

Review

Integration of Electrospun Scaffolds and Biological Polymers for Enhancing the Delivery and Efficacy of Mesenchymal Stem/Stromal Cell Therapies

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Abstract

Mesenchymal stem/stromal cells (MSCs) have emerged as a promising therapeutic approach for a variety of diseases due to their immunomodulatory and tissue regeneration capabilities. Despite their potential, the clinical application of MSC therapies is hindered by limited cell retention and engraftment at the target sites. Electrospun scaffolds, with their high surface area-to-volume ratio and tunable physicochemical properties, can be used as platforms for MSC delivery. However, synthetic polymers often lack the bioactive cues necessary for optimal cell-scaffold interactions. Integrating electrospun scaffolds and biological polymers, such as polysaccharides, proteins, and composites, combines the mechanical integrity of synthetic materials with the bioactivity of natural polymers and represents a strategic approach to enhance cell-scaffold interactions. The molecular interactions between MSCs and blended or functionalized scaffolds have been examined in recent studies, and it has been shown that integration can enhance MSC adhesion, proliferation, and paracrine secretion through the activation of multiple signaling pathways, such as FAK/Src, MAPK, PI3K/Akt, Wnt/ β -catenin, and YAP/TAZ. Preclinical studies on small animals also reveal that the integration of electrospun scaffolds and natural polymers represents a promising approach to enhancing the delivery and efficacy of MSCs in the context of regenerating bone, cartilage, muscle, cardiac, vascular, and nervous tissues. Future research should concentrate on identifying the distinct characteristics of the MSC niche, investigating the processes involved in MSC-scaffold interactions, and applying new technologies in stem cell treatment and biofabrication to enhance scaffold design. Research on large animal models and collaboration among materials scientists, engineers, and physicians are crucial to translating these advancements into clinical use.

Keywords: electrospinning; mesenchymal stem/stromal cell; polymer; polysaccharide; protein; secretome

1. Introduction

Mesenchymal stem/stromal cells (MSCs) have attracted significant interest as a therapeutic option for various clinical conditions due to their immunomodulatory and tissue-regenerating capabilities [1]. However, MSC therapies are challenged by the limited retention of administered cells at the targeted delivery site [2]. Systemic delivery is a reasonable approach, especially for diseases with a more extensive pathology. However, it requires MSCs to go through a series of steps to exit the circulation and migrate to the injury site. Intravenous injection typically causes an initial buildup of MSCs in the lungs followed by redistribution to other organs, such as the liver, spleen, and kidneys [3]. To address this issue, recent studies have focused on local delivery methods, which enable more predictable delivery and higher retention of MSCs. Indeed, the majority of MSC-containing products that have been approved for use across the globe are delivered through local routes (e.g.,

intraarticular, intracoronary, intralesional, intrathecal, and intramuscular) [4]. Nonetheless, several factors that limit the engraftment, retention, and efficacy of locally delivered cells remain. These include inadequate cell-to-matrix interaction, disruptive mechanical forces, and the pathologic microenvironment at the site of delivery [5,6]. One strategy that can potentially mitigate these factors is scaffold-based delivery. Specifically, electrospun scaffolds, which can provide a three-dimensional (3D) fibrous framework that resembles a native extracellular matrix (ECM), have emerged as an attractive delivery platform. These scaffolds have a high surface area-to-volume ratio and tunable physicochemical properties that can be optimized to facilitate cell attachment and protect cells from both mechanical and biochemical injury [7]. Because synthetic polymers are comparatively inert, the combination of electrospun scaffolds and natural polymers presents an opportunity to generate a more conducive microenvironment for cell attachment and proliferation. Natural polymers exhibit natural domains



for cell recognition, but they often exhibit lower mechanical strength and faster degradation rates compared to synthetic polymers. Hence, recent investigations have explored the integration of natural polymers into synthetic polymers in the context of MSC delivery. In this review, we have summarized the recent progress in the integration of synthetic electrospun scaffolds and biological polymers for enhancing the delivery and efficacy of MSC therapies. Moreover, we have reviewed the molecular and cellular mechanisms that underlie the interactions between MSCs and electrospun scaffolds, as well as the impact of biological polymers in enhancing these mechanisms and interactions.

