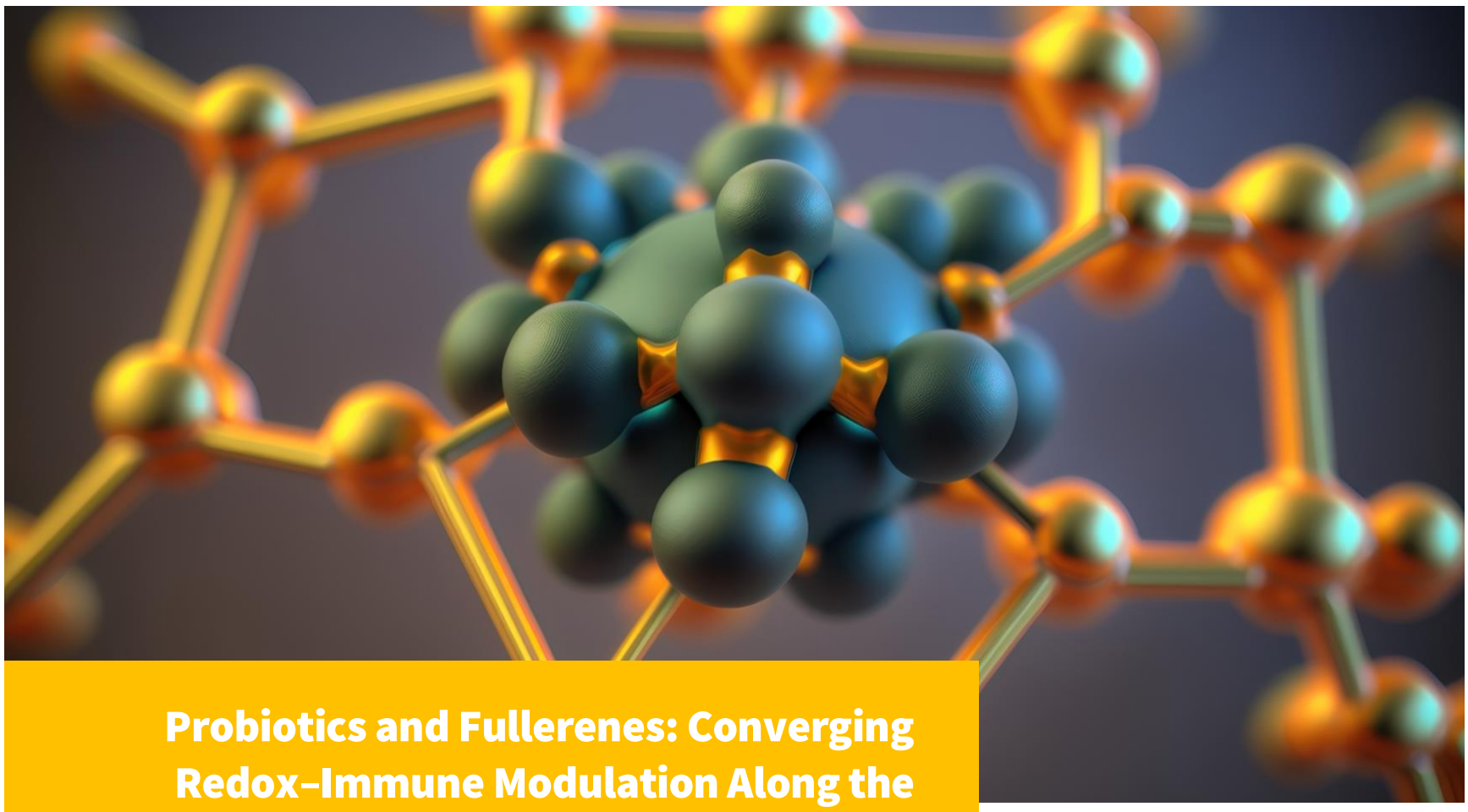
SCIENTIFIC DIGITAL JOURNAL

FRONTIERS OF BIOSCIENCE

ISSN # 3085-9069 AUG, 2025 ISSUE N. 11



Probiotics and Fullerenes: Converging Redox–Immune Modulation Along the Microbiota–Gut–Brain Axis for Neuroprotection

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https://doi.org/10.6084/m9.figshare.29885819

Neurodegenerative processes represent a growing challenge for global health systems, with prevalence increasing in parallel with population aging and changes in environmental exposures (Scheltens et al., 2021). These conditions are characterized by a gradual loss of neuronal integrity and function, leading to progressive impairment of motor, sensory, and cognitive capacities. Despite their clinical diversity, they share converging molecular mechanisms, including mitochondrial dysfunction, chronic neuroinflammation, oxidative stress, and failure of proteostasis systems (Hou et al., 2021).

