric digestion and maintained its TPC after intestinal digestion (26.82 mg GAE/g). DPPH inhibitory activity decreased with digestion in both FS and unFS, however, the decrease was greater in unFS (316%) compared to FS (77%). Gastrointestinal digestion increased the TEAC for both FS and unFS. In contrast, digestion

caused a decrease in the ACE-I activity of both FS and unFS, whereas FS was able to conserve the activity obtained at the gastric digestion stage after the intestinal digestion step as well. Overall, FS maintained its activity better than the unFS products. Regarding the different fractions obtained as a result of ultrafiltration, the fraction 5-10 kDa showed good bioactive properties for TPC and DPPH inhibitory activity for both unFS and FS while the higher molecular weight fraction >10 kDa had the highest ACE-I activity for both products. While the smallest fraction <5 kDa had the highest TEAC for FS (793.2 mM Trolox/g), higher MW fractions (>10 kDa and 5-10 kDa) showed better TEAC values (189.7 and 204.3 mM Trolox/g, respectively) for unFS. Thus, this study emphasized the importance of fermentation as an important strategy to conserve bioactive properties of products during in vitro digestion. Finding the effective fractions can lead to purification of active compounds.

Keywords: alternative proteins, Spirulina, fermentation, in vitro digestion, ultrafiltration, bioactivity

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SS-23
AI-2 (autoinducer-2) based spoilage mechanism of *Pseudomonas* species isolated from meat

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Quorum Sensing (QS) is a system that bacteria use to sense their population density via signals called autoinducers. Gram-negative bacteria can produce autoinducer-1 (acylated homoserine lactones, AHL) as QS signal molecules, and another type of signal molecule group known as autoinducer-2 (AI-2) is seen in both Gram-positive and Gram-negative bacteria. These signal molecules are produced and detected by spoilage-causing microorganisms to modulate various mechanisms, including biofilm formation, proteolytic activity, and motility. *Pseudomonas fragi* is a significant meat spoiler that causes deterioration under refrigerated conditions resulting in an unacceptable product associated with economic losses. *Pseudomonas fragi* cannot produce AHL, but this species has been shown to recognize and respond to Autoinducer-2. The effect of the *P. fragi* AI-2 molecule on meat spoilage has yet to be examined in detail. In this study, 337 *Pseudomonas* species were isolated from beef and minced meat samples collected from 12 local butchers. Using P. fragi-specific primers fraF and carA, 100 isolates were determined to be putative *P. fragi*. Phylogenetic analysis using *rpo*D gene sequences indicated that 57% of the isolates were *P. bubulae*, a recently defined species close to *P. fragi*. While 13% of the isolates were *P. fragi*, there were also isolates forming separate clades different from *P. fragi* and *P. bubulae*, indicative of possible new species. The isolates were tested for QS-related spoilage activities: biofilm and motility. Biofilm formation was tested using the Congo red agar (CRA) test, the tube method, and the microtiter plate assay. The bacterial motility of all isolates was examined on semi-solid agar; twitching, swarming, and swimming movement zones were measured. Thirty-eight strains that formed black colonies in CRA were accepted as biofilm positive, and their biofilm production was measured spectrophotometrically at 4° and 25°C for 1, 4, and 7 days. It was observed that biofilm production was increased with increasing incubation times. Generally, higher biofilm formation was detected at 25°C than at 4°C; however, certain isolates produced more biofilm at 4°C. The biofilm production ability was parallel to the motility of the isolates. The AI-2 production of the isolates was screened using the luminescence created by the biosensor Vibrio harveyi strain, and the relation of the signal molecule with the spoilage activities of the isolates was analyzed. The findings of this study will be the first endeavor to elucidate

the spoilage behavior of meat *Pseudomonas* strains related to AI-2 signal molecule and QS-dependent biofilm formation and bacterial motility.

Keywords: Autoinducer-2, biofilm, meat spoilage, Pseudomonas, Quorum Sensing

SS-24

Molecular characterization of genes responsible for formation of petroleum odor within mold and yeast species isolated from kashar cheese

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Kashar cheese is one of the most consumed cheeses in Turkey after white cheese. Molds and yeasts are one of the main causes of spoilage in cheese production and ripening, resulting in quality and health problems and economic losses. Although the hygiene rules and vacuum packaging are applied during the production, these problems cannot be completely prevented. That's why, manufacturers prefer the usage of potassium sorbate as a legal preservative additionally. However, undesired petroleum/kerosene odor is formed in sorbate-treated kashar cheeses, which is caused by the 1,3-pentadiene formed as a result of the decarboxylation of sorbic acid by sorbate resistant molds and yeasts. In this study, we aimed to reveal the genes/enzymes that is responsible for petroleum odor formation in mold and yeast genomes. The isolated mold and yeast species from 24 commercial sorbate-treated kashar cheese samples, with and without petroleum odor, collected from 5 different enterprises were used for the characterization of petroleum odor related genes/enzymes. The studied molds belonged to Penicillium, Aureobasidium, Byssochlamys and Cladosporium genera, and the yeast taxa belonged to the Candida genus besides Deboraymyces hansenii. The genomes of yeast and mold species caused petroleum odor were examined for the presence of PAD1/padA1 and ohbA1 genes encoding enzymes that degrade sorbate. PAD1 gene encodes phenylacrylic acid decarboxylase. A similar decarboxylation enzyme had been found in Aspergillus niger and named padA1. Additionally, padA1, another decarboxylase gene, ohbA1, was thought to be important in the decarboxylation of sorbate. The PAD1/padA1 gene was observed in Penicillium commune and D. hansenii isolated from cheese samples showing strong odor, and Penicillium chrysogenum, Penicillium roqueforti, Penicillium raistrickii. Any PCR product for PAD1/padA1 could not be obtained neither Penicillium brevicompactum and Penicillium solitum nor Candida genus, which do not cause a sense of petroleum odor. ohbA1 gene was seen in P. commune and D. hansenii. Each PCR product was sanger sequenced, then multiple DNA sequence alignment was performed by UGENE and ClustalW2 programs. P. commune and P. roqueforti were found to be closest to each other in the PAD1/padA1 gene with 96% similarity. When D. hansenii and P. commune ohbA1 gene were compared, 42% similarity was found. In the sorbate treated cheese samples, P. commune was more common than P. roqueforti. In following stages, a molecular test kit will be developed to test in a short time whether the presence of yeast and mold that causes odor formation is in the cheese. Flavor defects caused by microorganisms leads to food-waste because it creates a spoiled food perception for consumers. With these findings, it will be possible to control the factors that trigger odor formation in case of using sorbate. Acknowledgment: The authors are grateful for the financial support provided for the project 120 O 480 by the Scientific and Technological Research Council of Turkey (TUBITAK).

Keywords: genotyping, kashar cheese, mold and yeast, petroleum odor, sorbate

SS-26

Effect of Solanum lycopersicum and Citrus limon–Derived Exosome-Like Vesicles on Chondrogenic Differentiation of Adipose-Derived Stem Cells

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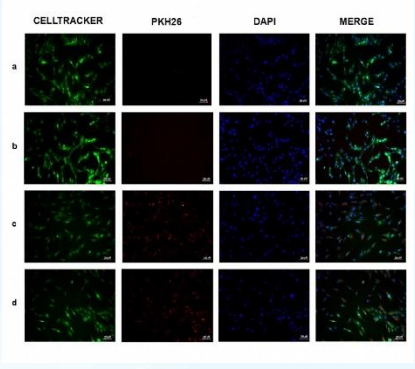
Articular cartilage defect treatment is a very important problem because its therapeutic optionsare not successful enough. Due to the weak self-repairing capacity of the avascular cartilage, even minor damage can progress and cause joint damage leading to osteoarthritis. Although various treatment strategies have been developed to repair damaged cartilage, cell- and exosome-based therapies are promising. Plant extracts have been used for decades, and their effects on cartilage regeneration have been studied. Exosome-like vesicles, which are secreted by all living cells, are involved in cell-to-cell communication and cell homeostasis. The differentiation potential of exosome-like vesicles isolated from S. lycopersicum and C. limon, which are known to have antiinflammatory and antioxidant properties, was investigated in the differentiation of human adipose-derived mesenchymal stem cells (hASCs) into chondrocytes. In order to obtain tomatoderived exosome-like vesicles (TELVs) and lemon-derived exosome-like vesicles (LELVs) Aquous Two- Phase system was performed. Characterisation of isolated vesicles based on size, shape were achived via Zetasizer, NTA, FAME analysis, and SEM techniques. These results showed that TELVs and LELVs increased cell viability and did not show any toxic effects on stem cells. Although TELVs triggered chondrocyte formation, LELVs downregulated. The expression of ACAN (Aggrecan), SOX9 (SRY-Box Transcription Factor 9), and COMP (Cartilage oligomeric matrix protein), known as chondrocyte markers, was increased by TELV treatment. In addition, protein expression of the two most important proteins, COL2 (Collagen type 2) and COLXI (Collagen type XI), found in the extracellular matrix of cartilage, increased. These findings suggest that TELVs can be used for cartilage regeneration, and may be a novel and promising treatment for osteoarthritis.

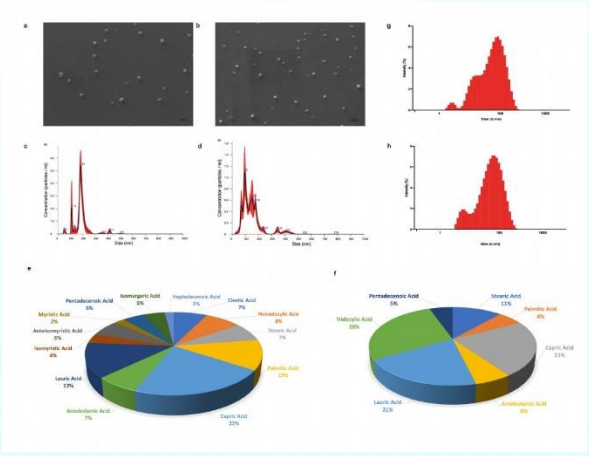
Keywords: Citrus limon, Exosome, Osteoarthirit, Plant-derived exosome, Solanum lycopersicum

Celluler Uptake



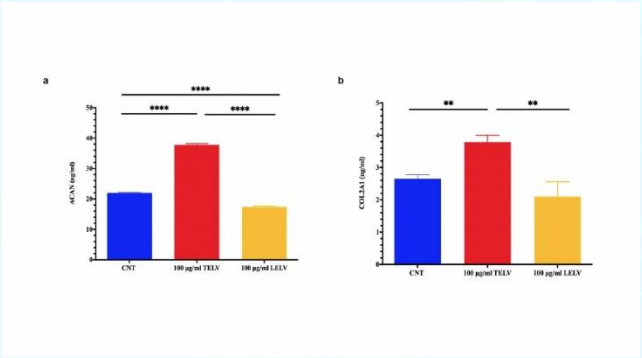
a S. lycopersicum 0-h, b C. limon 0-h, c S. lycopersicum 6-h, and d C. limon 6-h incubation of exosome-like vesicle uptake of human adipose-derived stem cells at 20 × magnification

Exosome Characterization



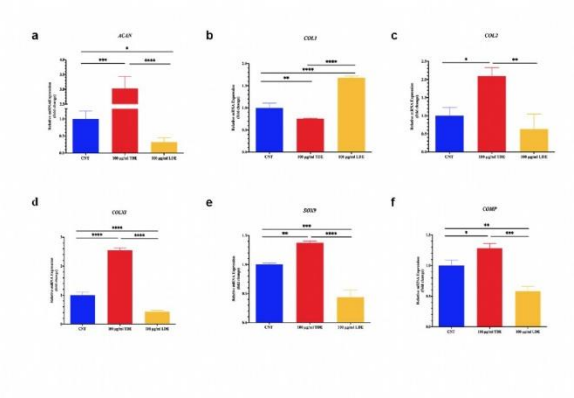
a. Scanning electron microscope image of S. lycopersicum-derived exosome-like vesicles b. Scan- ning electron microscope image of C. limon-derived exosome-like vesicles with a scale bar of 1 µm c. Size distribution of S. lycopersicum d. Size distirbution of C. limon-derived exosome-like vesicles e. FAME analysis of S. lycopersicum-derived exosome like vesicles f. FAME analysis of C. limon-derived exosome- like vesicles g. Size distribution and density of S. lycopersicum h. Size distribution and density of C. limon- derived exosome-like vesicles i. Exosome surface marker protein (CD63, CD81 and CD9) expressions were detected by Simple WesternTM

Protein expressions



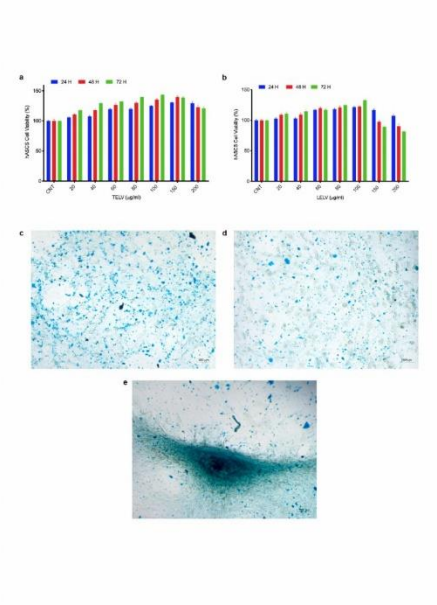
*Expression levels of a ACAN and b COL2A1 of control cells without no treatment and differentiated cells which were treated with 100 µg/mL of LELV and TELV (*P≤0.05, **P≤0.01, ***P≤0.001, ****P ≤ 0.0001)*

RNA analysis



*mRNA expression levels of differentiated cells for a ACAN, b COL1, c COL2, d COLXI, e SOX9, and f COMP genes upon control; 100 µg/mL of TELV and LELV treatment (*P≤0.05, **P≤0.01, ***P ≤ 0.001, ****P ≤ 0.0001).*

Viability and Chondrogenic Differentiation



*Effects of different doses of a S. lycopersicum and b C. limon exosomes on hASC proliferation for 24 h, 48 h, and 72 h. c Alcian Blue staining for control cells which were not treated with any ELVs at the end of 21 days of differentiation. d Alcian Blue staining for LELV at the end of 21 days of differentiation. e Alcian Blue staining for TELV at the end of 21 days of differentiation (*P < 0.05)*

SS-27

***In vitro* Evaluation of Cytotoxic, Genotoxic and Antigenotoxic Activity of Bee Bread on HCT116 Human Colon Cancer Cells**

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INTRODUCTION: Apitherapy is the therapeutic use of bee products such as honey, bee pollen, royal jelly, bee venom, propolis and bee bread. As a result of the use of synthetic substances for therapeutic purposes, various side effects have been encountered in the human body, and for this reason, traditional, natural treatment methods have been trended. Due to the fact that our country is suitable for beekeeping, studies on apitherapy, which is a sustainable method, have increased. Bee bread is formed by the addition of honey and enzymes involved in the digestive system of bees to the pollen collected by the bees, packaging with the help of beeswax in the honeycomb parts of the hive, and lactic acid fermentation as a result of packaging. It is thought that bee bread, which is beneficial for bees as well as for humans, will be used in the treatment of many diseases, especially cancer. Although studies on the biological activities of bee bread have increased in recent years, they are still not sufficient.In this study, it was aimed to determine the cytotoxic, genotoxic and antigenotoxic effects of bee bread on HCT116 colon cancer cells.

MATERIALS-METHODS: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used to determine the cytotoxic activity of bee bread, and the Comet assay was used to determine the genotoxic and antigenotoxic activity.

RESULTS: According to the results of the MTT test; cell viability was decreased in all tested concentrations (500, 1000, 1500, 2000, 2500, 3000, 3500 ppm) of bee bread extract, compared to the control as concentration dependentlySince cell viability was observed to be below 70% in general and the IC50 value was found to be 2320,7 ppm. According to the comet test results for genotoxicity, it was determined that all tested bee bread concentrations increased DNA damage compared to the control, but this increase was statistically significant only at 250 and 500 ppm ($p<0,05$). A comet assay was also performed on cells treated with hydrogen peroxide (H2O2) to determine the antigenotoxic effect, and the results showed that bee bread increased DNA damage at all concentrations. At 125 and 500 ppm, this increase is statistically significant. According to these results, it was determined that bee bread increases the DNA damage in colon cancer cells both alone and when applied together with H2O2.

CONCLUSION: Based on findings of MTT and Comet assay, it was determined that bee bread has cytotoxic and genotoxic activity and no antigenotoxic activity on HCT116 colon cancer cells. Acknowledgement: This study is supported by TUBITAK 2209-A, Project No: 1919B012221919.