2. MSC Biology and Niche Interactions

2.1 MSC Biology and Therapeutic Applications

MSCs are multipotent cells that can be readily isolated from certain compartments in the body, such as adipose tissue, bone marrow, and umbilical cord. Nonetheless, all other tissues in the body have been shown to contain MSCs as part of the microvasculature [8]. The minimum criteria for isolated MSCs include (a) adherence to plastic when maintained in standard culture conditions, (b) expression of CD105, CD73, and CD90, and lack of expression of CD45, CD34, CD14/CD11b, CD79 α /CD19, and human leukocyte antigen-DR isotype (HLA-DR), and (c) ability to differentiate to osteoblasts, adipocytes, and chondroblasts *in vitro* [9]. It is also well-known that MSCs produce a multitude of growth factors, cytokines, and microRNAs, which can influence the behavior of various cells and tissues. Presently, therapeutic studies on MSCs capitalize on their regenerative and immunomodulatory properties [8]. These properties have enabled the translation of MSCs into products with regulatory approval for a wide variety of indications requiring tissue repair and mitigation of inflammation, such as acute myocardial infarction, amyotrophic lateral sclerosis, Crohn's fistula, critical limb ischemia, graft versus host disease, knee articular cartilage defects, spinal cord injury, and subcutaneous tissue defects [4]. It is anticipated that ongoing preclinical and clinical investigations will further expand the therapeutic application of MSCs [10,11]. Nonetheless, the degree to which extracted or cultured MSCs resemble tissue-resident MSCs remains unknown. MSC interactions with the ECM and other cells have profound effects on their multipotency and secretion profile. Hence, recent studies aim to recreate the MSC niche or native microenvironment to enhance the efficacy of MSC treatment.

2.2 MSC Niche Components and Interactions

The MSC niche refers to all the components surrounding the stem cells, such as ECM, soluble factors, and other niche cells, maintaining the MSCs in their undifferentiated state [12]. Cell adhesion molecules (CAMs), which are transmembrane proteins that help anchor cells to surfaces, play an important role in cell-to-cell and cell-to-ECM

interactions in stem cell niches. The two major families of CAMs involved in stem cell niche interactions are cadherins and integrins. Cadherins are Ca²⁺-dependent CAMs that maintain homotypic interactions with neighboring cells [13]. These CAMs, which are linked to the actin filaments of the cytoskeleton through catenins, not only facilitate cell adhesion but also modulate cell signaling in response to various external stimuli. Different types of cadherins have been shown to play a role in promoting MSC adhesion, proliferation, stemness, and paracrine functions [14]. On the other hand, integrins, which are also linked to the cytoskeleton through actin and intermediate filaments, are composed of two glycoprotein subunits (α and β) that maintain heterotypic interactions with ECM components and other proteins. Multiple integrins are abundantly found on the surface of many stem cell types. Subunit β 1 is often linked to the stem cell phenotype as it aids in homing to the niche, maintaining stemness, and promoting proliferation [15]. In the case of MSCs, it has been shown that the interaction of human bone-marrow-derived MSCs (BMSCs) with type I collagen through α 2 β 1 and α 11 β 1 integrins promotes survival and proliferation [15,16]. Aside from integrins, MSCs have also been shown to interact with ECM components through discoidin domain receptors, CD44, syndecans, and mechanosensitive ion channels [15]. These receptors interact with a wide variety of biological polymers in the ECM, which is composed of a variety of fibers and ground substance (i.e., glycoproteins, proteoglycans, and glycosaminoglycans [GAGs]). All molecules in the ECM share common domains, but the exact matrix composition and architecture that are optimal for maintaining the beneficial properties of MSCs remains unknown.