A central pathological feature in many neurodegenerative contexts is the abnormal accumulation of misfolded proteins, a phenomenon collectively known as proteinopathy**.** These aggregates are closely linked to glial activation, where microglia and astrocytes adopt sustained pro-inflammatory phenotypes, releasing cytokines and reactive oxygen and nitrogen species that amplify neuronal damage. In parallel, this neuroinflammatory state contributes to the compromise of two essential barriers for brain homeostasis: the blood–brain barrier (BBB) and the intestinal epithelial barrier (Montagne et al., 2015).

In recent decades, the microbiota–gut–brain axis has emerged as a key regulator of central nervous system (CNS) health. This bidirectional communication network connects neural, immune, endocrine, and metabolic pathways, allowing the gut microbiota to influence brain physiology and behavior (Cryan & Dinan, 2012). Dysbiosis—an imbalance in microbial composition—has been associated with changes in short-chain fatty acid (SCFA) production, systemic inflammation, and increased permeability of intestinal and cerebral barriers, which can exacerbate neural vulnerability (Kowalski & Mulak, 2019).

Within this framework, probiotics—defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as *"live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"* (Hill et al., 2014)—have demonstrated the ability to modulate key physiological pathways relevant to neuroprotection. Experimental and clinical evidence supports their role in reinforcing barrier integrity, reducing systemic inflammatory markers, influencing neurotransmitter synthesis, and improving behavioral parameters (Barichella et al., 2016; Akbari et al., 2016; Tan et al., 2021).

Concurrently, nanotechnology has enabled the development of advanced therapeutic tools capable of crossing biological barriers and acting directly on neural tissue. Among these, C_{60} fullerenes, spherical carbon allotropes first described by Kroto and colleagues in 1985, stand out for their unique redox and interfacial properties (Kroto et al., 1985). Their conjugated carbon structure enables exceptional radical-scavenging capacity, neutralizing oxidative agents

Mechanistic node	C60 contribution	Probiotic contribution	Anticipated combined effect
Oxidative stress	Multielectron ROS/RNS scavenging; Nrf2 activation	SCFA-driven antioxidant enzyme upregulation	Greater redox buffering and reduced lipid/protein oxidation
Mitochondria	Membrane potential preservation; ATP support	Butyrate-signaled biogenesis and metabolic flexibility	Improved neuronal bioenergetics under stress
Microglia/Inflammation	Downregulates pro-inflammatory mediators	Reduces gut-derived LPS/cytokines; fosters homeostatic microglia	Lowered neuroinflammatory tone
Proteostasis	Potential interference with amyloid aggregation	Less systemic inflammation that accelerates aggregation	Reduced proteotoxic burden
Barrier/BBB	Indirect protection via anti-oxidative milieu	Strengthens gut barrier, lowering endotoxins	Decreased systemic triggers of CNS inflammation

Mechanistic elements are supported by canonical sources on fullerenes and microbiota–brain signaling, and by experimental evidence of C60-OH effects on microglia and probiotic effects on tight junctions.

with high efficiency (Dugan et al., 1997; Yin et al., 2023). Functionalization strategies have improved their solubility, biocompatibility, and pharmacokinetics, expanding their potential for clinical applications in neuroprotection (Moussa et al., 2017).

The conceptual convergence of these two therapeutic fronts—microbiota modulation via probiotics and targeted neuroprotection using C_{60} fullerenes—offers a promising and underexplored pathway. By combining peripheral modulation of inflammatory and metabolic processes with direct antioxidant and anti-inflammatory action in neural tissues, this approach may open new avenues for the prevention and management of neurodegenerative processes.