Keywords: antigenotoxicity, bee bread, colon cancer cell, cytotoxicity, genotoxicity

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SS-28

Identification and investigation of GLUT4-associated miRNAs and their target genes potentially responsible for tissue damage in *Diabetes mellitus*

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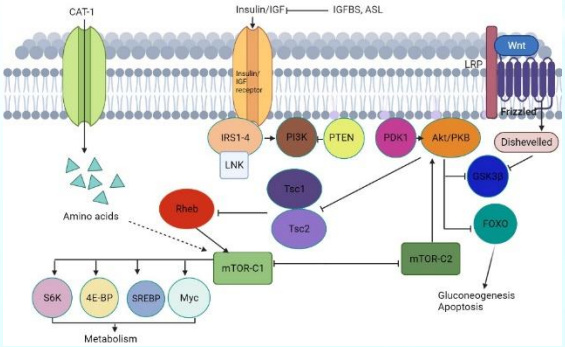
INTRODUCTION: *Diabetes mellitus* (DM) is a complex metabolic disorder characterized by chronic hyperglycemia and lipotoxicity, which can lead to tissue damage and various complications. Among the crucial factors involved in glucose homeostasis, the glucose transporter 4 (GLUT4) protein plays a vital role in insulin-mediated glucose uptake within cells. Dysregulation of GLUT4 expression and function has been linked to diabetic complications. microRNAs (miRNAs) are small non-coding RNA molecules that post-transcriptionally regulate gene expression, and they are known to play a critical role in numerous physiological and pathological processes, including diabetes. In diabetes, they are involved in the production, secretion and insulin signaling in target tissues. miRNAs have been being defined as leading GLUT4 regulators in the recent years. This study aims to identify potential miRNAs associated with diabetic tissue damage and their involvement with GLUT4 by employing bioinformatic methods to analyze their interactions with target genes.

MATERIALS-METHODS: Initially, the sequences encoding the Human GLUT4 gene and protein sequence information were downloaded from the "National Center for Biotechnology Information (NCBI)" database. The promoter analysis of the GLUT4 gene was determined from the “Eukaryotic Promoter Database (EBD)” database. One of the comprehensive miRNA target prediction tools, TargetScanHuman, was used to identify miRNAs that could target the GLUT4 gene. The targets and associated pathways of potential miRNAs were found with the mirPathDB tool. Results and DISCUSSION: As a result, For GLUT4, 137 transcription factors with a 5% dissimilarity rate were found. Twelve miRNAs associated with the GLUT4 gene and their targets were identified. In the target and signal pathway analysis, it was found that the identified miRNAs were associated with INSR, ASL, IRS4, PTEN, PDK1, FOXO1, FOXO3, WNT, TSC1 and GSK3B targets, and we depicted the locations of these targets within the metabolic process pathways which are linked to the insulin pathway. Although there have been a number of recent reivews compiling information on the effects of GLUT4-associated miRNAs in diabetic tissue damage, we find that the miRNAs that we focused on in this study have not yet been addressed. Hence, our results provide a new perspective to GLUT4-associated miRNA role in DM tissue damage.

CONCLUSIONS: The findings from our study will provide valuable insights into the molecular mechanisms underlying diabetic tissue damage and its association with GLUT4. Understanding the further regulatory roles of miRNAs concerning GLUT4 expression and function may lead to the development of novel approaches for diabetes management and prevention of associated tissue complications.

Keywords: Bioinformatic analysis, {Diabetes mellitus} (DM), tissue damage, microRNAs (miRNAs), glucose transporter 4 (GLUT4)

Insülin signaling pathway.



miRNAs associated with the GLUT4 gene and their targets

miRNAs	Targets
miR-372-3p	, IRS4, PTEN, PDK1, FOXO1, FOXO3, TSC1, ASL, WNT
miR-302e	, IRS4, PTEN, PDK1, FOXO3, TSC1, ASL, WNT
miR-520a-3p	, PTEN, PDK1, GSK3B, FOXO3, TSC1, ASL, WNT
miR-302a-3p	, IRS4, PTEN, PDK1, FOXO3, TSC1, ASL, WNT
miR-302b-3p	, IRS4, PTEN, PDK1, FOXO3, TSC1, ASL, WNT
miR-520d-3p	, ASL, IRS1, IRS4, PTEN, PDK1, FOXO3, TSC1, ASL, WNT
miR-373-3p	, ASL, IRS1, IRS4, PTEN, PDK1, GSK3B, FOXO3, WNT, TSC1
miR-520c-3p	, ASL, IRS1, IRS4, PTEN, PDK1, FOXO3, WNT, TSC1
miR-302c-3p	, ASL, IRS4, PTEN, PDK1, FOXO1, FOXO3, WNT, TSC1

SS-29

Contribution of Exosomes Released from Cisplatin-Resistant Ovarian Cancer Cells to Tumorigenesis In Vivo

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INTRODUCTION: In the cancer mechanism, it is necessary to determine the mechanisms responsible for the differentiation of normal cells and the transfer of oncogenic signals between cancer and normal cells. It is predicted that the reprogramming of the tumor microenvironment for the formation, invasion and migration of cancer cells is provided by oncogenic signals released from cancer cells and carries these signals in their exosomes. In the present study, it was evaluated whether exosomes released from drug-resistant ovarian cancer cells were associated with tumor formation in the primary and secondary tumor microenvironment in vivo.

MATERIALS-METHODS: Ovarian cancer xenograft models has been used in the Balb/c nude mice to evaluation whether tumor formed or not. Two groups of mice were formed as subcutaneous (sc) and intraperitoneal (ip) injections. 5 x10^6 and 10x10^6 of A2780cis cells were injected to create a subcutaneous tumor model and followed for 3-4 weeks. To observe the second group, intraperitoneal tumor formation, A2780cis cells+exosome and A2780cis (control) cell groups were created. Peritoneal membrane, ovary, liver, lymph node of the mice sacrificed at 8, 10 and 12 weeks were collected, and their histopathological evaluations were made.

RESULTS: Tumor formations were observed in the sc injected with both cell groups and tumor parenchyma cell morphology was determined to be similar. While degeneration and apoptotic cells to be observed in the liver tissues no metastasis has been determined. Nevertheless, the ovaries of mice injected specifically with 10x10^6 A2780cis cells (sc) were completely tumor tissue. In the IP injection group studies, tumor formation was observed 10 weeks after the "A2780cis+exosome" injection compared to the control group. It was observed that the tumor was adherent to the liver and stomach. Moreover, degeneration was observed in the liver, necrosis started in some hepatocytes and follicle phase and corpus luteum were observed in the ovum together with the reactive lymph node.

CONCLUSIONS: It was observed that the lymph nodes doubled in mice (ip) with tumor formation by giving the exosome. The contribution of exosome released from A2780cis cells to tumor formation in the abdominal cavity and lymph enlargement was observed.

Keywords: Exosome, A780cis cell, ovarian cancer

SS-30

Preparation and Characterization of Dextran Coated Iron Oxide Nanoparticles Loaded with Doxorubicin and MDR1-siRNA for Use in the Treatment of Breast Cancer

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Introductıon: Cancer diseases are one of the most prominent disease groups in terms of fatal diseases today. Breast cancer is a leading subgroup in terms of the incidence of the cancer disease. The most common applications in breast cancer treatment are traditional methods such as surgery, chemotherapy and radiation therapy. But in all these approaches, there are some destraction effect possibilities to the cancer tissues as well as healthy tissues. For this reason, there is a need for a new generation of therapeutics and treatment applications in the treatment of those cancer diseases.

Materials & Methods: In this presented study; it was tried to provide MDR1-siRNA transfection with a special nanocarrier system containing dextran coated magnetic nanoparticle in the MCF-7 cell line, which is resistant to Doxorubicin and has high P-glycoprotein, P-gp activity especially for the destruction of cancer cells that are resistive to the active substance of Doxorubicin, which is widely used in chemotherapeutic applications. In this context, iron oxide nanoparticles were prepared by using precipitation of iron chloride salts and these nanoparticles were coated with dextran polymer to get functional nanostructures. Then, MDR1-siRNAs were added to the magnetic nanoparticles loaded with doxorubicin and the necessary gene silencing process was performed to overcome the drug resistance mentioned above. During these studies, first dextran coated iron oxide nanoparticles were synthesized with co-precipitation method with a spherical structure and Doxorubicin loading was performed on the dextran coated nanoparticles by using impregnation method and Doxorubicin loading were confirmed by FTIR and spectrophotometric techniques. During the loading experiments, higher loading efficiency values were obtained with the long term preparation formulations where the loading time of doxorubicin is so long. In the part of the study performed with genetic materials, optimization studies were performed using MDR-1 primer primarily in preparation for MDR1-siRNA loading and transfection studies. Thus, according to the obtained values, the concentration of MDR1-siRNA, which should be loaded into the nanoparticles developed to reduce the targeted P-gp activation, has been successfully determined.

Results & Discussions: Dextran coated iron nanoparticles produced by the co-precipitation method were characterized by SEM, DLS and FTIR, and their dimensions were measured as approximately 10 nm (± 2nm), and the size of doxorubicin loaded nanoparticles was measured as 18 (± 5nm) nm on average. The size of the drug-loaded nanoparticles increased as expected. In addition, as a result of 48 hours loading time for drug loading and optimization studies for loading genetic material, 25 nmol concentration was found to be more efficient for loading MDR-1 siRNA. It has been observed that the developed dextran-coated doxorubicin and MDR-1 siRNA-loaded iron nanoparticles can be used as an effective nanocarrier in MCF-7 cancer cells.

Keywords: Gene silencing, nanoparticles, MDR-1 siRNA, iron-oxide, RNAi

SS-31

Development and Characterisation of Biocompatible Structures for Use in Endovascular Embolization of Intracranial Aneurysms

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Introduction: An intracranial aneurysm is a focal dilation and weakening of the arterial blood vessels in the brain[1]. Aneurysms can grow unpredictably, and even small aneurysms (<15 mm) are at risk of rupture, which can cause life-threatening bleeding and brain damage. Although there are surgical and endovascular methods of treating aneurysms, endovascular methods are the most commonly used in the clinic. The aim of endovascular methods is to block the blood flow to the aneurysm and the most commonly used endovascular treatment in the clinic is endovascular filling using polymer or metal based fillers, coils and fibres[2]. The aim of this study was to develop and characterise biocompatible fillers for use in aneurysm treatment.

Materials & Methods: Biocompatible polymeric structures consisting of micro-nanofibres with a diameter that can pass through the catheter were prepared by electrospinning, taking into account the actual dimensions of the structures used in the clinic.
In order to produce the most suitable micro-nanofibre structure, the collector types (iron, brass, etc.), electrospinning system parameters (applied voltage, needle-electrode distance, spiral formation techniques) and polymer material parameters (polymer and solvent type, concentration, etc.) were optimised. As a result of the parameter optimisations, the type of polymer to be used was evaluated as PCL (Polycaprolactone) and tubular structures of different diameters (0.8 mm,1 mm, 2 mm, 3 mm etc.) in PCL micro-nanofibre structure were produced. The PCL micro-nanofibres were then placed in hydrogels prepared with alginate at different viscosities and concentrations to impart swelling properties to the microfibre structures.Within the scope of hydrogel preparation, 3%, w/v alginate solutions prepared at different concentrations were crosslinked with different amounts of $CaCl_2$. Then the hydrogels were dried and their swelling behaviour was investigated in pH 7.4 PBS (Phosphate-buffered saline) solution.

Results & Discussions: The morphological properties of the microstructures of the polymeric fillers were evaluated by SEM and the swelling behaviour of the filler was determined gravimetrically and volumetrically. The mean diameters of the microfibers obtained were measured to be in the range of 2-5 µm. The results of the observed swelling behaviour showed that all samples showed an increasing swelling trend. The swelling rate increased 5 to 10 times gravimetrically and at least 3 times volumetrically.The tendency of the samples to swell in volume with water uptake also demonstrated the ability to fill and occlude an aneurysm.

Resources

[1] Cerebral aneurysm (2022) JHM. Available at: https://www.hopkinsmedicine.org/health/conditions-and-diseases/cerebral-aneurysm (Accessed: 08 August 2023).
[2] Bederson, J. B., Connolly, E. S., Batjer, H. H., Dacey, R. G., Dion, J. E., Diringer, M. N., Duldner, J. E., Harbaugh, R. E., Patel, A. B., & Rosenwasser, R. H. (2009). Guidelines for the Management of Aneurysmal Subarachnoid Hemorrhage. Stroke, 40(3), 994–1025. https://doi.org/10.1161/strokeaha.108.191395

Keywords: Endovascular Embolization, Intracranial Aneurysms, Rod Electrospinning

SS-32

Beyond Conventional Therapies: Polymeric Micelles as a Cutting-Edge Arsenal to Combat Triple Negative Breast Cancer

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Introduction: Triple-negative breast cancer (TNBC), a distinct form of breast malignancy, is characterized by the absence of ER, PR, and HER-2 receptors within the tumor. This highly aggressive subtype, TNBC, exhibits initial indications of resistance to chemotherapy. A significant drawback associated with this variant lies in its unfavorable prognosis relative to other breast cancer subtypes, manifesting as diminished overall survival rates, frequent relapses, and heightened mortality rates.

Materials And Methods: In our study, we have effectively engineered polymeric micelles using a novel negatively charged SPMA/PMMA copolymer and synthesized via RAFT (Reversible addition−fragmentation chain-transfer) polymerization. These micelles stand poised to act as a proficient carrier system, strategically poised to surmount the obstacles encountered in addressing TNBC. Utilizing the nanoprecipitation technique, we synthesized polymeric micelles, subsequently forming a micelle-doxorubicin (DOX) complex via electrostatic interactions.

Results: By employing Dynamic Light Scattering (DLS) and ζ-potential analysis, we established that our nanoparticles exhibit a dimension of 180.8 ± 1 nm, accompanied by a surface charge of -41.2 ± 3 mV.

Conclusions: These measurements facilitated an evaluation of size distribution and stability in the micelle-DOX complex, as well as an assessment of surface charge attributes through ζ-potential assessments. Ongoing investigations explore the therapeutic potential of these polymeric micelles in in vitro models.

Acknowledgments
Berrin Chatzi Memet is supported by the TUBITAK (121C221).

SS-33
Development of dopamine-conjugated polymeric micelles against breast cancer

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Polymeric micelles have been utilized for the controlled delivery of anti-cancer drugs and gene therapeutics to enhance their efficiency and minimize systemic side effects. Nevertheless, non-specific drug/gene release has been a challenge, limiting the therapeutic efficacy of these systems. Active targeting strategies employing ligands have gained considerable interest to address this issue. On the other hand, dopamine receptors are known to be overexpressed in breast cancer cells, making them an attractive target for active drug delivery. In this study, diblock copolymers were synthesized using reversible addition−fragmentation chain transfer (RAFT) polymerization. The hydrophobic block consisted of biocompatible poly (methyl methacrylate) (PMMA), while the hydrophilic block comprised statistical copolymers of biocompatible oligo (ethylene glycol)-methacrylate (OEGMA) and a dichloromaleimide functional monomer (DCMMA). Self-assembly of the synthesized block copolymer library was achieved using the nanoprecipitation technique. To enable active targeting, we conjugated dopamine on the surface of nanoparticles using the DCMMA monomer, known for its rapid reactivity with amine-bearing molecules.
The resulting dopamine-conjugated polymeric micelles (DCPMs) were thoroughly characterized using dynamic light scattering (DLS) and transmission electron microscopy (TEM). According to the obtained results, their size was determined to be 157 nm with a zeta potential of -28 mV. The DCPMs exhibited excellent stability and monodispersity. The DCPMs showed cellular uptake efficiency of up to 75% by breast cancer cells.
Our study successfully demonstrated the incorporation of dopamine conjugation offers the potential to enhance the therapeutic efficacy of anti-cancer drugs by enabling specific uptake of breast cancer cells. This approach holds

promise in overcoming the limitations of non-specific therapeutics release and fostering the advancement of more effective breast cancer treatments.

• This work was funded by Erciyes University Scientific Research Projects (BAP) Grant No: TKB-2021-11075.

Keywords: Breast Cancer, Dopamine, Polymeric Micelles

SS - 35

Structure Activity Relationship for Antifungal Activity of Chalcone Derivatives

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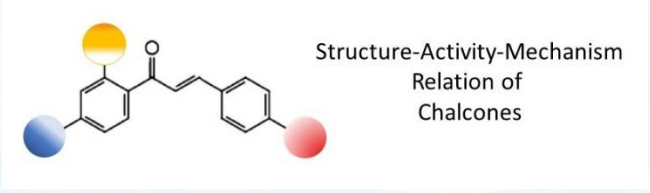
Fungal infections are described as infections that are caused by fungal cells or fungi. These types of infections can be serious or life threatening in severe cases. For this reason and due to the increased number of people suffering from fungal infections, the need to discover and find new and novel antifungal agents has been raised. In addition, the frequent use of the existing antifungal drugs and agents, has gave the fungal cells resistance against them. Chalcones are phenolic organic substances that exist naturally in plants. These substances can be extracted from fruits and vegetables to be used in pharmaceutical drugs industry as they have properties such as having good anticancer, antibacterial, anti-inflammatory features. Chalcones participate in the biosynthesis of flavanones, which give them biological properties similar to the chalcones.

Most of the existing antifungal agents target the cellular membrane of the fungal cell. Cell membrane acts as the external barrier that controls substances entering or leaving the cell. When cell membrane is disrupted by such agents the cell loses its viability. The mechanism of fungal cellular membrane disruption differs from agent to another. An example for this are agents that inhibit the biosynthesis of ergosterol which is involved in the integrity and permeability of the fungal cell.

The studies on the link between chalcone derivatives with different chemical structures and the fungal cell viability are very few. Thus, in this study, we aim to clarify the relationship between chalcones and its derivates on yeast cells S. cerevisiae. Many tests are applied to S. cerevisiae treated with chalcones to investigate the exact effect of these substances on yeast cells. Our study will take part in the improvement of the current knowledge regarding antifungal agents, and it will help in the discovery and development of new and novel antifungal agents.

