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Engineered Microbiota and Bacterial Extracellular Vesicles: Programming the Gut-Brain Axis for Precision Therapies

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INTRODUCTION

The microbiota-gut-brain (MGB) axis is a complex and dynamic communication system that connects the gut, its vast microbial community, and the central nervous system. This bidirectional axis plays a fundamental role in regulating physiological and pathological processes, including metabolism, immunity, neural development, and behavior. Dysbiosis, an imbalance in the composition or function of the gut microbiota, has been associated with a wide range of conditions, from gastrointestinal disorders to neuropsychiatric and neurodegenerative diseases (CRYAN; DINAN, 2012).

Traditionally, the modulation of the gut microbiota has been sought through the use of probiotics, prebiotics, and fecal microbiota transplantation (FMT). However, these approaches present significant limitations, such as the low survival rate of probiotics in the gastrointestinal tract, inconsistency in colonization, and difficulty in obtaining precise and reproducible therapeutic effects (SANDERS et al., 2019). The need for more effective and targeted therapies has driven the development of innovative approaches, such as engineered microbiota and bacterial extracellular vesicles (EVs).

Engineered microbiota, which utilizes synthetic biology principles, allows for the genetic modification of bacterial strains to confer them with new therapeutic functionalities, such as the optimized production of beneficial metabolites or the delivery of bioactive molecules (LIM et al., 2020). In parallel, bacterial EVs emerge as a promising platform for therapeutic delivery, offering advantages such as reduced immunogenicity, greater stability, and the ability to cross biological barriers, including the blood-brain barrier (BBB) (WANG et al., 2019).

This article aims to explore the transformative potential of engineered microbiota and bacterial EVs in programming the MGB axis for the development of precision therapies. We will address advances in synthetic biology applied to microbial engineering, the biogenesis and advantages of EVs, the mechanisms by which these approaches modulate the MGB axis, their potential clinical applications, and the crucial regulatory and ethical considerations for their translation into clinical practice.

1. SYNTHETIC BIOLOGY IN MICROBIOTA ENGINEERING

Synthetic biology offers a powerful set of tools to design and reprogram microorganisms, transforming them into biological "factories" capable of performing specific therapeutic functions in the gut. This approach aims to overcome the limitations of conventional probiotics, allowing for more precise control over microbial activity and the delivery of therapeutic agents (DAEFFLER et al., 2020).

1.1 CRISPR-Cas Systems for Precision Editing

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated proteins) systems have revolutionized genetic engineering, enabling precise and efficient genomic edits in a wide range of organisms, including bacteria (DOUDNA; CHARPENTIER, 2014). In the context of engineered microbiota, CRISPR-Cas is used for:

- Targeted gene editing: Inserting, deleting, or modifying specific genes in probiotic strains to optimize the production of beneficial metabolites (e.g., short-chain fatty acids - SCFAs, neurotransmitters) or to eliminate undesirable virulence factors (GOMAA et al., 2019).
- Gene expression regulation: Controlling the expression of therapeutic genes in response to specific stimuli from the intestinal environment, ensuring the release of therapeutic agents only when and where needed (MIMEE et al., 2018).
- Microbial community engineering:

Potentially, CRISPR-Cas can be employed to modulate the composition of complex microbial communities, eliminating pathogens or enriching beneficial species (BIKARD et al., 2014).

1.2 Genetic Circuits and Kill-Switch Mechanisms

Engineering genetic circuits in probiotic bacteria allows for the creation of sophisticated control systems for therapeutic function. These circuits can be designed to:

- Sensors and Responders: Detect specific disease biomarkers (e.g., inflammation, abnormal metabolites) in the gut and, in response, activate the production and release of therapeutic molecules (KOTULA et al., 2014).
- Feedback Systems: Regulate the activity of the engineered bacterium itself, ensuring that therapeutic production is maintained within optimal levels and avoiding adverse effects (RIGLAR; SILVER, 2018).
- "Kill-Switch" Mechanisms: Incorporate safety systems that induce the death of the engineered bacterium under specific conditions (e.g., absence of an external inducer, detection of an environment outside the gut), preventing uncontrolled dissemination and ensuring biological containment (CALIANDO; VOIGT, 2015). These mechanisms are crucial for the safety and regulatory acceptance of live microorganism-based therapies.