C_{60} fullerenes and derivatives: chemistry to neurobiology

The C_{60} cage supports dense surface functionalization that tunes solubility, bio-distribution, and protein interactions while retaining a high electron-affinity "radical sponge" core (Kroto et al., 1985; Dugan et al., 1997). Strategic derivatization (e.g., carboxylation, hydroxylation, amino-substitution) affords water-soluble species with brain-relevant activity: some cross the blood–brain barrier (BBB), protect neurons, or exhibit antitumor effects depending on linkage chemistry (Hsieh et al., 2017). Hydroxylated and carboxylated C_{60} attenuate microglial inflammatory programs (iNOS/COX-2, TNF-α, IL-1β, IL-6) via Nrf2-linked antioxidant responses (Ye et al., 2016). At the protein-aggregate interface, pristine and functionalized C_{60} inhibit Aβ fibrillization by binding key hydrophobic motifs (Kim et al., 2003) and deform amyloidogenic β-structures (Sun et al., 2016). Collectively, these data support a multimodal fullerene pharmacology radical scavenging immune re-programming, and anti-amyloid interactions—highly germane to NDD pathophysiology (Dugan et al., 1997; Hsieh et al., 2017; Ye et al., 2016; Kim et al., 2003; Sun et al., 2016).

Microbiota–gut–brain axis and probiotics

Germ-free and antibiotic-perturbed models reveal that the microbiota controls microglial maturation and tone (Erny et al., 2015) and influences BBB permeability from fetal to adult life (Braniste et al., 2014). In humans, stressors impair intestinal barrier function, whereas several probiotic combinations enhance barrier integrity and reduce permeability in stress/disease contexts (Camilleri, 2021). Seminal preclinical work showed probiotics modulate CNS signaling via vagal and GABAergic pathways (Cryan & Dinan, 2012), complementing clinical signals in PD constipation (Barichella et al., 2016; Tan et al., 2021) and mild cognitive impairment (MCI) cohorts using *Bifidobacterium breve* A1 (Xiao et al., 2020). Together, these findings establish tractable, human-relevant levers, barrier integrity and microglial immune set-points by which probiotics may influence neurodegeneration-linked biology (Hill et al., 2014; Camilleri, 2021).

Convergence: Why combine probiotics with C_{60} fullerenes?

Rationale 1: Redox–immune complementarity. Fullerenes provide rapid, catalytic-like quenching of reactive oxygen species (ROS) and direct interference with misfolded-protein interfaces (Dugan et al., 1997; Kim et al., 2003), whereas probiotics induce host-encoded resilience (tight junctions, mucus, SCFAs, tryptophan–kynurenine balance) and reshape peripheral immune tone that feeds forward to microglia (Erny et al., 2015; Camilleri, 2021).

Rationale 2: Two-barrier model**.** Probiotics address intestinal barrier dysfunction and endotoxemia; fullerenes act across the BBB–parenchyma compartment to dampen glial reactivity and amyloid seeding (Braniste et al., 2014; Ye et al., 2016; Sun et al., 2016).

Rationale 3: Motor and non-motor PD symptoms**.** RCTs show multistrain probiotics improve constipation in PD—an outcome linked to reduced systemic inflammation and improved quality-of-life metrics (Barichella et al., 2016; Tan et al., 2021). C_{60} derivatives could, in principle, address central inflammatory and proteostatic stressors that co-drive motor and cognitive decline.

Rationale 4: Formulation synergies. Fullerenes can be formulated or conjugated to biomolecules; engineered co-delivery with probiotic-derived postbiotics (e.g., butyrate donors) or micro-encapsulation with symbiotics is technologically feasible (Hsieh et al., 2017; Hill et al., 2014).

Evidence base

Fullerenes—Preclinical neuroprotection

Carboxyfullerenes reduced excitotoxic neuronal death and improved survival in SOD1-mutant ALS mice (Dugan et al., 1997). Hydroxylated/aminated C_{60} down-regulated microglial inflammatory mediators via Nrf2 activation in BV-2 microglia challenged with prion peptide; mitochondrial dynamics contributed to the anti-inflammatory phenotype (Ye et al., 2016). Surface-engineered, water-soluble C_{60} showed BBB penetration and linkage-dependent neuroprotective vs antitumor profiles (Hsieh et al., 2017). At the proteome interface, C_{60} and derivatives inhibited Aβ aggregation in vitro and in silico (Kim et al., 2003; Sun et al., 2016).

Probiotics—Human signals

Two Class-I-evidence RCTs in PD demonstrated that daily multistrain probiotics (with/without prebiotic fiber) significantly increase weekly complete/ spontaneous bowel movements vs placebo

over 4–8 weeks (Barichella et al., 2016; Tan et al., 2021). In MCI, *B. breve* A1 improved cognitive composites and reduced brain atrophy over 24 weeks in a randomized, double-blind, placebo-controlled trial (Xiao et al., 2020), consistent with open-label pilot findings.