Keywords: Antifungal, Chalcones, Cell membrane.

structure activity mechanism relation of chalcones



SS-36

Symbiosis with Beneficial Soil Fungi Promotes Growth and Affects Abiotic Stress Tolerance of Wheat Grown in Soilless Culture

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Boron (B) is an essential microelement with several exceptional traits, including differences in phloem mobility among species, a narrow range of adequacy between deficiency and toxicity, and variations in responses to these conditions. B toxicity affects a variety of processes in vascular plants including root and shoot growth, photosynthesis, and reproductive development. Plants have evolved various mechanisms for avoiding or tolerating the detrimental effects of B toxicity such as reduced B uptake and exudation of excess B from roots, induced production of B-chelating compounds, regulation of antioxidative response, and partitioning of excess B into tissues where it will be less damaging. Recently, symbiosis with beneficial soil microorganisms is gaining interest due to their ability to increase abiotic stress tolerance in plants as well as promote growth and increase yield in crop plants. In this study, the effects of root colonization by arbuscular mycorrhizae (AM) or the non-mycorrhizal but AM-like endophytic symbiont *Piriformospora indica* on the root morphology and stress tolerance of bread wheat *(Triticum aestivum)* and durum wheat *(Triticum durum)* subjected to B toxicity and/or phosphorus (P) deficiency in soilless culture were studied. The responses related to growth and stress tolerance varied depending on the wheat species and symbiotic partner. Colonization with *P. indica* was particularly effective for promoting growth although B toxicity symptoms were not completely eliminated through symbiosis. In contrast to *P. indica*, which did not influence root length or surface area but increased average root diameter and tip number, colonization with AM caused significant changes in root architecture, including reductions in root length, surface area, and tip number. Significant effects of AM colonization on P uptake in plants under P deficiency were observed. There were also major variations in the effects of the studied fungal symbionts on mineral nutritional status and antioxidative enzyme activities. Results confirmed that microbial partners can be critical agents for sustainable agriculture under stressful conditions and indicated that *P. indica* as well as AM fungi can also work under B toxicity.
Acknowledgement: This study was supported by THE SCIENTIFIC AND TECHNOLOGICAL RESEARCH COUNCIL OF TURKIYE (TÜBİTAK) 1001 Project 118Z984 with the title “Investigation of the application potential and physiological effects of *Piriformospora indica* as a biological agent against boron toxicity in wheat”.

Keywords: Boron toxicity, beneficial soil fungi, phosphorus deficiency, soilless culture, root morphology.

SS-37

Symbiosis with Arbuscular Mycorrhizae Protects Bread Wheat against the Combined Impacts of Boron Toxicity and Salinity Stress

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Salinity and boron (B) toxicity are abiotic stress factors that reduce agricultural output and threaten the economy and global food security. They frequently co-occur in fields in arid and semi-arid regions, especially where agriculture depends on irrigation with poor quality water with high salt and B content. Because of their complex

interactions, the combined effects of salinity and B toxicity on crops may differ from those of either stress factor alone. Although there is substantial research on this interaction, the results have been inconsistent, and the underlying physiological mechanisms have not been fully elucidated. Arbuscular mycorrhizal (AM) fungi colonize roots of host plants, can promote plant growth and increase resistance to abiotic stressors, including salinity and drought. Mycorrhizal colonization is often highly correlated with soil phosphorus (P) content. High P levels in the soil may inhibit mycorrhizal colonization although extremely low P availability may also limit the potential benefits of AM symbiosis.

In a controlled greenhouse experiment, bread wheat (*Triticum aestivum* cv. Nusrat) plants were grown at different levels of salinity, B toxicity, and P supply with or without AM inoculation. Under autoclaved and AM-inoculated conditions, the main effects of salinity and B supply as well as their interaction on vegetative development, the distribution of specific nutrients among sink and source tissues, oxidative damage, and yield parameters were investigated. Additionally, mycorrhizal colonization was assessed by staining and microscopy. Both salinity and B toxicity significantly reduced shoot DW 27 days after sowing (DAS) whereas the effect of their interaction on vegetative growth parameters was insignificant. Interestingly, AM inoculation and high soil P increased the shoot biomass by 35% and 32%, respectively. Shoot B accumulation tended to decrease in response to salinity and AM inoculation, indicating an antagonism between salinity and B toxicity and suggesting a protective role of AM against excess B. Moreover, Na concentration was reduced by AM symbiosis. Salinity significantly reduced membrane damage under B-toxic conditions. While low P supply limited grain yield, AM inoculation significantly enhanced different grain yield parameters. Moreover, AM symbiosis enhanced grain P concentration by approximately %17.

The findings of the present study suggest that symbiosis with AM can alleviate the combined effects of salinity and B toxicity on bread wheat and contribute to sustainable wheat production in stress-prone areas. Higher P availability does not necessarily suppress these benefits of AM. The interactions between salinity, B toxicity, P supply and AM colonization deserve a closer look under field conditions.

Keywords: arbuscular mycorrhiza, B toxicity, bread wheat {Triticum aestivum} mineral homeostasis, salinity

SS-38

Biofortification of Lettuce with Selenium and Zinc and Interactions with Sulfur Nutrition

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Selenium (Se) and zinc (Zn) are two minerals that are essential for humans and whose deficiencies endanger human health. Agronomic biofortification is a way of increasing the micronutrients needed by humans. Se and Zn can be used in combination for agronomic biofortification purposes. Sulfur (S) chemically resembles Se and has been studied separately in various species in terms of its physiological interactions with Se and Zn. This study aimed to biofortify lettuce with Se and Zn simultaneously and investigate the interactions between Se, S, and Zn by supplying different levels of Se, S, and Zn. Increasing Se levels resulted in higher Se concentrations in lettuce without causing visible phytotoxicity even in the high-Se treatment groups which contained excessively high Se concentrations for human nutrition. The low-dose Se application resulted in nutritionally relevant and safe target concentrations of Se. The high S applications significantly reduced tissue Se concentration under all conditions. Zn application at high level significantly reduced tissue Se concentration only at low S and high Se conditions. High levels of S, Se, and Zn applications significantly increased tissue S concentrations and their

combination resulted in the highest S concentration. While high S increased tissue Zn concentrations only under high Zn conditions, high Se significantly decreased tissue Zn concentrations again only under high Zn conditions. With respect to nutritional quality, higher applications of all three elements increased total antioxidative capacity while Zn and Se treatments increased vitamin C concentration.
Results indicated that simultaneous biofortification of soil-grown lettuce with Zn and Se is practically possible but interactions of these elements with each other as well as with S must be considered for optimizing the treatments and achieving safe target concentrations which can be make a difference in public health. These findings will pave the way for applications in soilless and vertical systems.
Acknowledgment: This study was supported by Plant Factory (https://plantfactory.company).

Keywords: biofortification, lettuce, nutritional quality, selenium, sulfur, zinc

SS-39
The Potential Effect of Seaweed Extract *Cystoseira barbata* on Improving Early Growth of Wheat

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The seed germination and seedling growth stages are essential for healthy plant growth. However, during these stages, plants are very sensitive to various environmental factors. For this reason, the use of biostimulants obtained from renewable natural resources, which are increasingly important in agricultural applications, is a practical, simple, and sustainable approach in pre-sowing seed applications. Thanks to these applications, plants can perform better both during and after germination periods. It is well recognized that seaweed formulations containing rich bioactive compounds are used as biostimulants in crop production and that seaweed-based products have growth promoting properties. In this study, *Cystoseira barbata* (*C. barbata*), a brown seaweed species common along the Mediterranean coasts, was subjected to various extraction techniques (hot water, alkali, acidic), and the biostimulant activity of these extracts was tested using two different application method (seed or substrate applications) by using wheat (*Triticum durum* cv. Saricanak-98) seeds. These studies were carried out in a growth chamber using perlite media. In this study, the effects of seaweed extracts on growth parameters, root system morphology, and mineral concentrations of wheat seedlings were evaluated. The findings show that seaweed extracts can act as a biostimulant to increase the performance of wheat seedlings and have a positive effect on several growth parameters. According to the results, the addition of seaweed extracts to the growing medium had a greater effect on improving seedling performance compared to direct seed treatment method. The findings obtained from the study support that seaweed extracts obtained from *C. barbata* can be used as a biostimulant in seed applications in agriculture and thus reduce economic losses in wheat production and support sustainable agricultural practices.
This study was supported by TUBITAK (Project number: 121Z215).

Keywords: Biostimulant, (Cystoseira barbata), Seaweed Extract, Seed Treatment, Sustainable Agriculture, Wheat.

SS-40
The Biostimulant Effects of Brown Seaweed (*Cystoseira barbata*) Extracts on Tomato

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It is important to implement the concept of sustainable farming systems instead of conventional agricultural techniques because of the food crisis as a result of population growth around the world. Nowadays, people give importance to consuming functional and nutritional foods, so manufacturers have to consider the quality of crops as well as higher yields. Biostimulants have the capability to stimulate nutrient uptake independent of their nutrient content, whose essential goal is to ameliorate the characteristics of the plant like nutrient use efficiency, abiotic stress tolerance, quality characteristics, and the intake of limited nutrients from the soil or rhizosphere. Even at low concentrations, the use of seaweed extracts as "plant biostimulants" can cause a variety of beneficial physiological plant responses with the help of their rich ingredients like polysaccharides, minerals, and phytohormones. In Turkey, the brown seaweed *Cystoseira barbata* can be a promising biostimulant candidate for the cultivation of high-value crops like tomatoes. One of the most important things in tomato production is the improvement of yield and organoleptic quality. In this research, three different seaweed extracts were applied to tomato plants grown in soil under greenhouse conditions with the aim of analyzing their agronomic, nutritional, and physiological effects. Biostimulant application leads to the improvement of both growth parameters and secondary metabolites in tomatoes. Different biostimulant treatments increased both the level of total phenolic content and the total antioxidant content by 21% and 40%, respectively. The application of different extracts affects the nutrient concentrations like potassium and anti-oxidative agents like carotenoids and vitamin C in the fruits. That's why it can be contributed that biostimulant-treated fruits have improved in terms of food quality compared with the control. It has also increased the marketable yield of tomatoes with biostimulant applications. Our findings from the greenhouse experiment suggested that applying three distinct seaweed extracts would be advantageous for the enhancement of vegetative and reproductive parameters in tomatoes. Especially, the application of hot water extract and alkali extracts in greenhouse conditions encouraged the synthesis of phenolics, antioxidant activity, as well as vitamin C in tomato fruits while both of them resulted in significant increments in potassium concentration in fruit which are parallel with the literature. In order to create ecologically safe biostimulant goods for the market, additional partnerships are needed. In this way, the national bio-economic sources of Turkey can be turned into innovative products to be used in agriculture. This study is supported by TUBITAK collaborated with GUBRETAS (Project number: 119C030).

Keywords: Biostimulant, {Cystoseira barbata}, Fruit Quality, Secondary Metabolites, Seaweed Extract, Tomato

SS-41
The Investigation of Watercress as Affected by Selenium Biofortification

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Watercress, Nasturtium officinale R. Br., belongs to the Brassicaceae family. Watercress is recognized as a nutritionally safe and edible plant. It is rich in minerals and vitamins including folic acid, carotenoids, glucosinolates, vitamins B, C, and E, and pro-vitamin A, in addition to essential minerals such as calcium, iron, iodine, and sulfur. Moreover, it also contains remarkable qualities of elevated dietary fiber, and it is a low-caloric plant. Selenium (Se) is an essential mineral that our bodies need. It is part of special proteins called selenoproteins which help with things like controlling thyroid hormones, boosting our immune system, and protecting against

damage from harmful molecules. Many people globally suffer from micronutrient deficiencies, also known as a "hidden hunger". Among the micronutrients, Se is the one most connected to global micronutrient problems. Biofortification of Se is a promising functional agricultural strategy to increase the uptake and accumulation of Se in nutrition. In this study, watercress plants were grown hydroponically for five weeks using a soilless farming device (Arzum x Vahaa Smart Garden). The nutrient solution was changed every two weeks using Vahaa Plant Nutrition, which contains essential micro and macronutrients for the plant. Sodium selenate (Na2SeO4) was added to the nutrient solution at concentration of 0, 0.25, 0.50 mg/L in the second week of the growing period. After the growing period, watercress plants were harvested. Se applications did not cause any negative effect on watercress fresh weight, total phenolic content, chlorophyll and carotenoid concentration, antioxidant capacity. Moreover, the application of 0.25 mg/L Se and 0.50 mg/L Se resulted in Se accumulation of 135 µg/kg and 306 µg/kg in watercress, respectively. Thus, one portion (34 g) of biofortified watercress could meet 3-6 % of the Recommended Dietary Allowance (RDA) for Se, which is 55 µg/day for adults [1]. By applying of Se as Na2SeO4 to plants in soilless agriculture, it can contribute to public health in the production of Se-biofortified watercress. It is predicted that functional green plants can be successfully used in this way for Se biofortification in soilless agriculture.

Acknowledgment: We extend our gratitude to Vahaa Vertical Farming Solutions for providing the smart garden used in the Se biofortification studies within the context of this project.

Keywords: biofortification, hydroponic smart garden, nutritional quality, selenium, soilless

SS-42
Selenium Biofortification of Commercial Greenhouse Tomatoes Grown in a Soilless System

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Selenium (Se) is not an essential micronutrient for plants, but it is essential for vertebrates, including humans. Among human populations, deficiency in Se represents a prevalent health concern and is a significant part of the global 'hidden hunger' problem. Se biofortification by agronomic biofortification emerges as a promising approach to obtain Se-enriched crops and reduce related health issues in human populations. As tomato is the most widely consumed vegetable globally, it presents a good candidate for Se biofortification. Despite the considerable variation in the Se concentrations of soils, there is typically no Se at all in nutrient formulations used in soilless farming systems, which focus mainly on yield and sensory quality but neglect the nutritional quality parameters such as concentrations of minerals critical for human health. In this study, Se biofortification applications were conducted on five tomato cultivars within a commercial soilless tomato greenhouse located in Çan/Çanakkale. In summer 2022, half of the plants were subjected to foliar application with a solution containing 30 mg/L of sodium selenate every 21 days, while the remaining half served as an untreated control group. The application of foliar Se did not significantly affect plant yield and growth parameters, including plant height, leaf dimensions, stem diameter, fruit set, and fruit size. In addition, other minerals relevant for plant performance or human nutrition were unaffected by foliar Se treatments. Throughout the growing season, Se concentrations in harvested tomatoes remained negligible in the control group whereas they demonstrated an increasing trend in the treatment group. Depending on the cultivar, Se levels reached 15–30 µg per kg of fresh weight in the biofortified tomatoes. Consequently, a single portion (200 g) of these Se-biofortified tomatoes could contribute 6–10% of the recommended dietary allowance (RDA) for Se, which stands at 55 µg/day for adults. The results highlight the

potential for successful usage of optimized foliar Se applications in commercial soilless greenhouses to cultivate high-quality biofortified tomatoes, offering a means to enhance public health through the consumption of nutritionally enhanced tomatoes.

Acknowledgement: This study was supported by Mavruz Tarım and KSV (Kaleseramik Dr. (h.c.) İbrahim Bodur Eğitim, Sağlık ve Sosyal Yardım Vakfı).

Keywords: biofortification, soilless agriculture, tomato, greenhouse, selenium

SS-43

İn Silico Araştırmaların Biyoteknolojik İlaç Geliştirmedeki Rolü: Ferula Comminus Esansiyel Yağının Aflatoksin B1'e Karşı Potansiyel İlaç Etkileri

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Bu çalışma, biyoteknolojik ilaç geliştirmede in silico araştırmalarının önemini tartışıyor. Spesifik olarak, Ferula Comminus bitkisinde bulunan Bulnesol, α-Pinen ve β-Pinen'in potansiyel olarak ilaç etkilerini araştırmadaki rolünü vurgulamaktadır. Bilgisayar destekli yöntemler kullanılarak, in silico çalışmaları moleküler etkileşimleri, bağlama affinitelerini ve farmakolojik özellikleri tahmin edebilir. Bu yöntemler, aday maddeleri hızlı ve etkili bir şekilde tarayarak erken aşama ilaç geliştirme süreçlerini hızlandırır. Ferula Comminus bitkisi, doğal olarak bulunan Bulnesol, α-Pinen ve β-Pinen gibi biyoaktif bileşiklere sahiptir. Bu çalışmanın amacı, in silico teknikleri kullanarak bu bileşiklerin Aspergillus flavus küf mantarının salgıladığı Aflatoksin B1' in sebep olduğu kanser ile alakalı KEAP1, AKR7A1, AKR7A3, p53 tümör baskılayıcı gen ve Nrf2 sinyal yolağı üzerine olası etkilerini değerlendirmektir. Sonuç olarak, bu çalışma, doğal kaynaklardan gelen bileşiklerin potansiyelini açığa çıkarmak ve biyoteknolojik ilaç üretimini hızlandırmak için in silico araştırmaların önemini vurgular. Ferula Comminus'un zengin kimyasal içeriğinin hesaplamalı biyolojinin lensinden keşfedilmesi yeni terapötik olasılıkları ortaya çıkarabilir.