1.3 Safety and Containment Strategies

Safety is a paramount concern in microbiota engineering. In addition to "kill-switches," other containment and biosafety strategies include:

- Auxotrophy: Modifying bacteria to depend on specific nutrients not available in the external environment, limiting their survival outside the host (STEIDLER et al., 2000).
- Minimal genome engineering: Removing non-essential genes to reduce complexity and the potential for undesirable interactions (HUTCHISON et al., 2016).
- Use of GRAS (Generally Recognized As Safe) strains: Prioritizing the engineering of bacterial strains that already have a history of safety in humans, such as *Lactobacillus* and *Bifidobacterium* (FOOD AND DRUG ADMINISTRATION, 2010).
- Rigorous monitoring: Developing tracking and monitoring methods to detect the presence and activity of engineered bacteria in the host and the environment (PIRES et al., 2020).

2. BACTERIAL EXTRACELLULAR VESICLES (EVS)

Bacterial extracellular vesicles (EVs), including outer membrane vesicles (OMVs) from Gram-negative bacteria and cytoplasmic membrane vesicles (CMVs) from Gram-positive bacteria, are nanovesicles actively released by bacteria. They represent a mechanism of intercellular communication and have emerged as a promising platform for therapeutic delivery (YÁÑEZ-MÓ et al., 2015).

2.1 Biogenesis and Structural Composition

The biogenesis of bacterial EVs involves the budding of the outer membrane (for OMVs) or the cytoplasmic membrane (for CMVs), encapsulating cytoplasmic and membrane components. The composition of EVs is highly diverse and reflects the bacterial origin, containing:

- Proteins: Enzymes, virulence factors, adhesion proteins, and proteins involved in immune modulation (LIU et al., 2018).
- Nucleic acids: DNA (plasmids, genomic) and RNA (mRNA, tRNA, sRNA), which can be transferred to host cells and influence their gene expression (GHOSAL et al., 2015).
- Lipopolysaccharides (LPS): Present in OMVs of Gram-negative bacteria, acting as potent immunomodulators (KULP; KUEHN, 2010).
- Metabolites: Small molecules that can have biological effects on the host (KIM et al., 2015).

2.2 Advantages Over Live Bacteria

Bacterial EVs offer several significant advantages compared to the administration of live bacteria:

- Lower immunogenicity: Being acellular, EVs generally induce a less robust immune response than live bacteria, reducing the risk of adverse reactions (VAN DER POL et al., 2016).
- Greater stability: EVs are more stable under various environmental conditions (pH, temperature) and during storage, facilitating formulation and administration (LIEW et al., 2019).
- Ability to cross barriers: Their nanometric size and lipid composition allow EVs to cross biological barriers, such as the intestinal barrier and the blood-brain barrier (BBB), facilitating the delivery of therapeutic payloads to hard-to-reach sites (HOSHINO et al., 2020).
- Targeted delivery: The surface of EVs can be modified to express ligands that allow specific targeting to cell types or tissues, increasing therapeutic efficacy and reducing off-target effects (FAN et al., 2018).
- Safety: They lack replication capacity, eliminating the risk of unwanted colonization or horizontal gene transfer (TOYOFUKU et al., 2019).

2.3 Cargo Engineering for Therapeutic Delivery

The ability to carry and deliver bioactive molecules makes bacterial EVs ideal vehicles for therapies. Cargo engineering can be performed in several ways:

- Parental bacterial engineering: Genetically modifying the EV-producing bacterium to produce and encapsulate therapeutic proteins, RNAs, or metabolites in EVs (GUJRATI et al., 2019).
- Post-purification loading: Loading purified EVs with therapeutic molecules (e.g., drugs, siRNA, proteins) through methods such as electroporation, extrusion, or incubation (KIM et al., 2017).
- Surface modification: Anchoring therapeutic molecules or targeting ligands on the EV surface to improve specificity and efficacy (CHEN et al., 2020). These strategies allow EVs to deliver a wide range of therapeutic agents, from small molecules to complex proteins and nucleic acids, to modulate the MGB axis and treat various pathologies.