Barriers and microglia as shared endpoints

Germ-free conditions cause leaky BBB and immature microglia; microbiota reconstitution normalizes both (Braniste et al., 2014; Erny et al., 2015). Human data indicate probiotics can tighten the intestinal barrier under stress and disease (Camilleri, 2021). Given that activated microglia amplify ROS and cytokine cascades that damage neurons (Dugger & Dickson, 2017), the pairing of barrier-centric (probiotics) and redox–microglia-centric (fullerenes) interventions targets two causally connected hubs.

Safety and Characterization

The development of probiotic–fullerene hybrid therapeutics demands a dual-framework approach to safety and quality characterization, integrating established microbiological standards with the specialized requirements of nanomedicine. In the probiotic domain, adherence to the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus guidelines remains essential, encompassing strain-level taxonomic identification through genomic sequencing, verification of viability and potency across the product's declared shelf life, and validation of clinically relevant endpoints directly linked to strain-specific mechanisms of action (Hill et al., 2014). These parameters ensure that probiotic components meet reproducibility, stability, and efficacy benchmarks recognized by both scientific and regulatory communities.

For the fullerene-based nanomaterial component, safety evaluation must begin with rigorous physicochemical characterization**,** including particle size distribution and morphology (e.g., via dynamic light scattering, TEM/SEM), zeta potential (ζ-potential) to assess colloidal stability, protein corona formation profiling to predict biological interactions, and endotoxin quantification to exclude pyrogenic contaminants. Such characterization must precede in vivo studies to ensure reproducibility and interpretability of biological data (Hsieh et al., 2017).

Toxicological assessment should comply with Good Laboratory Practice (GLP) principles, incorporating acute and sub-chronic toxicity**,** immunotoxicity**,** and genotoxicity evaluations, along with biodistribution and clearance profiling using sensitive detection methods such as ICP-MS or radiolabel tracking. These studies are particularly critical in nanomedicine, where particle size, shape, and surface chemistry dictate tissue accumulation, blood–brain barrier penetration, and long-term biopersistence (Fadeel et al., 2018).

Extensive reviews on carbon nanomaterial toxicology emphasize that surface functionalization is a primary determinant of biocompatibility and pharmacokinetics. Hydrophilic derivatization, such as hydroxylation or carboxylation, frequently reduces cytotoxicity, enhances aqueous dispersion, and modulates opsonization, thereby influencing immune clearance pathways (Hsieh et al., 2017; Moussa et al., 2017). Optimizing these surface chemistries is thus not only a matter of formulation stability but a prerequisite for achieving a favorable safety profile in translational applications.

When integrated, these frameworks create a comprehensive safety strategy: the probiotic component undergoes strain-specific quality control and efficacy validation**,** while the fullerene nanomaterial is subjected to nanotoxicological and pharmacokinetic scrutiny**.** This dual-path characterization ensures that the combined platform meets the stringent safety expectations required for advanced therapeutic development in neurological and systemic applications.

Phase-Appropriate Research Agenda

The development of probiotic–fullerene combination therapies necessitates a staged research strategy that integrates mechanistic preclinical mapping with biomarker-driven clinical translation. This phased approach allows for iterative optimization of both the biological rationale and the safety-efficacy profile prior to large-scale trials.

Mechanistic Causal Mapping in Preclinical Models

Initial work should employ gnotobiotic or antibiotic-perturbed murine models to dissect the causal interplay between microbiota modulation and fullerene-mediated neuroprotection. Experimental arms would include: (i) probiotic alone, (ii) functionalized C_{60} derivative alone, and (iii) combination therapy. Primary readouts would span multiple biological compartments:

- **Intestinal barrier function** assessed by FITC-dextran translocation assays and plasma lipopolysaccharide (LPS) quantification.
- **Microbial metabolic output** profiling, particularly short-chain fatty acids (SCFAs), using targeted GC-MS metabolomics.
- **Blood–brain barrier (BBB) integrity** measured via Evans Blue dye extravasation or dynamic contrast-enhanced MRI.
- **Neuroimmune activation states** characterized through single-cell RNA sequencing of microglia and astrocytes, enabling high-resolution mapping of pro- and anti-inflammatory transcriptional phenotypes.
- **Proteostatic stress** quantified by immunohistochemical and biochemical assays of pathogenic protein aggregates.
- **Behavioral phenotyping** using validated motor and cognitive assays relevant to neurodegeneration models.