Keywords: In Silico, Ferula Comminus, Aspergillus

POSTER BİLDİRİLER

PP-01
Physicochemical Characterisation of IgG1 Monoclonal Antibodies

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Monoclonal antibodies are immunoglobulins (or fragments of immunoglobulins) with a precise target, produced from a single cellular clone. They are proteins composed of 4 chains (2 light chains and 2 heavy chains) linked together with disulfide bridges. Antibodies are divided into 5 isotypes (IgG, IgA, IgM, IgE, and IgD), different in structure and function. The IgG isotype can be further divided into subtypes, differing in the number of disulfide bonds, especially in hinge region. IgG1 is the most commonly used subtype for drug manufacturing (Le Basle et al. 2019). Due to the increasing number of approved therapeutic proteins in the pharmaceutical area and the number of biosimilars, the need for analytical techniques for their detailed characterization has increased. In general, the identity, heterogeneity, impurity content,and activity of each new batch of therapeutic proteins has to be thoroughly investigated before release (Fekete et al. 2016).
In this study, we aimed to physicochemical characterization of two commercial IgG1 monoclonal antibodies with different lots. The characterisation of antibodies was performed by Enzyme ELISA, HIC-HPLC, IEX-HPLC and SDS-PAGE. So we compared characteristics of two different IgG1 and particular lots of each one. As a result, the two antibodies we examined had different concentrations. However, small concentration differences were detected between individual lots of each antibody. It may means that one lot is more concentrated than the other or the antibody in one lot became inactivated (denatured, degraded) more than in the other batch. Particular lots of antibodies showed similer profiles among themselves in HIC-HPLC, IEX-HPLC and SDS-PAGE.
The combination of different analytical methods is used to monitor the product quality in different stages of the bioprocessing as well as in the formulated products. Database from manufacturing and from stability testing should be carefully maintained to ensure the consistency in the manufacture process and the product stability profiles of the therapeutic mAbs (Wang et al. 2018).

Keywords: Monoclonal antibody, IgG1, Characterisation, Elisa, IEX, HIC

PP-02
Bio-Cosmeceuticals: Innovative, Sustainable, Effective Technology in Cosmetics

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The beauty and personal care industry is a dynamic industry that is rapidly growing globally with the development of innovative ingredients, cutting-edge technology and new formulation techniques. Biotechnology, which is used to produce compounds through sources such as microorganisms or plant stem cell technology, also serves the beauty industry as a sustainable alternative to natural ingredients. On our planet with limited resources, biotech-based cosmeceuticals offer sustainable solutions in the field of beauty. Biotechnology in skincare create high-quality, high-performance ingredients that target specific skin needs. It also diverts the biotechnology supply chain

from depleting natural resources and reduces energy use. What makes biotechnology so exciting in the beauty industry is not only sustainability, but also sophistication and willingness to leverage biomimicry performance. However, the increased safety, purity and efficacy of products and new product categories also make this technology interesting. This technological transformation also means moving from energy-intensive industrial production to microbial factories and fermentation.

The use of biotechnology in the beauty industry has some challenges. First of all, biotechnology requires high financial investment and therefore may not be an accessible production technology for boutique producers. Second, consumers may be concerned about using biotech products. Unlike conventional chemical-based beauty products, biotechnology-based beauty products are quite new. Experiencing a new product that is not widely used may discourage them from adopting that technology. This is causing many consumers to choose simpler and more familiar ways to care for their skin. Another challenge is that biotechnology has yet to prove its effectiveness in the beauty industry to the extent of its potential. Research is needed to show how each product works in humans and whether it is 100% safe. This makes it difficult to trust and use technology. Finally, regulatory issues can be seen as a significant disadvantage. The beauty industry does not have uniform rules for regulating its products and services.

Biotechnology is a powerful trend that can truly transform those who experience beauty. Market forecasts predict that biotechnology-based ingredients will increase even more in the cosmetics industry of the future. Considering the developments in formulation technology, it is obvious that this is not far from utopian. Incredible potential has been demonstrated to achieve safer and more sustainable products – but there is still a lot of work to be done. It can help increase product compliance, but it has challenges to overcome. What we know about the importance of biotechnology in the beauty industry seems to be just the tip of the iceberg. This study discusses the different classes of biotechnology-based cosmetics and the biomaterials used for their formulation. This study also highlights and discusses the key drivers of the biocosmetics industry. Finally, the most significant achievement of this study encourages a refocus on biotechnological advances in cosmetics while adhering to sustainable techniques and ecological principles.

Keywords: cosmeceuticals, biotechnology, sustainability, innovation, formulation

PP-03
CHO Cell Line Development and Investigation of DHFR Gene Amplification on Cellular Diversity in a Single Cell Clone

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Introduction: Cell line development (CLD) constitutes a pivotal step in biotherapeutic production having some bottlenecks including lengthy, labor-intensive workflows for isolating desirable clones, limited reproducibility, and potential protein quality issues [1]. In the study, a CLD workflow was developed for a recombinant CHO cell (rCHO) expressing mAb. The Dihydrofolate reductase (DHFR) gene of a single rCHO cell clone was amplified by Methotrexate (MTX), and the mAb production and specific productivity (Qp) of subclones of amplified-rCHO cells were examined to understand clonal diversity.

Materials and methods: Light and heavy chain expressing plasmids (Invitrogen) and dhfr- CHO DG44 cells (in

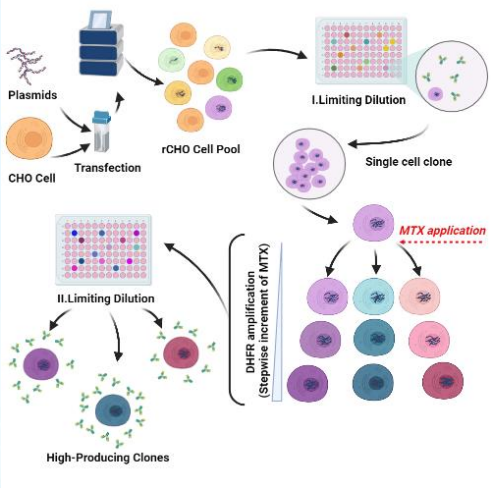
αMEM) were utilized for mAb synthesis. Transfection of plasmids was performed by Lonza 4D-Nucleofector (in SF buffer/DG-208 program). Single-cell clones were obtained by Limiting Dilution (LD) after DHFR selection of the stable rCHO cell pool. The highest productive single-cell clone (C28) was selected, and DHFR amplification of C28 was achieved by adding MTX up to 500 nM and 1000 nM as a gradient. LD was applied on the amplified-C28 cell to obtain its subclones. Qp (pg protein/cell/day) and mAb titer of each subclone were measured after 4-days of cultivation. The three highest C28 subclones were selected, then shake flask-batch and fed-batch cultures were performed in a Chemically Defined (CD) medium. Western Blot was performed to compare expressed mAb with the reference product.

Results: After the transfection and DHFR selection, a stable rCHO cell pool (mAb Titer: 0.3±0.04 mg/L and Qp: 0.3±0.03) was acquired. 34 single-cell clones were obtained after the LD of the cell pool. While the mAb production of the clones was determined between 0.1-3.5 mg/L for 4-days cultivation, the highest mAb expression was observed at the C28 clone (mAb Titer: 3.6±0.1 mg/L, Qp: 2.6±0.1). After DHFR amplification, C28 (500MTX) reached (mAb Titer: 8.3±1.2 mg/L (Qp:4.6±0.1) while C28 (1000MTX) achieved mAb Titer: 5.8±1.0 mg/L (Qp: 3.9±0.4). Subclones of amplified-C28 were obtained via second LD. 22 subclones of C28 (500 MTX) had mAb Titer: 2.3-13.8 mg/L and Qp: 2.3-13.1, and also 10 subclones of C28 (1000 MTX) had mAb Titer: 0.8-14.2 mg/L and Qp: 0.6-11.1 after 4-days of cultivation. Three higher subclones including C28-500-9 (Qp: 11), C28-500-16 (Qp: 7.5) and C28-1000-9 (Qp: 9.5) were selected for shake flask batch and fed-batch culture in the CD medium. mAb Titer of the subclones were between 143.7-234.1 mg/L (Qp: 7.8-12.0) for batch culture (11-13 days) while they were between 546.2-827.4 mg/L (Qp: 8.6-19.5) for fed-batch culture (15-17 days) in non-optimized condition. Besides, expressed mAbs were compatible with the reference product according to Western Blot results.

Conclusions: The study demonstrated the clonal diversity-enhancing effect of DHFR amplification on a single rCHO clone. The results of subclones (in shake flask batch and fed-batch culture) were similar to the existing literature results, and they have a potential for 1-3 g/L mAb production in a bioreactor [1,2,3].

Keywords: Biopharmaceuticals, Cell Line Development, mAbs, DHFR Amplification, CHO Cells,

CHO cell line development and effect of DHFR gene amplification on cellular diversity in a single cell clone



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PP-04

Comparison of Antibody Expression Levels of Bicistronic Plasmids Having Different IRES Locations and Signal Peptides

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Introduction: Antibody (Ab)-based drug production has gained increasing significance in the biopharmaceutical field. Achieving efficient Ab production requires precise control of the heavy chain (HC) and light chain (LC) ratios in the Ab. Moreover, the selection of suitable HC and LC signal peptides of Ab is a critical parameter for robust and effective production due to their translocation into the endoplasmic reticulum lumen [1, 2]. This study investigated the effects of cistron positions of HC and LC in the internal ribosome entry site (IRES)-mediated plasmids on the Ab expression. Furthermore, we compared Ab (DEM-Ab) production levels of three different signal sequences for HC and LC of the DEM-Ab.

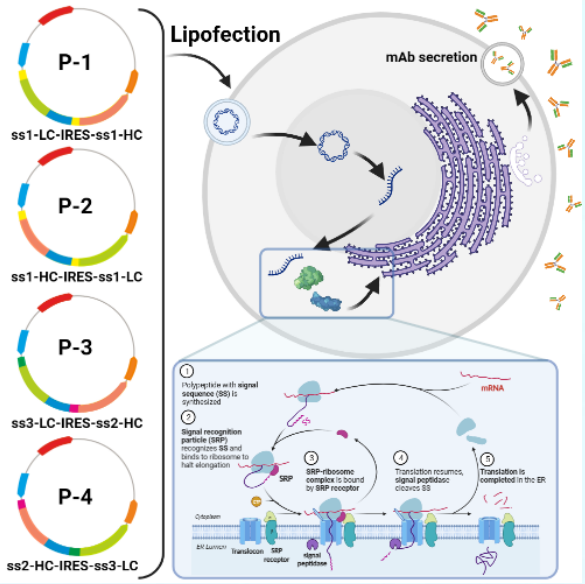
Materials and Methods: Four different IRES-mediated plasmids (ATUM) were designed to compare the DEM-Ab productions. HC and LC genes were arranged as either the first or the second cistron in the plasmids. To examine the effects of different signal sequences (ss) on the DEM-Ab expression, ss1 (for HC and LC), ss2 (for HC), and ss3 (for LC) were placed before LC and HC gene sequences. The plasmids were constructed as ss1-LC-IRES-ss1-HC (P-1), ss1-HC-IRES-ss1-LC (P-2), ss3-LC-IRES-ss2-HC (P-3) and ss2-HC-IRES-ss3 (P-4). The presence and location of signal peptide cleavage sites in the amino acid sequences of HC and LC were predicted by SignalP 6.0 Server (Artificial neural networks). Transfections of plasmids expressing DEM-Ab and a plasmid expressing GFP (Control) into CHO-DG44 cells were performed with Lipofectamine 3000 (Invitrogen). On the third day post-transfection, supernatants of the transfected cells were collected to determine the Ab titer by BiaCore T200. Three replicates of each group were performed and data were analyzed by one-way ANOVA (Post Hoc Tukey HSD).

Results: In the study, the groups expressing the highest DEM-Ab were P-1 (182.3±26.6 ng/uL) and P-3 (163.3±36.5 ng/uL), which had LC-IRES-HC. The lowest Ab titers were determined in P-2 (98.0±26.8 ng/uL) and P-4 (52.7±40.1 ng/uL) with HC-IRES-LC arrangement. The difference in the Ab production yield between P-3 (ss3-LC-IRES-ss2-HC) and P-4 (ss2HC-IRES-ss3LC) was statistically significant ($p=0.015$).
Cleavage sites of signal peptides may vary depending on Ab types, and selecting correct peptide sequences are essential for biosimilar production [3]. SignalP 6.0 server predicted cleavage sites of signal sequences in HC and LC of DEM-Ab occurred at the appropriate locations, and the selected signal sequences were compatible with DEM-Ab.
In signal sequence comparisons, P-1 (ss1-LC-IRES-ss1-HC) exhibited higher production than P-3 (ss3-LC-IRES-ss2-HC). Moreover, P-2 (ss1-HC-IRES-ss1-LC) provided higher Ab titer than P-4 (ss2-HC-IRES-ss3-LC).

Conclusions: In the study, the four different plasmids were compared according to their cistron arrangement and signal sequences of HC and LC. LC-IRES-HC resulted in higher DEM-Ab expression than HC-IRES-LC which aligns with existing literature [1]. Moreover, we investigated the effects of different signal sequences on DEM-Ab expression, and plasmids containing ss1 showed higher Ab expression compared to plasmids with ss2 and ss3.

Keywords: Biopharmaceuticals, mAb, Signal Sequence, IRES, Chinese Hamster Ovary Cell

Comparison of antibody expression levels of bicistronic plasmids having different IRES locations and signal peptides



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PP-05
Creation of an Innovative Liposomal Formulation to Target Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) poses significant therapeutic challenges due to limited treatment options. In this study, we propose a novel approach for TNBC treatment by developing a liposomal formulation consisting of sunflower lecithin, cholesterol, and quercetin molecules.

The liposomes were thoroughly characterized using advanced techniques. Scanning transmission electron microscopy (STEM) imaging confirmed the spherical morphology of the liposomes, while nanoparticle tracking analysis (NTA) revealed a size range of 100-110 nm, indicating their potential for targeted drug delivery. The Zeta sizer analysis demonstrated a surface potential of approximately -22 mV, signifying favorable stability and colloidal dispersion properties.

To assess the efficacy of the liposomal formulation, TNBC cell lines were subjected to a cell viability assay. Encouraging results were observed, with a significant reduction in cell viability upon treatment with liposomes, as compared to free drug treatment. These findings highlight the potential of our liposomal formulation as an effective therapeutic strategy for TNBC.

In conclusion, our study introduces a promising approach for TNBC treatment through the use of liposomes composed of sunflower lecithin, cholesterol, and quercetin. This liposomal formulation exhibits excellent potential for targeted drug delivery and warrants further investigations to elucidate the underlying mechanisms and optimize its formulation for eventual clinical translation.

Keywords: Breast cancer, TNBC, drug delivery, nanotechnology, liposomal formulation

PP-06

Assessing the Management of Hospitalized Cystic Fibrosis Patients: Recommendations for Optimizing Pharmacists' Roles

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INTRODUCTION: Cystic fibrosis (CF) is a complex rare genetic disease primarily affecting the respiratory, gastrointestinal, and endocrine systems. CF directly affects many organ systems, and the clinical manifestation is usually severe. Therefore, the treatment of CF patients requires the use of many drugs. Polypharmacy in CF management increases the risk of adverse drug events and drug-related problems (DRPs), particularly in children. In addition to conventional medicines, Dornase alfa (DA), a synthetic form of human deoxyribonuclease I is one of the cornerstones of CF treatment. Our objective was to investigate the prevalence, types, and causes of DRPs, with a focus on optimizing the roles of clinical pharmacists.

METHODS: This prospective, observational study was conducted on hospitalized pediatric patients with CF. This study has been approved by the local Clinical Research Ethics Committee with decision number of 11/05. A structured medication review was conducted by a clinical pharmacist to identify, classify, and analyze the causes of DRPs. Additionally, we aim to explore potential areas for optimizing the roles of pharmacists in the management of CF.

RESULTS: The study included fifteen children, with 73% (n=11) of them being female, and an average age of 12 years. CF exacerbation (n=13) was the most common reason for hospitalization. A total of 285 drugs were prescribed, with inhaled drugs (23%), antimicrobials (16.5%), and vitamins (14%) being the most frequently prescribed. Of the patients, 53% experienced at least one DRPs. The most common type of DRP was related to dose selection and frequency (n=9). DA use was present in all participants (n=15). Notably, a drug interaction requires therapy modification, between Elexacaftor/tezacaftor/ivacaftor and fluconazole was observed in one patient. Additionally, a discrepancy with the dosage form of vitamin supplement was detected. The patient had been consistently using a vitamin supplementation, but due to its unavailability locally, they independently obtained an alternative dosage form, which they continued to use throughout their admission. As a consequence of this substitution, there arose a significant difference in the content of the vitamin supplement, leading to a variation in the dosage the patient needed to take. However, neither the physician nor the patient was initially aware of this discrepancy.

CONCLUSION: Dose selection and frequency is common problem in pediatric CF patients. Given the prevalence of

polypharmacy in this population, pharmacists must be cautious in handling potential drug interactions, particularly with novel drugs for CF. One of the most common problems experienced by CF patients is access to medicines. The high cost of DA, produced with recombinant DNA technology leads to the need to act carefully in drug supply which may take time for patients to reach DA with high benefit due to the regulations applied. In addition, long-term antimicrobial therapies which may be required against common infections. Pharmacists should be aware about the DRPs that may arise in such situations. Raising awareness and actively monitoring medication regimens for CF patients can significantly contribute to improving treatment outcomes and patient safety.