3. Integration of Electrospun Scaffolds and Biological Polymers for MSC Delivery

3.1 Electrospinning Technique

Electrospinning is a highly tunable method of fabricating nanofibrous scaffolds with a porous structure and high surface area mimicking the three-dimensional (3D) architecture of endogenous ECM [17,18]. Several products incorporating electrospun fibers have advanced into human use or testing [19]. Compared to most bottom-up fabrication techniques, the electrospinning process is economically advantageous because it is inexpensive, simple, and typically does not require additional purification techniques. Using high-voltage power, polymer solutions are charged and ejected through a spinneret following the direction of the electric field to form nanofibers on the collector plate. The interior bulk composition and physicochemical properties of nanofibers can be customized for specific applications by modifying the electrospinning parameters and implementing integration techniques. The electrospinning variables that influence the structure and function of nanofibers include solution parameters (e.g., molecular weight, concentration, solvent

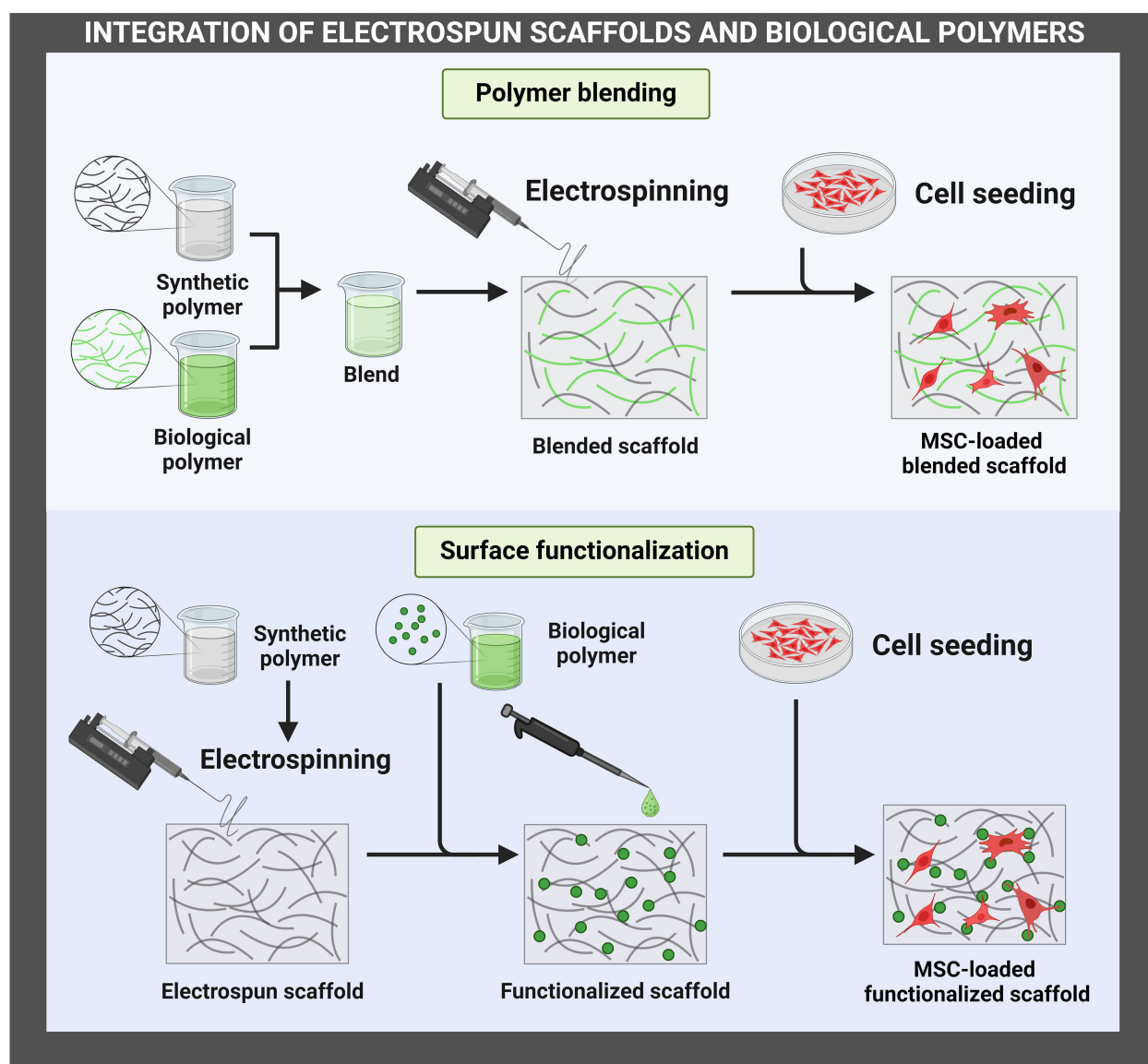


Fig. 1. Integration of electrospun scaffolds and biological polymers for enhancing MSC therapies. The figure has been created using <https://www.biorender.com/>. MSC, Mesenchymal stem/stromal cell.

type, surface tension, conductivity, and viscosity), processing parameters (e.g., applied voltage, flow rate, and spinning distance), and environmental parameters (e.g., temperature and relative humidity) [20]. Optimization of the physicochemical characteristics of the scaffolds is crucial since these have a substantial influence on cell behavior and function. For example, highly aligned fibers are needed to support the directional growth of seeded cells through contact guidance, and the stiffness of the scaffolds can promote the differentiation of MSCs toward specific cell types [21]. MSCs could be differentiated into neuronal cells on soft substrates, while musculoskeletal cells could be generated on more rigid scaffolds [21]. Examples of synthetic polymers that have been used for the production of electrospun scaffolds for MSC delivery include poly(aniline) (PANI), poly(ϵ -caprolactone)

(PCL), poly(ethylene oxide) (PEO), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(glycidyl methacrylate) (PGMA), poly(tetrahydrofuran) (PTHF), and poly(vinyl alcohol) (PVA).