3. PROGRAMMING THE MGB AXIS

Engineered microbiota and bacterial EVs offer innovative mechanisms to modulate the MGB axis, influencing communication between the gut and the brain at multiple levels.

3.1 Neurotransmitter Modulation

The gut microbiota is a significant producer of neurotransmitters and their precursors, which can directly influence brain function. Microbiota-based therapies can:

- Increase beneficial neurotransmitter production: Engineered bacteria can be designed to optimize the production of neurotransmitters like GABA (gamma-aminobutyric acid) and serotonin, which play crucial roles in mood and anxiety regulation (STRANDWITZ et al., 2019).
- Modulate neurotransmitter precursors: Influence the availability of precursors such as tryptophan, which is metabolized into serotonin, thereby affecting neurochemical pathways (O'MAHONY et al., 2015).
- Reduce harmful neurotransmitters: Inhibit the production of microbial metabolites that can be neurotoxic or pro-inflammatory (SAMPSON; MAZMANIAN, 2015). EVs can deliver enzymes or RNAs that modulate neurotransmitter synthesis or degradation in both intestinal cells and neuronal cells after crossing the BBB (HOSHINO et al., 2020).

3.2 Epigenetic Reprogramming

Epigenetics, which involves modifications in gene expression without altering the DNA sequence, is a key mechanism by which the environment, including the microbiota, can influence cellular function. Microbiota therapies can:

- Influence DNA methylation: Microbial metabolites, such as SCFAs (butyrate, acetate, propionate), can act as histone deacetylase (HDAC) inhibitors, promoting histone acetylation and altering gene expression in host cells, including neurons and immune cells (STILLING et al., 2016).
- Modulate microRNA (miRNA) expression: Bacterial EVs can carry miRNAs that, upon delivery to host cells, can regulate post-transcriptional gene expression, influencing processes such as neuroinflammation and synaptic plasticity (LIU et al., 2016). These epigenetic modifications can have long-lasting effects on brain function and resilience to disease.

3.3 Immune and Barrier Function

The integrity of the intestinal barrier and the modulation of the immune system are essential for the health of the MGB axis. Microbiota therapies can:

- Strengthen the intestinal barrier: Engineered bacteria can produce proteins or metabolites that promote the integrity of tight junctions between intestinal epithelial cells, reducing "gut permeability" and the translocation of toxins and microorganisms (HIIPPALA et al., 2018).
- Modulate the immune response: Bacterial EVs and microbial metabolites can interact with immune cells in the gut and systemically, modulating the production of pro-inflammatory and anti-inflammatory cytokines, and influencing the differentiation of regulatory T cells (ROOKS; GARRETT, 2016).
- Reduce neuroinflammation: By modulating intestinal and systemic immunity, these therapies can indirectly reduce neuroinflammation, a key factor in many neuropsychiatric and neurodegenerative diseases (ERNY et al., 2015).

4. CLINICAL APPLICATIONS

The therapeutic potential of engineered microbiota and bacterial EVs in the MGB axis is vast, encompassing various clinical conditions.

4.1 Neuropsychiatric Disorders

Disorders such as depression, anxiety, autism spectrum disorder (ASD), and schizophrenia have been associated with gut dysbiosis and MGB axis dysfunction.

- Depression and Anxiety: Engineered bacteria that produce GABA or serotonin can alleviate symptoms of anxiety and depression by modulating brain neurochemistry (BRAVO et al., 2011). EVs can deliver molecules that reduce neuroinflammation and oxidative stress, contributing factors to these disorders (HOSHINO et al., 2020).
- Autism Spectrum Disorder (ASD): Microbiota modulation can improve social behaviors and reduce gastrointestinal symptoms in individuals with ASD, possibly by reducing toxic microbial metabolites and restoring intestinal barrier integrity (SHARON et al., 2019).

4.2 Neurodegenerative Diseases

Diseases such as Alzheimer's, Parkinson's, and multiple sclerosis are characterized by neuroinflammation and abnormal protein accumulation, with increasing evidence of MGB axis involvement.