Keywords: Cyctic fibrosis, Dornase alpha, Drug-related problems, Clinical pharmacist

PP-07
The Effect Of Environmental Conditions On The Study On Experimental Animals

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If the environmental conditions in which the experimental animals are housed cannot be met, there are negative effects on the final results of the pharmaceutical tests performed on the experimental animals. Inadequate ventilation, failure to provide air circulation, equipment used not to meet international standards, not cleaning the cages on time and not using feed suitable for the species in feeding have negative effects on many systems, especially the respiratory and digestive systems. These effects can cause misleading results in the evaluation of the results obtained in preclinical studies.

Keywords: deney hayvanları, ortam koşulları, hastalık

PP-08
Development of Liposomal Formulation for the Treatment of Triple-Negative Breast Cancer

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INTRODUCTION: Breast cancer is a common type of cancer that affects women worldwide. In Turkey alone, it is estimated that between 24,000 to 30,000 cases are diagnosed each year. On a global scale, the number of breast cancer cases reaches 2.3 million annually. Among various subtypes of breast cancer, triple-negative breast cancer (TNBC) is particularly aggressive and challenging to treat. To overcome this issue we have developed cost-effective anti-cancer agent liposomal formulation.

METHOD & RESULTS: The liposomes were thoroughly characterized using advanced techniques. Scanning transmission electron microscopy (STEM) imaging confirmed the spherical morphology of the liposomes, while

nanoparticle tracking analysis (NTA) revealed a size range of 100-110 nm, indicating their potential for targeted drug delivery. The Zeta Size analysis demonstrated a surface potential of approximately -22 mV, signifying favorable stability and colloidal dispersion properties.

To assess the efficacy of the liposomal formulation, TNBC cell lines were subjected to a cell viability assay. Encouraging results were observed, with a significant reduction in cell viability upon treatment with liposomes, as compared to free drug treatment. These findings highlight the potential of our liposomal formulation as an effective therapeutic strategy for TNBC.

CONCLUSION: In conclusion, our study introduces a promising approach for TNBC treatment through the use of liposomes composed of sunflower lecithin, cholesterol, and quercetin. This liposomal formulation exhibits excellent potential for targeted drug delivery and warrants further investigations to elucidate the underlying mechanisms and optimize its formulation for eventual clinical translation.

Keywords: Breast cancer, TNBC, Liposome, cancer treatment

PP-09

Deletion Of Membrane-Integrated Region OF A PD-1 Membrane Protein For Production And Use In Diagnostic Applications

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INTRODUCTION: Validation of the biological activities of these produced mAbs is as important as the production of mAbs and is critical in terms of production costs. Production of Programmed cell death 1 (PD-1) protein is on the agenda of various biotechnological pharmaceutical companies in our country in order to validate the antigen-binding properties of anti-PD-1 antibodies such as nivolumab and pembrolizumab[1]. Programmed cell death 1 (PD-1) protein is a membrane protein that is naturally expressed by cells of the immune system and binds to the cell membrane proteins PD-L1 and PD-L2 ligands. The interaction of PD-1 with these ligands expressed on tumor cells inhibits T cell-mediated lymphocyte activation [2]. For this reason, many anticancer drug development studies focus on blocking the interaction between PD-1 and PD-L1, and anti-PD-1 or anti-PD-L1 monoclonal antibodies are being developed in our country as well. Thus, the study aims to delete the gene region coding for the membrane-integrated part of PD-1 protein in order to produce a soluble extracellular domain of PD-1 with its signal peptide which can help translocation through the cell membrane. Therefore, the extracellular domain of PD-1 can be coated on ELISA plates to be used as an antigen-mAb interaction assay platform.

MATERIALS-METHODS: To this end, the Q5 Site-directed mutagenesis kit was used for the deletion of the region coding for the intracellular and transmemberane domains of the PD-1 protein. The expression plasmid pCMV3-PDCD1-GFPSpark encoding the PD-1 protein was obtained from the company "Sino Biological Inc". The full Q5 Site-Directed Mutagenesis Protocol is followed with designed primers to obtain the targeted region of PD-1. Confirmation of the desired deletion on PD-1 coding region is achieved with designed primer sets. After Q5 site-directed mutagenesis, colonies on the incubated selection plates were used to isolate the mutant plasmid and deletion was confirmed with a PCR protocol. After PCR amplification, PCR products are loaded into the 1 % agarose

gel to check PCR products based on their size. Further confirmation of deletion was achieved by sequencing the mutated region.

RESULTS: Results have indicated that the gene regions corresponding to the intracellular and transmembrane domains on the PD-1 protein have been successfully deleted. Desired deleted plasmids are verified by both PCR amplification and sequencing of the region of interest. According to agarose gel results of PCR products, the amplified deleted mutant PD-1 PCR product band was observed at ~1.4 kb, and the wild type PD-1 PCR product band was observed at ~1.7 kb. Furthermore, selected plasmids are confirmed by sequencing results.

CONCLUSIONS: We have successfully generated deletion plasmids that only code for the extracellular domain of PD-1. In the next step, we aim to use these deletion plasmids to produce the extracellular domain of the PD-1 protein in mammalian cells. The resulting deleted PD-1 (PD-1-ECD) will be used to coat multiwell plates in order to construct an ELISA-based antigen-mAb assay platform.

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Keywords: PD-1 protein, ELISA, mutation, deletion, sequencing

PP-10

Synthesis, Characterization and Micelle Formation Behavior of Amphiphilic Copolymers Containing Uracil Side Groups

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Introduction: In recent years, there has been an increasing interest in new drug delivery systems for the needs arising from difficulties such as dosage, targeting, solubility, stability, and side effects encountered in traditional drugs [1]. Modern drug delivery technology has been made possible by advances in polymer science which resulted in polymers with unique properties [2,3]. Polymeric micelles, structures formed by the self-assembly of amphiphilic copolymers, are used to improve solubility, stability, release and bioavailability of hydrophobic drugs [4]. In some cases, crosslinkers are used to improve the structural stability of polymeric micelles. It has been determined that this crosslinking, which can take place in the shell or core part of the micelle, stabilizes the micelles in dilute solutions and even in organic solvents [5]. Photo-crosslinkers stand out from others because they are non-toxic, economical, and do not produce any by-products. In addition, drug carriers, especially those sensitive to light stimulation, are able to minimize early release in delivering encapsulated drugs to the target area with their controlled release kinetics [6].

Materials and Methods: The aim of this work was to synthesize photosensitive amphiphilic copolymers containing 6-methyluracil side groups, to characterize their structures and to investigate their micelle formation behavior. For this purpose, uracil functional methacrylate monomer (UMA) was synthesized and its structure was characterized by 1H NMR, 13C NMR and FT-IR. mPEG-b-P(MMA-co-UMA) amphiphilic copolymers containing 5% and 15% UMA units were synthesized by RAFT polymerization using mPEG Macro-RAFT agent and their structures were characterized by spectroscopic, chromatographic and thermal analyses. Micelles were formed by dialysis

method with these amphiphilic copolymers and the sizes of the micelles were measured with DLS. The dimerization behavior of uracil side groups was investigated by UV-Vis and DLS measurements upon irradiation at 254 nm for a certain period of time.

Results and Discussion: In conclusion, we have successfully synthesized amphiphilic copolymers containing uracil side groups and demonstrated their self-assembling behaviour in aqueous medium. The light responsive behaviour make them attractive as functional drug carriers.

Keywords: Polymer micelles, RAFT polymerization, amphiphilic copolymers, uracil

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PP-11
Replication Defective Adenoviral Vectors do not induce Apoptosis and Autophagy in endothelial cells

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INTRODUCTION: Adenoviral vectors, especially replication-deficient adenovirus vectors, have been widely used in vaccines as they induce potent humoral and T-cell responses against transgenes expressed by the vector. The mechanism of action of replication-defective adenoviral vector cancer vaccines is based on the expression of tumor-associated antigens (TAAs) into non-tumor tissue or antigen-presenting cells (APCs). Adenovirus infecting its host causes cell death by inducing the apoptosis pathway, which cells undergo in their usual physiological process to prevent various diseases, including cancer. Autophagy, a cytoprotective mechanism, also causes cell death during adenoviral infection. Replication-defective adenoviruses are used in gene therapy studies commonly. This study investigated the effects of recombinant adenoviral vectors developed as a COVID-19 vaccine and cancer vaccine on apoptosis and autophagy in benign and malignant cells.

TOOLS AND METHODS: For these purposes, HUVEC, A549, and MDA-MB-231 cells were infected with viral vectors at low and high MOIs (Multiplicity of Infection) (1 and 1000). Cell viability assay was performed to obtain healthy cell number, and transduction of cells with viral vectors at MOI: 0,1,100 were performed at 24h, 48h, and 72h. RNA isolation, cDNA synthesis, and qRT-PCR experiments were performed to quantify gene expression levels. The Western Blot method also determined the protein expression level at 72h.

RESULTS: While there are some differences in the MOI:1 and MOI:100 values of the CoVacHG and AdCDGMCSG replication-defective adenoviral vectors, in general, the CoVacHG vaccine suppresses the expression of apoptotic genes at the mRNA level, increased autophagy level in benign cells, AdCDGMCSF cancer vaccine was found to increase the expression of both apoptotic and autophagic genes in cancer cells but not in benign cells.

DISCUSSION: The protection mechanism was activated in healthy cells infected with CoVacHG,

but the cells did not die. These results suggest that replication-deficient adenoviral vectors may be safe as vaccine vectors in healthy individuals. In benign cells infected with AdCDGMCSF, designed as a cancer vaccine, the protection and death mechanisms were activated, and the cells died. The effects of adenoviral vectors on apoptosis and autophagy differed significantly among cancer cells. It was found that the cells in lung cancer cells were more sensitive to the vaccine, but breast cancer cells were more resistant. Therefore, the apoptosis-inducing effect of adenoviral vector vaccines arising from the vector, regardless of the therapeutic gene, should be optimized at different doses.

Keywords: apoptosis, autophagy, cancer, COVID- 19, replication defective adenoviral vector, vaccine

PP-12
Replication Defective Adenoviral Vectors Decreases The Immunosuppresive Cytokine Expression In Tumor Cells

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Introduction: Gene therapy has been tried in many diseases including cancer, for the last 30 years, and several products have been routinely used in recent years. In gene therapy, mainly viral vectors are often used. During the Covid-19 pandemic process, adenoviral vector-based vaccines have been routinely introduced. In particular, the vaccines use the replication- defective adenoviral vectors. Along with the effects of therapeutic genes within the vectors, the adenoviral vectors can also affect the cellular functions. In particular, the effects of recombinant vectors on immune responses and inflammation are not well known. This study aimed to investigate the effect of the replication-defective adenoviral vector administration on the secretion of immunosuppressive and inflammatory cytokines in tumor cells.

Tools and Method: We transduced the tumor cell lines, HeLa (human cervical cancer cell line) and MCF7 (human breast cancer cell line), and HUVEC (human umbilical cord and endothelial cell) by defective type 5 Adenoviral vectors at low (1 MOI) or high (100 MOI) doses; one has a SarsCov-2 Spike gene, another contains cytosine deaminase and granulocyte macrophage-colony stimulating factor (GM-CSF). Following 24, 48, and 72 hours of infection, the RNA was isolated from all cells. We assayed the messenger-RNA and protein expression of immune suppressive and inflammatory cytokines, including interleukin (IL) 1-Beta, IL-6, IL-8, IL-10, Tumor Necrosis Factor-alpha (TNF-alpha) and Transforming Growth Factor-beta (TGF-Beta) by quantitative polymerase chain reaction (qPCR) and western blotting.

Results: We found that proinflammatory genes have similarly demonstrated a change in expression at mRNA and protein levels with both vectors independent of the transgenes that they carry. Both vectors at low or high doses increased the expression of proinflammatory cytokines but suppressed the expression of immunosuppressive IL-10 and TGF-Beta.

Discussion: Our results suggest that adenoviral vectors support the use of those vectors as vaccines and suggest that adenoviral vectors may be a good vehicle in cancer treatment.

Keywords: Ad-CD-GMCSF, cancer gene therapy, CoVacHG, immunosuppression, inflammation, Type 5 adenoviral vectors

PP-13

In Vitro Angiogenic And Osteogenic Effects of Exosomes Derived from Mesenchymal Stem Cells

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OBJECTIVE: Bone fractures and bone diseases are a global public health issue and pose a serious economic burden. Accumulating evidence suggests that mesenchymal stem cells (MSCs) derived exosomes are closely related to bone regeneration and rearrangement of bone microenvironment. This study investigated the role of exosomes derived from Wharton jelly mesenchymal stem cell cultures on osteogenesis and angiogenesis.

MATERIALS-METHODS: Wharton jelly mesenchymal stem cells (MSCs) were isolated from umbilical cord samples obtained during the cesarian section.

Surface antigens of the isolated MSCs were analyzed by flow cytometry (Yeditepe University). Besides flow cytometric analysis, three lineages (adipogenic, chondrogenic, and osteogenic) differentiation potential of the cells was investigated.

The 3rd passage Mesenchymal stem cell cultures were grown in %5 CO2 and %3 O2 condition without serum addition for 48 hours at 37°C. After that, the supernatant of the cell cultures was collected. The extracellular vesicles were isolated by a total exosome isolation reagent (Thermo Fisher). The isolated extracellular vesicles' morphology was visualized by electron microscopic analysis (Yeditepe University). Nanoparticle Tracking Analysis (NTA) (Yeditepe University) determined the exosome concentration and size. Surface antigens of the exosomes were characterized by flow cytometric analysis (Yeditepe University).

The MSCs were cultured in an osteogenic medium with or without 200 µL exosome suspension for 14 days. The osteogenic medium with or without the exosome suspension was changed every two days. The cultures in an osteogenic medium without exosome suspension were considered the control group. On the 14th day of the study, total RNA was isolated from the control and assay groups. Real-Time PCR analysis determined RUNX2, collagen, and alkaline phosphatase expression patterns in control vs. assay groups. Calcification in cultures was shown by Alizarin Red S (Thermo Fisher) staining.

Human umbilical vein endothelial cells (HUVEC) (Thermo Fisher) were seeded on Geltrex Matrix (Thermo Fisher) coated 24 wells plates at 25x103 cells/cm2 density with Medium 200 (Thermo Fisher) supplemented with Large Vessel Endothelial Supplement (Thermo Fisher) and 200 µL exosome suspension. The plates without being treated with the exosome suspension were regarded as control groups. The plates were incubated overnight at 37°C in a humidified atmosphere of 5% CO2. Tube formations in control and assay groups were observed and photographed under an inverted microscope.

RESULTS: The isolated cells were determined positive for CD73 (%83), CD90 (%81), and CD105 (%93) and negative for CD34 (%2) and CD45 (%0,63) surface antigens. The isolated cells differentiated into adipogenic, chondrogenic, and osteogenic lineages. The average size of the isolated exosomes was 174.3 nm. The concentration of the

exosome 4.20x10[9]±2.05x10[8] particle/mL. The particles were carrying CD9, CD63, and CD81 surface antigens. The cells treated with the exosome suspension and osteogenic induction medium had relatively high Alkaline phosphatase, collagen, and RUNX2 gene expression compared to control groups treated with only osteogenic induction medium. Also, the cells with the exosome suspension and osteogenic induction medium were strongly stained with Alizarin Red S compared to control groups treated with only osteogenic induction medium. The HUVEC cells treated with Large Vessel Endothelial Supplement (Thermo Fisher) and the exosome suspension had an increased tube formation.

CONCLUSIONS: This preliminary in vitro study shows the potency of mesenchymal stem cells derived exosomes to treat bone fractures and bone disease.

Keywords: Angiogenesis, Exosomes, Mesenchymal Stem Cells, Osteogenesis

PP-14

Advances in Biomedical Engineering: Polymeric miRNA Delivery Vehicles Revolutionizing Breast Cancer Therapy

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Breast cancer, which accounts for 15% of all cancer-related deaths in women and 30% of all female cancer cases, is a significant global health issue. Breast malignancies are typically treated with surgery, radiation, chemotherapy, hormone therapy, and anti-Her2-targeted monoclonal antibody treatments. Although breast tumors can be treated with normal methods, some breast cancer cells can develop resistance to those methods. The development of this resistance can be brought on by genetic alterations or modifications to the tumor microenvironment, which renders the cancer cells less vulnerable to the impacts of therapy. Because of this, conventional treatment is insufficient to completely cure breast cancer. Gene therapy has a big chance to overcome these difficulties.

Due to their capacity to control gene expression, microRNAs (miRNAs) have become recognized as possible therapeutic agents for the treatment of cancer. Particularly, miRNA has been linked to the spread and metastasis of breast cancer. Effective miRNA delivery to breast cancer cells is still difficult, though. The molecular structure of miRNAs is unstable in the physiological environment, and their small size, negative charge, and poor cellular absorption are obstacles to their usage.