Biomarker-Driven Pilot Randomized Controlled Trial (RCT)

Following preclinical validation, a dose-escalation safety and feasibility trial should be conducted in patients with a clearly defined clinical phenotype—for example, individuals with neurodegenerative disease-associated constipation**.** Participants would be randomized to receive probiotic monotherapy or probiotic plus functionalized C_{60} derivative**.**

- **Primary outcomes**: changes in **Colonic Transit Biomarkers (CBMs)** and **Patient Assessment of Constipation Quality of Life (PAC-QOL)** scores.

- **Secondary outcomes**: modulation of the **gut microbiome** (shotgun metagenomics) and **metabolome**, plasma **LPS-binding protein** levels, **fecal zonulin** as a marker of gut permeability, **fecal calprotectin** for intestinal inflammation, and **serum neurofilament light chain** as a marker of neuroaxonal injury.

Cognitive Decline Intervention in Mild Cognitive Impairment (MCI)

A subsequent study could evaluate the cognitive and neurostructural impact of Bifidobacterium breve A1 with or without a functionalized fullerene derivative. This should employ composite cognitive batteries sensitive to early decline and high-resolution MRI volumetrics to assess hippocampal and cortical atrophy, building on findings such as those of Xiao et al. (2020).

When the microbiome meets nanocarbon, the brain will never be the same.

Translational Pharmacokinetic /Pharmacodynamic (PK/PD) Integration

Where feasible, in vivo neuroimaging approaches such as positron emission tomography (PET) or intracerebral microdialysis should be incorporated to correlate fullerene brain penetration with functional endpoints. Such studies could quantify the relationship between fullerene exposure**,** brain oxidative tone**,** microglial activation states**,** and downstream clinical or cognitive outcomes. This PK/PD coupling would be essential to validate mechanistic hypotheses and guide dose optimization.

By following this phase-appropriate, biomarker-integrated pathway**,** the translational pipeline ensures that candidate interventions are de-risked at the mechanistic level, supported by human-relevant biomarkers, and optimized for regulatory and clinical acceptance.

These results are summarized in Table 1 in the page 2.

These findings suggest a clear trend: multistrain formulations, especially those containing *Lactobacillus* and *Bifidobacterium* species, tend to yield more consistent benefits in both glycemic and inflammatory outcomes. Importantly, none of the reviewed studies reported adverse effects, reinforcing the favorable safety profile of probiotics during pregnancy.

Despite these encouraging results, most trials still rely on small sample sizes and short follow-up periods and rarely assess obstetric outcomes such as insulin use, birth weight, or mode of delivery. Therefore, further studies are necessary to validate these findings in broader clinical contexts.

Conclusion

The strategic integration of C60 fullerenes and probiotics represents a promising advancement in the management of neurodegenerative diseases, acting synergistically on multiple central and peripheral pathophysiological targets. While C60 fullerenes provide direct and multifaceted protection against oxidative stress, mitochondrial dysfunction, and neuroinflammation — all central elements in the neurodegenerative cascade — probiotics play an essential modulatory role along the gut–brain axis, restoring microbial homeostasis, reinforcing blood–brain barrier integrity, and reducing the systemic inflammation that perpetuates neuronal damage.

This combination builds on the growing evidence that neurodegeneration is not confined to the central nervous system but is the result of complex interactions between the brain and peripheral organs, particularly the gastrointestinal tract. By acting in an integrated manner on these interdependent systems, this approach has the potential not only to slow disease progression but also to improve cognitive and motor function as well as overall patient quality of life.

From a translational perspective, this strategy aligns with the emerging paradigm of precision medicine, enabling personalization based on microbiome profiles, inflammatory markers, and individual genetic parameters. However, its clinical implementation requires overcoming technical and regulatory barriers, including formulation standardization, long-term safety assessment, optimal dose and delivery route definition, and multicenter controlled clinical trials to validate efficacy and safety across diverse populations.

By combining nanobiotechnology with microbiological interventions, this approach positions itself as a hybrid therapeutic model capable of redefining the treatment of neurodegenerative diseases, paving the way for more effective, safer, and globally relevant therapies in the twenty-first century.

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