- Alzheimer's Disease: Engineered microbiota can be designed to produce enzymes that degrade amyloid-beta aggregates or to reduce neuroinflammation, slowing disease progression (MINTER et al., 2017). EVs can deliver neuroprotective or anti-inflammatory agents directly to the brain (HOSHINO et al., 2020).
- Parkinson's Disease: Microbiota modulation can influence alpha-synuclein aggregation and inflammation, which are characteristics of PD. Microbiota-based therapies can aim to reduce inflammation and promote neuronal protection (SAMPSON et al., 2016).

4.3 Gestational Health

Maternal microbiota health during pregnancy has significant implications for fetal development and long-term offspring health.

- Prevention of gestational complications: Engineered microbiota can be used to prevent or treat conditions such as gestational diabetes, pre-eclampsia, and preterm birth, by modulating maternal inflammation and metabolism (KOREN et al., 2012).
- Fetal neural development: Modulation of the maternal microbiota can influence fetal neural development, potentially reducing the risk of neurodevelopmental disorders in the offspring (SGRITTA et al., 2019). Maternal EVs can transfer bioactive molecules that influence fetal brain development (HOSHINO et al., 2020).

5. REGULATORY AND ETHICAL CONSIDERATIONS

The translation of therapies based on engineered microbiota and EVs into the clinic requires a robust regulatory framework and careful consideration of ethical implications.

5.1 FDA and EMA Frameworks

Regulatory agencies, such as the Food and Drug Administration (FDA) in the US and the European Medicines Agency (EMA) in Europe, are developing guidelines for complex biological products.

- Live Biotherapeutic Products (LBPs): Engineered microbiota falls under the LBP category, requiring rigorous safety, efficacy, purity, and potency evaluations. Challenges include complete characterization of the modified microorganism, genetic stability, and containment (FOOD AND DRUG ADMINISTRATION, 2016).
- Advanced Therapy Medicinal Products (ATMPs): EVs, especially if loaded with genetic material or drugs, may be classified as ATMPs or biological products, depending on their composition and mode of action. This implies complex regulatory requirements, including good manufacturing practices (GMP) and extensive pre-clinical and clinical testing (EUROPEAN MEDICINES AGENCY, 2018). Standardization of production, quality control, and clinical trials is essential for regulatory approval.

5.2 Ethical Challenges

Microbiota-based therapies raise several ethical questions:

- Access and Equity: Ensuring that these innovative therapies are accessible to all who need them, avoiding health disparities (KNOPPERS et al., 2019).
- Informed Consent: The complexity of these therapies requires a comprehensive informed consent process that explains the risks, benefits, and uncertainties to patients (RESNIK, 2019).
- Ecological Impact: The release of engineered microorganisms into the environment, even if contained, raises concerns about potential ecological impacts and the horizontal transfer of genes to the native microbiota (WATTERSON et al., 2019).
- Dual-Use Potential: The ability to manipulate the microbiota to influence behavior and cognition raises concerns about misuse or unethical use (DOUGLAS, 2013).

5.3 Biosafety and Risk Mitigation

Rigorous biosafety strategies are crucial to minimize the risks associated with engineered microbiota:

- Containment Mechanisms: Implementation of "kill-switches" and auxotrophy to ensure that engineered microorganisms do not survive or replicate outside the therapeutic environment (CALIANDO; VOIGT, 2015).
- Complete Characterization: Exhaustive genomic and phenotypic analysis of engineered strains to ensure their stability and absence of undesirable characteristics (PIRES et al., 2020).
- Post-Market Monitoring: Continuous surveillance to detect any long-term adverse effects or unexpected interactions with the host microbiota or the environment (STEIDLER et al., 2000). For EVs, risks are generally lower due to their acellular nature, but purity, immunogenicity, and the

potential for delivering toxic payloads still need careful evaluation (YÁÑEZ-MÓ et al., 2015).

6. BROAD CLINICAL APPLICATIONS

The breadth of clinical applications for engineered microbiota and bacterial EVs is vast, extending beyond neuropsychiatric and neurodegenerative conditions.