It is well known that miRNAs require carrier systems in order to fully carry out their functions, pass through the cell membrane, and reach the proper intracellular region. In addition, carrier systems enable miRNAs to provide greater molecular stability and efficient protection against nuclease-mediated cleavage. For the delivery of miRNAs with precision, nanocarriers present a promising option. In this study, we analyze the role of miRNA as an anticancer agent and explore the increased delivery of miRNA to breast cancer cells utilizing nanocarriers. To encapsulate and transport miRNA to breast cancer cells, we developed β-CD nanocarriers. This nanocarrier is made to shield miRNA from destruction and promote its effective uptake by cells. According to DSL measurements, the size of our developed delivery system is about 200 nm. Additionally, although our nanocarriers have a positive charge, it starts to lose strength when it interacts with miRNA. To enable efficient delivery and

controlled release of miRNA, the physicochemical characteristics of the β-CD nanocarriers, including size, charge on the surface, and stability, are optimized.
At a 2/1 N/P ratio, our inhibitor miRNA and β-CD nanocarriers can be complexed. Additionally, our mimic miRNA can form an 8/1 complex with β-CD nanocarriers. Finally, with an 8/1 N/P ratio, negative miRNA and β-CD nanocarriers can get complicated. The in vitro testing of this well-characterized polymer system is presently underway.
This study has been supported by the Erciyes University Scientific Research Projects (BAP) under Grant No: TSAÜ-2022-11895.

Keywords: miRNA, breast cancer, nanomedicine, nanocarriers. gene delivery, gene therapy

PP-16
Advances in Biomedical Engineering: Polymeric miRNA Delivery Vehicles Revolutionizing Breast Cancer Therapy

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Introduction: Breast cancer, which accounts for 15% of all cancer-related deaths in women and 30% of all female cancer cases, is a significant global health issue. Breast malignancies are typically treated with surgery, radiation, chemotherapy, hormone therapy, and anti-Her2-targeted monoclonal antibody treatments. However, these treatments can cause resistance in a while because of genetic alterations or modifications to the tumor microenvironment, which renders the cancer cells less vulnerable to the impacts of therapy. Thus, gene therapy has a big chance to overcome these difficulties. Due to their capacity to control gene expression, microRNAs (miRNAs) have become recognized as possible therapeutic agents for the treatment of cancer. Effective miRNA delivery to breast cancer cells is still challenging, though. The molecular structure of miRNAs is unstable in the physiological environment, and their small size, negative charge, and poor cellular absorption are obstacles to their usage. It is well known that miRNAs require carrier systems in order to fully carry out their functions, pass through the cell membrane, and reach the proper intracellular region. In addition, carrier systems enable miRNAs to provide greater molecular stability and efficient protection against nuclease-mediated cleavage

Materials and methods: For the delivery of miRNAs with precision, nanocarriers present a promising option. In this study, we analyzed the role of miRNA as an anticancer agent and explore the increased delivery of miRNA to breast cancer cells utilizing smart nanocarriers.

Results and Discussion: To complex and transport miRNA to breast cancer cells, we have developed "smart" polymeric nanocarriers. At a 2/1 N/P ratio, our inhibitor miRNA and nanocarriers can be complexed. Additionally, our mimic miRNA can form an 8/1 complex with smart nanocarriers. Finally, with an 8/1 N/P ratio, negative miRNA and smart nanocarriers can be complexed. In addition, According to DSL measurements, the size of our developed delivery system is about 200 nm.

Conclusion: The smart nanocarriers are made to shield miRNA from destruction and promote its effective uptake by cells. To enable efficient delivery and controlled release of miRNA, the physicochemical characteristics of the smart nanocarriers, including size, charge on the surface, and stability, are optimized.

Keywords: Breast cancer, miRNA, gene delivery, gene therapy, nanomedicine, nanocarriers

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PP-17

Production of Bioactive Food by Spirulina platensis via Fermentation with Kluyveromyces marxianus

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The need for alternative food sources is increasing daily, becoming a critical issue due to the world's growing population. Additionally, due to the ongoing climate crisis, obtaining access to healthy food is expected to become progressively challenging, and the nutritional content of certain crops is anticipated to decline. As a solution to these concerns, the recognition of "functional foods" has significantly increased. Microalgae, specifically Chlorella and Spirulina species, are an exceptional and nutritious option for human and animal consumption. They possess numerous bioactive properties, including antihypertensive, antioxidant, anti-inflammatory, anticancer, and anti-diabetic effects. However, consumers do not find it desirable due to its strong taste and odor characteristics. Fermentation is a relatively low-cost, easy-to-implement method that preserves and enhances the existing flavor characteristics of food products while enhancing bioactive properties. Studies have been conducted on the fermentation of Spirulina using different bacteria. However, there is limited research on fermentation of Spirulina using yeasts, which have numerous benefits for human health and act as probiotics when consumed. This study aimed to determine the changes in bioactive properties resulting from Spirulina fermentation with a yeast species. Moreover, the impact of Spirulina cell disruption induced by the homogenizer, as well as the influence of an additional carbon source in the medium, on cell growth and the bioactivity of the end product were examined. After examining the impact of lysis on cell growth, it was found that the cells in the lysed Spirulina medium had a growth increase of 5.3 log/cfu after 72 hours of fermentation. This growth increase was slightly better than the fermentation with unlysed Spirulina cells which only had a growth increase of about 5 log/cfu. When examined in terms of the amount of total soluble protein (TSP), the lysed group resulted in higher protein content (3.12 mg/mL) than the unlysed group (2.51 mg/mL). The lysed group also showed a higher total phenolic content (TPC) compared to the unlysed group with 0.17 and 0.11 mg/mL, respectively. The remainder of the study was continued with the lysed group, as the higher amount of phenolic compounds indicated higher bioactivity. In the other step of the study, glucose was added as an extra carbon source to the lysed Spirulina medium, and cell growth and various bioactive properties were monitored. The cell growth in the group with extra glucose was

higher (3 log/cfu after 144 hours of fermentation) than in the control group without extra glucose (1.2 log/cfu). Although there was a slight decrease in both groups in terms of TSP, it was observed that the glucose-added group has a higher TPC (0.15 mg/mL) than the control group (0.12 mg/mL) after 156 h fermentation. After considering the provided data, further investigation was conducted on the glucose group's ability to inhibit Angiotensin-converting enzyme (ACE). It was seen that Spirulina already had high ACE inhibitory property (89% ACE inhibition at T0), but this property did not face a large decrease with fermentation (85% ACE inhibition at T144). Finally, sensory analyzes of the final products were carried out in the study. Accordingly, it has been observed that fermentation almost eliminates the undesirable taste properties of Spirulina such as algae, and astringent/bitter taste, and adding an extra carbon source to the fermentation medium creates new features such as caramel / fruity flavors that make it attractive for consumption by the consumer. In conclusion, the product obtained as a result of the fermentation of Spirulina with yeast has been successfully brought into powder form, and important steps have been taken toward developing a sustainable and innovative functional food that has various potential benefits for human health. The study yielded results that will enhance our understanding of how to produce more nutritious and beneficial foods by incorporating bioactive ingredients into various product formulations in the future.

Keywords: Bioactive Foods, Fermented microalgae, Kluyveromyces marxianus, Spirulina platensis

PP-18

Evaluating the Potential Use of Different Types of Biomass for the Production of Protein Concentrates Assisted by Fermentation

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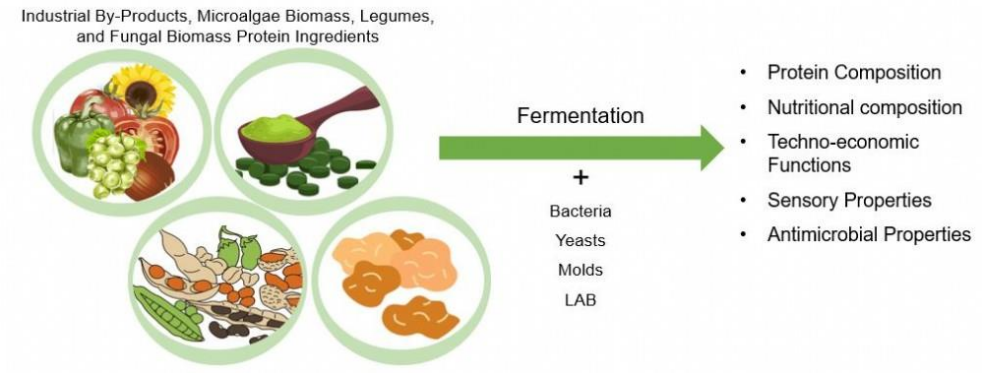
Global consumption of average animal protein has increased from 21 to 33 g per person per day over the past 50 years (FAOSTAT, 2020), and the overall consumption of animal proteins is expected to be on the rise in terms of daily availability per capita in the coming years. However, environmental concerns about large-scale livestock production, over-exploitation of the oceans and aquaculture are expected to limit the market for animal proteins. The objective of this review was to develop inside knowledge and increase the awareness about the plant protein isolates and the methodology of obtaining the protein concentrates through fermentation process.
The protein isolates obtained from a variety of biomass sources, such as legumes, industrial by-products, microalgae meal, and fungal meal, contain different amounts of proteins that are promising candidates to replace animal-based proteins. Their use is currently limited due to the presence of antinutritional substances, as well as their inferior technological capabilities and unpleasant sensory qualities. In order to avoid these problems, fermentation has traditionally been used. Compared to enzymatic or chemical modifications, fermentation using safe microorganisms with proteolytic activity is less expensive. Researchers are now interested in the fermentation of these resources to produce healthier, more flavorful, and more technologically advanced products. The fermentation of the protein isolates from plant species and biomass through the use of microorganisms (bacteria, yeasts, molds, and especially lactic acid bacteria (LAB)) enriches the potential of isolates to be used for industrial purposes. The biological modification of plant protein isolates improves the nutritional and sensory profile, as well as the increase of bioavailability and bio-accessibility of health promoting bioactive compounds. The fermentation of soybean protein isolates using L. helveticus has been proven to improve sensorial properties while the sensorial properties were negatively affected after the fermentation of isolates using B. subtilis, S. cerevisiae, and R. oryzae. Also, emulsifying capacity of the isolates decreased while the foaming

stability increased after the fermentation of isolates using L. helveticus (Meinlschmidt et al, 2016). In the study of Pon et al. (2023), sunflower protein concentrates were fermented with L. helveticus and extruded into meat analogues. After fermentation, glutamic acid content increased in the concentrate and the pH shifting to seven decreased sour taste in the analogues. Bao et al. (2018) studied the fermentation of spirulina biomass using LAB strains and B. subtilis. An unpalatable aroma was obtained from the fermentation with L. acidophilus while almost desirable sensory profile was obtained with Lb. plantarum strain. As a result, the mixed fermentation of this strain and B. subtilis was conducted to further improve the aroma and odor functions where creamy odor was obtained. These studies could be further evaluated and improved to produce meat and dairy analogues for the human consumption. To understand how fermentation affects the protein composition of these materials, as well as their functional, technical, and sensory qualities, this literature review has been conducted.

Keywords: Alternative sources, fermentation, improvement, protein, sustainability

Schematic representation of the effect of the fermentation on the protein concentrates and isolates of the different protein sources.



PP-19

Exopolysaccharides produced by lactic acid bacteria and its importance in the food industry

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Lactic Acid Bacteria (LAB) which includes several genera such as *Lactobacillus, Bifidobacterium and Streptococcus*, are gram-positive bacteria known for their ability to ferment sugars into lactic acid. LAB are bacteria that break down lactose into lactic acid and are capable of fermentation. They are commonly found in fermented foods, dairy products, and the gastrointestinal tract of humans and animals. LAB's unique metabolic capabilities contribute to its applications in numerous industries, including the food industry, probiotic formulation, pharmaceutical and agriculture. Exopolysaccharides (EPS) are high molecular weight, complex carbohydrates produced by a variety of microorganisms, including LAB. These substances serve many functions both in the natural environment and in industrial applications. EPS can contribute to the structural integrity of biofilms formed by microorganisms and can also protect against environmental stressors. In industrial settings, EPS has attracted attention for its unique rheological and emulsifying properties. In addition to emulsifying properties, EPSs also have gelling and stabilizing properties.The versatility of EPS has made it of great importance by using it

in various industries such as chemistry, medicine, detergent, cosmetics and packaging, especially in the food industry. In addition, its potential positive effects on human health have received great attention in recent years. Therefore, EPSs produced by LAB have extraordinary commercial potential, making them valuable for a variety of applications. EPS exhibit unique properties including the ability to form viscous solutions even at low concentrations and a pseudoplastic structure, making them highly desirable for a variety of food formulations. Unlike conventional polysaccharides, EPS is environmentally friendly with recycling potential. Microbial exopolysaccharides obtained from lactic acid bacteria are widely used in the dairy industry, especially in yogurt and other fermented milk products. The use of EPS-producing lactic acid bacteria is preferred to naturally eliminate structural defects such as loose structure and serum separation in yogurt. In addition, it is aimed to harden the ice cream by mixing the thickened milk with eps and pasteurized milk. In this way, it is aimed to improve the functional properties and quality of the final product by increasing the viscosity and water holding capacity of the product. Apart from this, EPS is also used in the production of fermented sausage. Advantages of EPS include improved viscosity, improved structure, reduced syneresis and better taste perception. In summary, EPS production includes growing LAB under controlled conditions, optimizing nutrient availability, and environmental factors to support EPS synthesis. Thanks to EPS produced from lactic acid bacteria, the consistency and texture of the food product is improved.

Keywords: lactic acid bacteria, exopolysaccharides, food industry, thickener

PP-20

Technological and Metabolic Properties And Volatile Compounds of *Penicillium Roqueforti* Isolated From Turkish Traditional Blue Cheeses

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Penicillium roqueforti is the principal mold that gives blue-green color to Turkish blue cheeses such as mold-ripened Konya Tulum and Erzurum Civil. This study aimed to determine the technological and metabolic properties and volatile compounds of *P. roqueforti* isolates obtained from Turkish blue cheeses. In previous studies, 120 *P. roqueforti* isolates were obtained from traditional Turkish blue cheeses (n=61). According to the sequence types determined by microsatellite analysis, 20 isolates representing the population were selected and used in this study. Mycelium growth at 12°C and 25°C, salt resistance (1%, 3%, 6% NaCl), proteolytic and lipolytic activities of the isolates were determined. While the colony diameters were between 29-77 mm on malt extract agar (MEA) without NaCl, at 1% of NaCl, they were in the range of 36-75 mm. As the NaCl ratio increased (3% and 6%), the colony diameters decreased to 19.9-68 mm and 8.1-49.2 mm, respectively. After incubating the isolates at 25°C for seven days on mycological agar containing 10% skimmed milk, a clear zone (1.5-5 mm) was observed in 11 isolates, indicating proteolytic activity. Lipolytic activity was detected in 16 isolates with an opaque zone (0.8-5.7 mm) around the colonies after seven days of incubation on Tween 80 agar at 25°C. The volatile compounds of the isolates were determined using GC-MS. Determining different technological, metabolic properties, and volatile compounds of *P.*

roqueforti isolates from Turkish blue cheeses will indicate their potential as secondary starters in mold-ripened cheese production.

Keywords: Blue cheese, *Penicillium roqueforti*, technological features, volatile compounds

PP-21
Pseudomonas diversity in raw beef and minced meat and characterization of new species

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Pseudomonas spp. are Gram-negative aerobic psychrotrophic bacteria capable of thriving in low-temperature-stored meat, fish, milk, and dairy products, causing undesirable odors, taste alterations, discoloration, gas production, and mucus formation. *Pseudomonas* species have intriguing characteristics, including the ability to degrade xenobiotics, fluorescent properties, plant growth-promoting capabilities, and the production of siderophores and antibiotics, which make them particularly interesting for scientific investigation. Recent developments in genome sequencing have contributed to resolving uncertainties among *Pseudomonas* species boundaries and discovering new species. Effective differentiation of *Pseudomonas* species is achieved through sequencing of specific genes such as the polymerase beta subunit *(rpoB)*, DNA gyrase B subunit *(gyrB)*, and protein-coding sigma factor *(rpoD)*. In this study, *rpoD* gene analysis was performed on raw beef and minced meat samples isolated from 12 different local butchers and markets, identifying 100 *Pseudomonas* isolates. The phylogenetic analysis revealed the presence of three distinct groups, *P. fragi* (n=13), a common spoilage agent in raw meat, and a recently identified species, *P. bubulae* (n=57), and eight isolates (YK24, YB92, YB55, YB144, YK16, YK50, T4, YK56) grouped in separate distinct clades having potential to be described as new species. To characterize these isolates, their biochemical and physiological characteristics, as well as their genomic sequences, were determined. The growth of the isolates at different temperatures (4, 12, 25, 30, 37, and 42°C) and various NaCl concentrations (0%-8%) in culture media, and at different pH values (4, 5, 6, 7, 8, 9, 10) based on turbidity formation were analyzed. All isolates could grow at 4, 12, 25, and 30°C, wheras all isolates except one (YK50) could grow at 37°C. At 42°C, only one isolate (YB144) could grow. Growth was observed in all isolates at NaCl concentrations ranging from 0% to 6%, while at 7% NaCl, two isolates (YK24 and YK50) could grow, and no growth was observed at 8% NaCl. No growth was observed at pH 4, but all isolates could grow at pH values 5, 6, 7, 8, and 9. At pH 10, no growth was observed except one isolate (YK56). Subsequent investigation will assess the growth behavior of the isolates under anaerobic conditions. Additionally, the isolates' ability to produce fluorescent pigments will be evaluated on King B agar, and biochemical characterization studies will be conducted using the API 20NE kit. The findings of this study will provide valuable information on the *Pseudomonas* diversity in raw meat and will enable the characterization of new species.