6.1 Integration Across Disciplines

The development and application of these therapies require an interdisciplinary approach, integrating knowledge from:

- Microbiology and Genetics: For the engineering and characterization of microorganisms and EVs (LIM et al., 2020).
- Neuroscience and Immunology: To understand the mechanisms of action in the MGB axis and the effects on the nervous and immune systems (CRYAN; DINAN, 2012).
- Biomedical Engineering and Nanotechnology: For the design of delivery platforms and optimization of formulation (FAN et al., 2018).
- Bioinformatics and Artificial Intelligence: For the analysis of complex microbiota data and the identification of therapeutic targets (FRANZOSA et al., 2015).
- Regulatory Science and Ethics: To ensure safety, efficacy, and social acceptance (KNOPPERS et al., 2019).

6.2 Emerging Clinical Evidence: Phase II and Advanced Trials for EVs and Engineered Microbiota in Neuroinflammation and MGB Disorders

While many of these approaches are still in preclinical phases, there is emerging clinical evidence demonstrating their potential:

- Phase II Clinical Trials: Several Phase II clinical trials are ongoing or have been completed, investigating the safety and efficacy of engineered probiotics and EVs in conditions such as irritable bowel syndrome (IBS), inflammatory bowel diseases (IBD), and more recently, in mood disorders and neuroinflammation (TILLISCH et al., 2013). For example, studies have explored the use of modified *Lactobacillus* or *Bifidobacterium* strains to produce specific metabolites that act on the MGB, showing promising results in modulating inflammatory responses and improving neuropsychiatric symptoms (AKBARI et al., 2016).
- EVs in Neuroinflammation: Advanced research with bacterial EVs has demonstrated their ability to reduce neuroinflammation in animal models of neurodegenerative diseases and, in some cases, are progressing to human studies. EVs, loaded with anti-inflammatory or neuroprotective molecules, can cross the BBB and modulate the activity of glial cells, such as astrocytes and microglia, which play a central role in neuroinflammation (HOSHINO et al., 2020).
- MGB Disorders: The application of engineered microbiota to modulate neurotransmitter production or to restore intestinal barrier integrity is being tested in trials targeting disorders such as treatment-resistant depression and chronic anxiety, with a focus on optimizing gut-brain communication (LIU et al., 2019).

These initial studies, while requiring large-scale validation, underscore the therapeutic potential of these platforms and the transition from basic research to clinical application.

6.3 Future Directions

Future directions include:

- Personalization: Development of personalized therapies based on the patient's individual genetic and microbiome profile, using precision medicine approaches (VALLES-COLOMER et al., 2019).
- Hybrid Platforms: Integration of engineered microbiota and EVs with nanotechnology and biosensors for real-time monitoring and controlled delivery (FAN et al., 2018).
- Longitudinal Studies: Conducting large-scale longitudinal clinical trials to evaluate long-term safety, efficacy, and health impacts (SANDERS et al., 2019).
- New Applications: Exploration of new applications in areas such as cancer (immunotherapy), autoimmune diseases, and healthy aging (ROUTY et al., 2018).

CONCLUSIONS

Engineered microbiota and bacterial extracellular vesicles represent an exciting frontier in precision medicine, offering the potential to reprogram the microbiota-gut-brain axis to treat a wide range of diseases. By overcoming the limitations of conventional probiotic therapies, these approaches allow for more precise and controlled modulation of microbial function and gut-brain communication.

Advances in synthetic biology, with tools like CRISPR-Cas and genetic circuits, enable the creation of microorganisms with optimized therapeutic functionalities and integrated safety mechanisms. Bacterial EVs, in turn, emerge as versatile delivery vehicles, capable of crossing biological barriers and delivering therapeutic payloads in a targeted manner, with lower immunogenicity and greater stability.

The ability to modulate neurotransmitters, induce epigenetic reprogramming, and restore immune and intestinal barrier function opens new avenues for the treatment of neuropsychiatric disorders, neurodegenerative diseases, and the promotion of gestational health. However, the translation of these therapies into the clinic demands rigorous attention to regulatory and ethical considerations, ensuring safety, efficacy, and equitable access.

Future perspectives point to interdisciplinary integration, the personalization of therapies, and the development of hybrid platforms that combine the best of synthetic biology, nanotechnology, and artificial intelligence. As research progresses and clinical trials provide more evidence, engineered microbiota and bacterial EVs are poised to revolutionize how we approach MGB axis health, paving the way for a new era of precision therapies.

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