Keywords: Meat, molecular identification, Pseudomonas

PP-22
Functional plant-based smoothie product to alleviate autism spectrum disorder

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INTRODUCTION: *ASD (autism spectrum disorder) is a combination of neurodevelopmental and neurobehavioral conditions including impaired social communication, repetitive/restricted patterns of behavior [1,2]. Recently, the gut microbiota is focused on due to its potential effects on mood disorders in the presence of gut dysbiosis which is a reason of neuropsychiatric and bowel dysfunctions [3]. Gut microbes have an influence on brain by producing neurotransmitters such as BDNF (brain-derived neurotrophic factor) via SCFA (short chain fatty acids), GABA (gamma amino butyric acid), tryptophan metabolites and serotonin which are reported to be effective to improve cognitive function [4]. In several studies, it was reported that lactic acid bacteria regulated central GABA receptor expression and emotional behaviors such as lowering anxiety and depression like behaviors [5]. Correcting dysbiosis, improving the gut microbiota, and reducing the severity of ASD symptoms can be considered as some of the beneficial properties of consuming supplementary or dietary ingested probiotics [6,7]. The gut microbiota can be modulated by diet and resulting effects on gut-brain axis. Decreased symptoms of ASD can be observed by applying modulations of specific plant-based diets such as gluten-free, casein-free, ketogenic, and curcumin containing diets [3]. Curcumin, a bioactive substance of turmeric, has a neuroprotective effect and tends to be useful in ASD treatment by reducing oxidative stress, inflammation and increasing the level of intracellular glutathione [3,8,9]. Therefore, the aim of this study is to develop a functional plant-based beverage suitable for the use of ASD individuals.*

MATERIAL-METHODS: *In this study, a functional plant-based smoothie was prepared with almond milk, date puree, banana puree, and turmeric which are suitable for recommended diets. A GABA producer probiotic Lactiplantibacillus plantarum was encapsulated with WPC (whey protein concentrate) and citrus pectin using water-in-oil emulsion technique. Free cells of Lb. plantarum (inoculum ratio: 3%) were also used to produce fermented smoothie. Microbial, total phenolic content, antioxidant activity (ABTS), color, and mineral analyses were carried out.*

RESULTS-DISCUSSION: *Phenolic and antioxidant activities were detected in plant-based smoothie products. Since there has been a relation between ASD and oxidative stress, the consumption of antioxidants was also suggested for ASD individuals [8,9]. WPC-citrus pectin complex was successfully applied to stabilize Lb. plantarum cells. In both encapsulated and fermented smoothies, the viable number of Lb. plantarum was found higher than 107 CFU/ml. In the literature, studies reported beneficial effects of Lb. plantarum on improving the microbiota and altering some of the autism symptoms [7], amending and ameliorating behaviors in the ASD children [4,10], and adolescents [11]. Consequently, a functional beverage has been developed for people with ASD.*

Keywords: *Autism spectrum disorder (ASD), functional beverage, gamma amino butyric acid (GABA), gut microbiota, lactic acid bacteria*

PP-23

Triggering *Akkermansia* growth with lactic acid bacteria in the simulated gut system

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INTRODUCTION: The gut microbiota can stimulate the host's immune system, plays an important role in establishing the natural immune response and enhancing gut barrier function [1]. Strains belonging to the *Bacteroides*, *Clostridium*, *Faecalibacterium* and *Akkermansia* groups have been reported as health-related gut bacteria [2]. Recently, there have been studies for the functions of *A.muciniphila*, which has been included in

the new generation super probiotic class [3], as a propionate-producing bacterium: immune regulation, increasing intestinal barrier function, reducing obesity, diabetes and adipose tissue inflammation, regulation of glucose and lipid metabolism and elimination of insulin resistance in the microbiota [4][5][6].These health problems can be solved by "modulation of the microbiota" [7].

Akkermansia, a microorganism breaks down mucin, needs other beneficial bacteria that can promote mucin/mucus thickening in the environment for its propogation [8]. Probiotic bacteria and certain food ingredients such as polyphenols may increase the abundance of *A.muciniphila* in the gut [6]. The beneficial effects of intestinal microbial balance on human health are a valuable probiotic property. Therefore, *Lactobacillus* strains have been characterized by their ability to adhere and colonize the intestinal mucin/mucus layer [9]. It has been reported in studies that probiotics promote mucin thickening, prebiotics affect weight management through SCFAs and target metabolic diseases, while polyphenols provide metabolic benefits to the host to support the presence of *Akkermansia* in the intestinal microbiota [6].

Adhesion of probiotics to intestinal layer is considered as an important criterion in the application of their beneficial effects to the host [10]. Furthermore, the adhesion of LAB (lactic acid bacteria) to the mucosa in the colon and promoting the secretion of mucus by HT-29 cell (intestinal epithelial cells) is another important aspect that supports the development of *Akkermansia* [6]. The subject of the study is based on the assumption that LAB having probiotic properties at GRAS (generally recognized as safe) status can modulate the microbiota or habitat for the development of *Akkermansia* group microorganisms for the mucin/mucus supporting roles of LAB in the simulated colon environment.

MATERIALS-METHODS: Artisanal strains of LAB including 6 olive isolates were selected for evaluated in terms of stability *in vitro* gastrointestinal conditions, bacterial adhesion on mucin/mucus layer, mucin/mucus sugars non-utilization properties, HT-29 cell binding for mucin/mucus thickness increment abilities.

RESULTS: All LAB strains were evaluated for their tolerance to gastrointestinal conditions. Most of them remained viable in the range of 10^5-10^8 CFU/mL in the simulated gastric juice and these bacteria continued their viability in the range of 10^4-10^7 CFU/mL in the simulated intestinal fluid. Additionally, those 6 strains adhered to mucin layer between 35.2-52.2%. All those strains adhered to HT-29 cell and they showed significant increase in mucin thickness by comparing the control groups which including varying conditions such as different amounts of lactate concentrations (20 mM & 50 mM) and presence of mucin initially added.

CONCLUSIONS: Adhesion of probiotic LAB to the colonic mucosa and promoting the secretion of mucus by HT-29 cell are important aspects that support the development of *Akkermansia*. All LAB strains have been determined as suitable candidates to promote the GIT (gastrointestinal tract) health since they have a role for thickening the mucin/mucus layer. These olive isolates did not use both fucose and N-acetylgalactosamine as mucin sugars. Therefore, the simulated GIT environment has been modeled so that mucin-degrading bacterium *Akkermansia* can be efficiently grown.

Keywords: {Akkermansia}, gut microbiota, HT-29 cell, lactic acid bacteria, mucin/mucus, probiotic

PP-24

Development of an Electroanalytical-Based Biosensor Platform for Use in Diagnosis of Breast Cancer

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Breast cancer, which constitutes approximately one-fourth of cancer cases, ranks second among the cancer types with the highest mortality rates worldwide. HER-2 (Human Epidermal Growth Factor Receptor), which is abnormally overexpressed in 25-30% of breast cancer cases, indicates a subtype of breast cancer (Howlader et al., 2018). In HER-2 cancer formation, it is highly expressed on the cell membrane (Schlessinger, 2004) and serves as an important biomarker used in the diagnosis of this type of cancer; its abundance in cells supports a poor prognosis (Wang et al., 2014).

Currently, the lack of early diagnosis mechanisms for breast cancer and its detection only in advanced stages contribute to increased mortality rates. Commonly used detection mechanisms such as mammography, X-rays, tomography, or biopsy have proven ineffective in detecting breast cancer at early stages (Tiwari et al., 2022). Developments in sensor technologies have offered new hope to this problem, resulting in the development of numerous sensor applications with high specificity for early diagnosis of breast cancer (Tiwari et al., 2022). Electrochemical sensors have shown superiority over other diagnostic methods for breast cancer due to their high sensitivity, stability, selectivity, low cost, versatility, portability, and user-friendly nature.

In this presented study, the aim is to develop a new generation electroanalytical-based biosensor system for the diagnosis of breast cancer. In this context, disposable electrodes were developed by modifying the surface of pencil graphite electrodes (PGEs) with manganese ferrite nanoparticles. Metal nanoparticles such as manganese ferrite have the potential for use in the manufacturing of electrochemical biosensors due to their high electrical conductivity and magnetic properties (Beitollai et al., 2019). In this study, MnFe nanoparticles with an average diameter of 30-70 nm were successfully synthesized. To increase the effective surface area of the electrodes, ensure easy binding, and immobilize the HER-2 protein on the surface, the electrodes were coated with chitosan, a polysaccharide. Manganese ferrite, with its high magnetic property, improved the signals and enhanced the sensitivity of the electrode, like other similar studies reported in the literature. The electrochemical performance of the modified electrode was evaluated using cyclic voltammetry and square wave voltammetry methods. As a result of the study, a new, rapid, inexpensive, and high-sensitivity electrochemical-based test platform for the diagnosis of breast cancer was successfully developed.

In conclusion, the development of this novel electroanalytical-based biosensor system holds promise for early breast cancer diagnosis. The implementation of such advanced sensor technologies may aid in detecting breast cancer at its treatable stages and thus improve the chances of successful treatment.

Keywords: Breast cancer,Chitosan,Electroanalytical-based biosensor, HER-2, Manganese ferrite nanoparticles

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Keywords: Breast cancer,Chitosan,Electroanalytical-based biosensor,HER-2,Manganese ferrite nanoparticles

PP-25

Evaluation of Phenotypic and Genotypic Methods for Carbapenemase Production and Typing in *Pseudomonas aeruginosa* Isolates from Various Clinical Samples

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Introduction: *Pseudomonas aeruginosa* is a multidrug resistant opportunistic pathogen that causes infections especially in hospitalized patients and immunocompromised individuals. It is on the list of "Bacteria that pose a serious threat to human health" by the American Center for Disease Prevention and Control [1]. Carbapenems are the main antibiotics used to treat infections caused by *P. aeruginosa*; however, carbapenem resistance in *P. aeruginosa* strains is increasing worldwide [2]. The development of resistance to carbapenems is mostly due to loss of OprD porin protein and increased activation of efflux pump proteins. In *P. aeruginosa*, resistance due to A, D class serine carbapenemases and molecular B class metallo-beta lactamases (MBL) has also been reported frequently in recent years. [3,4]. The time required to detect carbapenem resistance in the routine laboratory is about 48 hours. This delays the initiation of effective treatment, especially in critically ill patients. For this reason, researches focus on new methods to detect bacterial resistance earlier. In this study, it was aimed to investigate carbapenemase production by phenotypic and genotypic methods in clinical *P.aeruginosa* isolates resistant to carbapenems.

Materials and Methods: 100 carbapenem-resistant and 15 carbapenem-susceptible *P. aeruginosa* isolates obtained from patients and routinely tested for antibiotic susceptibility between January 2018 and February 2023 at the Microbiology Laboratory in M.U. Pendik Training and Research Hospital were included. In the investigation of carbapenemase production, a colorimetric method based on enzyme-substrate reaction was used as a phenotypic test. *P. aeruginosa* isolates were grown by overnight cultivation then suspended in Tris HCl for lysis of bacteria. Bacterial lysate was added to the solution containing imipenem and phenol red, then incubated for 1 hour at room temperature. Samples with color change from red to orange/yellow were recorded as carbapenemase positive. Carbapenem resistance was genotypically investigated by real-time PCR (RT-PCR).

Results: In this study, carbapenemase production was detected in 35 (35%) of 100 carbapenem resistant isolates by phenotypic and genotypic methods. Positive results were recorded within 1 hour using the phenotypic method. *P. aeruginosa* isolates found to produce carbapenemase enzyme were most commonly isolated from urine and catheter tip samples. The investigated genes were not detected in the isolates (N=65) that were found to be carbapenemase negative by phenotypic testing. In these isolates, carbapenem resistance can be attributed to other resistance mechanisms such as porin loss (OprD etc.), efflux pump activation and AmpC overexpression.

Conclusions: Starting an effective antibiotic treatment in the earlier phase is life-saving in antibiotic-resistant bacterial infections. Today, various rapid but costly commercial diagnostic methods such as PCR tests are used for the detection of antibiotic resistance. Reliable, rapid, and cost-effective tests to detect resistant bacteria or infections in the routine laboratory are needed for appropriate treatment and effective infection control measures. The phenotypic method based on the colorimetric detection of enzyme-substrate reaction used in our study can detect carbapenemase-producing isolates in a short time with low-cost.

Keywords: Antibiotic resistance, carbapenem, carbapenemase, phenotypic test, Pseudomonas aeruginosa

PP-26

Development of Manganese Ferrite Nanocarriers For Mr-Assisted Applications In Breast Cancer Diagnosis

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Introduction: According to World Health Organisation (WHO) data, breast cancer is the first or second leading cause of cancer deaths in women [1]. The WHO has set the goal of diagnosing and treating at least 60% of cases as early stage disease through early detection of breast cancer. It is possible to image tumourous tissue with magnetic resonance imaging (MR), a non-invasive method widely used in the clinic [2]. Metal oxide nanoparticles (NPs) are the most widely investigated MR contrast agents and it has been determined that MnFe2O4)/Mn-Ferrite NPs provide higher efficiency [3]. NP surfaces are used for active targeting of cancer cells with antibodies, peptides or aptamerylated receptor-selective agents that are deposited in tumour tissue for diagnosis/treatment [4].

Materials and Methods: Within the scope of the presented project, Mn-ferrite NPs were prepared by co-precipitation method[5]. For the synthesis, equimolar solutions of MnSO4 and FeCl3 were mixed in their stoichiometric ratios and homogenised at room temperature. The pH of the solution was first adjusted by adding 1M NH3 solution and the mixture was then heated at 80°C for about one hour and the NPs were washed several times with deionised water to remove unwanted salt residues and covalently bonded and modified using the precipitation method. Mn-Ferrit NPs, whose physicochemical tests and morphological evaluation studies were completed, were combined with antibody and interacted with MCF-7 breast cancer cell line. In this context, MR images of MCF-7 cells interacted with MCF-7 cells and cells not interacted with NPs were taken separately. In the evaluations, the usability of Mn-Ferrit NPs as a targeted nanocarrier in early diagnosis of breast cancer was evaluated.

Findings: It was determined that the obtained Mn-Ferrit NPs had an average diameter of 20 nm and the average size of the NPs after coating with BSA was around 60-70 nm. In the study conducted to selectively recognise breast cancer cells thanks to the HER-2 antibody on the NP surface as well as the MR contrast agent feature thanks to the Mn-Ferrite contained in the NPs, it was determined that MR signal was obtained only from cell culture dishes interacted with Mn-Ferrite NPs and no signal was obtained from cell culture dishes without NPs.

Results: It is thought that the developed HER-2 antibody coated Mn-Ferrit NPs, when selectively interacted with MCF-7 cell line, provide the necessary signal in MR applications and a nanocarrier with potential for use in early diagnosis of breast cancer has been developed with this study.

Keyword: Manganese ferritnanoparticle, albumin coating, HER-2 antibody, early diagnosis of cancer with MRI

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PP-27

Evaluation of Phenotypic and Genotypic Methods for Carbapenemase Production and Typing in *Klebsiella pneumoniae*Obtained from Various Clinical Specimens

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Introduction: Today, infectious diseases caused by "antibiotic resistant" bacteria are among the top ten problems threatening human health worldwide[1]. Antibiotic resistance causes treatment failure and high rates of mortality and morbidity. Therefore, early and rapid detection of resistance in infection causing pathogen is very important for early initiation of appropriate antibiotic treatment.

Klebsiella pneumoniae is an important gram-negative pathogen causing urinary tract infections, bacteremia, pneumonia and liver abscesses. In recent years, treatment success has significantly decreased for this pathogen due to increasing antibiotic resistance including carbapenem antibiotics. The main mechanism leading to carbapenem resistance is carbapenemase production [2].

Detection of carbapenem resistance in routine clinical laboratories takes about 48 hours; this delays the initiation of the correct treatment. The use of genotypic approaches for the detection of carbapenem resistance is not widespread due to its high cost and the need for experienced personnel[3]. Therefore, the development of phenotypic methods that provide rapid, accurate and reliable results is important. The presence of carbapenemase can be detected within 2 hours by enzyme-substrate reaction-based phenotypic method. The aim

of this study was to investigate the sensitivity and specificity of the enzyme-substrate reaction-based method for carbapenem resistance in clinical *Klebsiella pneumoniae* isolates.

Materials and Methods: In this study, 101 resistant and 25 susceptible *Klebsiella pneumoniae* isolates with previously determined carbapenem susceptibility by routine laboratory tests (disk diffusion method) were studied. The principle of the test is based on the detection of the pH change resulting from carbapenem degradation by a bacterial hydrolytic enzyme using a pH indicator (phenol red) [4]. In the first step, bacteria were lysed with Tris HCl for enzyme liberation and then incubated in medium containing imipenem and phenol red for 1 hour at room temperature. Samples with a color change from red to yellow/orange were recorded as carbapenemase positive. In the last step, carbapenemase resistance was analyzed by real-time PCR.

Results: As a result of the study, 94 of 101 carbapenem resistant *Klebsiella pneumoniae* isolates were also determined resistant by phenotypic method (93.1%). Five carbapenem resistant isolates (6.9%) were gave false negative results with the enzyme detection. All 25 susceptible isolates were found to be susceptible (100%). The PCR results were confirmed the phenotypic test results.

Conclusions: Today, infections with antibiotic resistant pathogens are increasing. It is very important to use accurate and rapid methods to start effective treatment at an early stage. In this study, the sensitivity and specificity of the phenotypic test used to detect carbapenem resistance was 93% and 100%, respectively, and was completed in an average of 1 hour. The use of this method, which provides accurate and reliable results in a short time, should be evaluated in routine laboratories.

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Ethical approval: Ethical approval was obtained from Marmara University Institute of Health Sciences Non-Interventional Clinical Research Ethics Committee (Date/No:19.09.2022/90).

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Keywords: carbapenemase,carbapenem resistance,Klebsiella pneumoniae,phenotypic tests

PP-28

Adhesive Biohydrels Inspired By Mussels With High Adhesion Strength And Hemostatic Properties Through Fe+3 ION And NaIO4 Crosslinking Of Dopamine For Seamless Wound Closure Of Internal Organs

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Introdaction: Gelatin-based hydrogels derived from extracellular matrices offer a promising solution for seamless wound closure surgery. However, these hydrogels often face limitations due to their low mechanical properties and weak adhesive characteristics. While tropoelastin-derived hydrogels provide a solution to brittleness, they come at a high cost. As a more cost-effective alternative, Gelatin Methacryloyl (GelMA), a functional gelatin, has gained prominence. By combining GelMA with methacrylate-modified alginate (AlgMA) and introducing reversible ion-induced crosslinking, hybrid hydrogels were rapidly developed to enhance their ability to withstand strain. Although these hybrid hydrogels significantly improved toughness by over 600% compared to GelMA, their adhesive strength was slightly reduced due to ionic crosslinking with Ca+2 ions.(1) Therefore, to further enhance adhesive strength without compromising toughness, we recommend the use of Fe+3 ions with dynamic and reversible crosslinking properties as an alternative to Ca+2 for ionic crosslinking. Additionally, drawing inspiration from mussels, we functionalized AlgMA with dopamine, creating AlgMAC as a crosslinker. Combining AlgMAC with Fe+3 and NaIO4 led to hydrogels with even better hemostatic and adhesive properties, demonstrating excellent biocompatibility in both laboratory and tissue-like settings. This study lays the foundation for the future use of bifunctional materials in wound closure applications.

Materials and Methods: To enhance the bioadhesive properties of the obtained hydrogels, GelMA and AlgMA materials were employed. Hybrid hydrogel formulations were created by mixing these materials, aiming to improve adhesive strength without compromising durability through the utilization of dynamic crosslinking with Fe+3 ions. The generated hydrogel formulations were subjected to photopolymerization by exposure to visible light. In this study, novel hybrid bioadhesive formulations were developed by incorporating bifunctional methacrylate and catechol-modified alginate (AlgMaC) into GelMA. The tissue adhesion properties, physical characteristics, biocompatibility, and ex vivo performance of the created hydrogels were examined. The effects of post-crosslinking ionically with Fe3+ ions and oxidatively with NaIO4 solution on the adhesion performance were compared for the bioadhesive compositions obtained via photopolymerization.

Results and Discussion: As a result of the experiments conducted, it was observed that the use of Fe+3 ions with AlgMA significantly improved adhesion, and an excessive increase in the content of catechol molecules in bioadhesive materials reduced the adhesive performance for typical wet adhesion. Furthermore, using AlgMAC in bioadhesive formulations together with Fe+3 and NaIO4 resulted in the creation of hydrogels that displayed excellent biocompatibility in laboratory and tissue-like settings, along with better hemostatic and adhesive properties. . This study paves the way for future bifunctional materials in wound closure applications.

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PP-29

Nanotechnology and Artificial Intelligence: A Promising Tool for Detection of Antimicrobial Resistance Bacteria

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Introduction: Antimicrobial resistance (AMR) in bacteria is a global health crisis due to the rapid emergence of multidrug-resistant bacteria and the lengthy development of new antimicrobials. The first step to limit antibiotic resistance is to reduce the use of antibiotics. For this, the bacteria causing the infection and their antibiotic resistance profiles must be detected quickly. Currently, culture-based diagnosis method, polymerase chain reaction (PCR) and antibody-based methods like enzyme-linked immunosorbent assays (ELISA) make up most of the techniques currently in use. However, these methods have several limitations, such as being expensive, time-consuming, and requiring specialized personnel. Therefore, there is an urgent need for new methods with rapid results to prevent the wrong and unnecessary consumption of antibiotics. Nanotechnology, with its ability to manipulate materials at the nanoscale, is a promising strategy to address the detection of antimicrobial resistance. A potent approach in this direction is Surface-enhanced Raman spectroscopy (SERS), a powerful technique based on the interaction of nanoparticles and analytes for sensitive label-free analysis of biological samples. SERS can be used for rapid detection and identification of bacterial strains. However, distinguishing the antibiotic-resistant and susceptible bacteria by SERS spectra is challenging due to the high molecular similarity of the bacterial strains. To overcome this challenge, we proposed to use artificial intelligence (AI) methods to assist SERS-based diagnostics of AMR bacteria.

Materials and methods: In this study, we used SERS to detect methicillin, erythromycin, and cefoxitin-resistant and susceptible Staphylococcus aureus, and applied a range of machine learning algorithms, including deep learning and traditional approaches, to distinguish between the bacterial species.
Results and Discussion: SERS spectra of the bacteria were collected by using silver substrate and compared by analyzing in range of 400-1800 cm-1. Collected spectra will be classified with artificial intelligence to highlight the difference between different antibiotic resistance bacteria.

Conclusion: In conclusion, the utilization of SERS and artificial intelligence holds promise as a sensitive and specific method for the detection of antibiotic resistance bacteria.

Keywords: Antimicrobial Resistance, Surface-enhanced Raman spectroscopy, Machine Learnings, S. aureus, Nanotechnology

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PP-30

Determining the Effects of Piroxicam and MMP-8 Inhibitor I on Collagenase Activity by Using Cell In Situ Collagen Zymography in 8505C Thyroid Cancer Cells

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INTRODUCTION: Collagenases (MMP-1, MMP-8, and MMP-13) are members of the matrix metalloproteinase family and are capable of cleaving collagenous extracellular matrix, facilitating tumor invasion and metastasis by creating spaces large enough for cell movement in the dense matrix. In addition, overexpression of collagenases is associated with various diseases such as periodontitis, atherosclerosis, asthma, autoimmune disorders, and corneal epithelial defects. Therefore, identification of MMP inhibitors has emerged as new drug approaches. In this study, we aimed to investigate the effects of MMP-1 inhibitor (piroxicam) and MMP-8 inhibitor I on collagenolytic activity in 8505C thyroid cancer cells by using cell *in situ* collagen zymography method.

MATERIALS-METHODS: The effects of piroxicam and MMP-8 inhibitor I on 8505C thyroid cancer cell viability were detected with crystal violet assay. Sandwich model containing dye-quenched collagen type I (DQ-collagen type I) was generated in 96-well plate. Approximately $1x10^4$ cells were seeded into the wells and then treated with piroxicam and MMP-8 inhibitor I. After the 48-hour incubation, collagenolytic activity was evaluated under the fluorescent microscope. The effects of the inhibitors on MMP-1 and MMP-8 protein expression levels were also analyzed with immunocytochemistry.

RESULTS: 100 µM piroxicam and 10 µM MMP-8 inhibitor I significantly reduced the collagenase activity in 8505C cells by 20.5% ($p<0.05$) and 36.4% ($p<0.05$), respectively. However, 100µM piroxicam and 10µM MMP-8 inhibitor I showed no significant effect on MMP-1 and MMP-8 protein expression levels.

CONCLUSIONS: Piroxicam and MMP-8 inhibitor I effectively reduced collagenolytic activity by binding to active forms of MMP-1 and MMP-8, respectively. Therefore, these inhibitors may have potency to be used as anti-invasive agents in the prevention of thyroid cancer metastasis.

Keywords: Collagenases, In situ zymography, MMP inhibitors, MMP-8 inhibitor I, Piroxicam, Thyroid cancer

PP-31

Recombinant Production of 1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase for Drug Development Studies

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Antimicrobial resistance (AMR) distinctly arising from the misuse and overuse of antimicrobial agents is ranked among the top 10 public health threats, and is presumed to cause 10 million deaths annually by 2050 [1], [2]. A drastic increase in AMR of sexually transmitted infections (STIs), especially gonorrhea, and consequent limitations

in treatment alternatives threaten humanity [3]. The aetiological agent of gonorrhea, multi-drug resistant Neisseria gonorrhoeae (Ng), is included in the list of prioritized infections in the worldwide strategic focus on ending STIs, and the targets for a 90% reduction of gonorrhea incidences by 2030 have been established [4]. The seven-stepped methylerythritol 4-phosphate (MEP) pathway has a vital role in the life cycle of most pathogens and its absence in humans makes it promising for drug-targeting studies [5]. The second enzymatic reaction step in MEP is committed by the 1-deoxy-d-xylulose 5-phosphate reductoisomerase (DXR) enzyme which catalyzes the reductive isomerization of 1-deoxy-D-xylulose 5-phosphate (DXP) to 2-C-methyl-D-erythritol 4-phosphate [6]. In this study, based on the main scope of the project, for the ultimate utilization in drug development studies, particularly in high-throughput screening, the recombinant production of soluble NgDXR by the C-terminal His-tagged pLATE31 expression vector system in Escherichia coli BL21(DE3) was aimed. Numerous strategies were applied for the production of NgDXR in soluble form, however, a sufficient amount of the recombinant protein for biochemical analysis was not obtained. Extensive literature research and a sequence identity analysis through sequence alignment of DXRs from E. coli and Ng revealed 100% identity of functional residues. Therefore, the EcDXR gene was cloned into the C-terminal 6-His tagged pLATE31 vector and protein expression was induced with 0,5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) in E. coli BL21(DE3) at 30^0C for 3 hours. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of expression samples revealed a high level of soluble EcDXR. A subsequent purification procedure was applied and EcDXR was obtained with purity over 95% for being used in biochemical analysis. Further studies for drug development are in progress.

Keywords: Antimicrobial resistance, Escherichia coli 1-deoxy-d-xylulose 5-phosphate reductoisomerase, Neisseria gonorrhoeae

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PP-32

Optimization of Concentration Step of Bacteriophage EA1T1.B3 Infect Erwinia Amylovora Causing Agent of Fire Blight By Using Response Surface Methodology

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Introduction: Erwinia amylovora is causative agent of fire blight disease and bacteriophages that infect Erwinia amylovora shows great promise to control of fire blight disease [1], [2]. Since phages can be used for control plant pathogens, production, concentration and purification of phages become important issue [3]. The aim of the present study is to optimize the concentration of phages with high recovery rate in a short time.

Materials and Method: We characterized Erwinia amylovora phage EA1T1.B3 and did optimization of concentration step of Erwinia amylovora phage EA1T1.B3 by using response surface methodology (RSM). A 5-level central composite design with 3 factors (PEG concentration (2-18%), NaCl concentration (0-4M) and incubation time (0-24 hours)) combined with RSM was used [4]–[7].

Results: TEM analysis revealed that phage belongs to Siphoviridae family and full length of phage was measured to be 174.71±2.7 nm. According one step growth curve experiments, latent period and burst size of phage were found to be 20 min and 11.46 PFU per infected cell, respectively. The optimum values of PEG and NaCl concentrations and incubation time for the maximum recovery of 85.4% were defined as 18%, 2.38, and 0 h, respectively. So, the concentration step, which was completed up to 18 hours with 65% recovery with the conventional method, could be achievehid in a much shorter time with 85±2.8 % recovery [8]. Discussion: Consequently, it has been stated that RSM can be used effectively in a short time and with higher recovery rates in finding the optimum conditions for phage concentration, a
step that should be specially designed for each bacteriophage.

Keywords: Phage concentration, fire blight, Erwinia amylovora, PEG/NaCl systems, response surface methodology, high recovery.

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PP-33

Analysis of the S-locus structure of apricots *Prunus armeniaca L.* in Azerbaijan

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Apricot is one of the important export products of Azerbaijan. Some studies showed that unproductiveness problem of apricots together with increasing production areas arises because of self-incompatibility. Apricot *Prunus armeniaca L.*, like other fruit species of the Rosaceae family, exhibits a gametophytic self-incompatibility system. In flowering plants, gametophytic self-incompatibility, controlled by a single locus with several allelic variants, is one of the major problems preventing self-fertilization. Among fruit trees, apricots show a high degree self-incompatibility, especially in Middle-Asian and Iranian-Caucasian eco-geographical groups. The aim of this study was to identify S-allele constitution of several apricot genotypes from apricot germplasm in Azerbaijan using polymerase chain reaction (PCR) with specific primer pairs.

In the present study, self-(in)compatibility characteristics of a total of 61 apricot genotypes found within the different regions of Azerbaijan was studied. Analyses were carried out by using four primer pairs (SRc-F and SRc-R, EM-PC2consFD and EM-PC3consRD, AprSC8-R and PaConsI-F, AprFBC8-F and AprFBC8-R). The primer pairs EM-PC2consFD/EM-PC3consRD was used for the amplification of the second intron region and the SRc-F/SRc-R for the first intron. Additionally, AprSC8R was used to selectively amplify the SC/S8-RNase allele and was used in combination with PaConsI F. A total of 9 S-RNase alleles (S2, S3, S6, S7, S8, S11, S12, S13 and Sc) were determined in the 61 apricot genotypes. One cultivar (İrevan eriyi) did not show PCR products at all. Consequently, for four Azerbaijan cultivars a fragment of 900 bp was detected that indicated the presence of allele S2. A fragment of 310 bp occurred in three cultivars (Shemsi, Agja Nabad 2, Goyje Nabad) confirming this allele as S3. Four cultivars (Hampa, Yay Sherefi, Gejyetishen, Ordubad Sherefi) yielded a fragment of 1300 bp, and hence this allele was labeled S6. The allele S7 occurred in seven cultivars (Maychicheyi, Yeni Forma 2, Teberze 1, Agja Nabad 2, Haqverdi 2, Alcha erik, Abu talibi) as a fragment of 820 bp. An 1700-bp fragment appeared in eight cultivars, indicating the presence of S11-allele. Fragment size characteristic for S12 allele was observed in three cultivars. A band of 1250 bp appeared in twenty seven cultivars, suggesting that the S13-allele is common to all. All apricot samples (except Forma 2, Mayovka 1) distributed in Azerbaijan used in the study showed self-incompatibility without SC-haplotype. As Azerbaijan apricot genotypes are determined to be mostly self-incompatible, the data obtained hereby might be of good use for apricot breeding programs and more practically, for new apricot plantations; thus pollinator cultivars should be considered when self-incompatible apricot cultivars are being used.

Keywords: Alleles, Prunus armeniaca, primers, self-(in)compatibility

PP-34

Are Nanobacteria in Kidney Stones and Atherom Plaques Different Lifeforms with Genetic Material other than Nucleic Acids?

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INTRODUCTION: Kidney stones are an important health problem seen in 10% of adults [1]. In recent years, especially in western countries, the incidence of urolithiasis has been increasing due to changes in lifestyle. Genetics, diet, metabolic disorders, inadequate fluid intake or fluid loss and urinary tract infections are among the causes thought to cause kidney stones [2]. Especially in the last 20 years, it is still a matter of curiosity whether calcified nanoparticles called "Nanobacteria" are important in the etiology of kidney stone formation [3]. Nanobacteria have been shown to be present in dental calculi, kidney stones, human and bovine blood samples [4]. It is suggested that nanobacteria, which are also seen in atheroma plaques, may cause atherosclerosis and lead to many diseases [5]. In this study, we investigated the presence of nanobacteria and the presence of nucleic acids in these formations cultured from kidney stones. Another aim was to grow nanobacteria from atheroma plaques. Since their first discovery, whether nanobacteria are living organisms or not is a matter of discussion. To investigate both their morphological structure and biochemical composition in detail and thus try to understand whether they are nucleic acid-based life form or not.

MATERIALS-METHODS: We collected 29 kidney stones and 20 atheroma plaques from human patients. The stones and plaques were homogenized by microbead homogenizer. The supernatant was filtered from a 0.2µm pore-size filter to remove any possible bacteria. The filtrate was cultured in DMEM media for growing nanobacteria without adding FBS albumin. Total nucleic acid isolation was done from the cultured samples, and they were measured by spectrophotometer. Macromolecules of the samples were separated by SDS-PAGE and their susceptibility to DNase, RNAse and Proteinase K were investigated. Samples were also analyzed by SEM and EDS. The nature of macromolecules was determined by LC QTOF MS.

RESULTS AND DISCUSSION: Spectrophotometric measurements of the samples indicated absence of nucleic acids. Spherical structures with sizes 80-600nm were detected in uncultured kidney stone samples and in nanobacteria cultured samples analyzed by SEM. These spherical structures were similar in size and appearance to nanobacteria described in other studies. EDS analysis of nanobacteria showed only trace levels of phosphorus indicating the absence of nucleic acids. In SDS-PAGE analysis of nanobacteria approximately macromolecules with various

molecular sizes were observed. LC QTOF MS analysis of these molecules showed that they are human proteins with albumin in abundance.

As a result of these findings, we can conclude that nanobacteria are a formation of human proteins and not a different life-form that lacks nucleic acids. Their importance in the pathology of kidney stones and atheroma plaques should further be investigated.

Keywords: Atherosclerosis, Kidney stone, Nanobacteria

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