3.2 Integration Techniques

The integration of electrospun scaffolds could be achieved through polymer blending or surface functionalization (Fig. 1). Polymer blending involves mixing two or more polymers in a common solvent to form a homogeneous solution, which is then electrospun into fibrous scaffolds. Solvents that are commonly used to blend synthetic polymers with biological polymers include acetic acid and hexafluoroisopropanol. On the other hand, surface functionalization involves the incorporation of biological polymers on the surface of electrospun synthetic scaffolds. Sur-

face functionalization may be accomplished through immersion of the scaffolds in a solution, plasma treatment, or direct printing onto the scaffolds. Polymer blending and surface functionalization may be employed simultaneously to enhance the integration of biological polymers with synthetic scaffolds.

A wide range of biocompatible synthetic polymers can be used to make electrospun scaffolds. The key advantages of synthetic polymers are spinnability, excellent mechanical integrity, and cost-effectiveness. However, modifications are typically needed for them to become bioactive. On the other hand, natural polymers, such as proteins and polysaccharides, are inherently bioactive and can often readily support cell adhesion and proliferation. Taking advantage of the benefits of both synthetic and natural polymers, recent investigations have focused on the integration of synthetic electrospun scaffolds and biological polymers to generate scaffolds with optimized physicochemical properties for MSC delivery. By improving the interaction between cells and scaffolds, biological polymers can support cell adhesion, stimulate cell proliferation, and promote the secretion of immunomodulatory substances.

3.3 MSC Seeding Techniques

3D culture conditions and scaffolds have been shown to enhance the survival of MSCs and amplify their immunomodulatory properties. Specifically, the use of polymer matrices to deliver MSCs has been shown to prevent the rapid loss of MSCs at the transplantation site by promoting cell-to-matrix adhesion and by protecting MSCs from mechanical disruption, biochemical stress, and immune clearance [8]. MSCs can be seeded onto the electrospun scaffolds through a variety of methods. These include passive seeding, electrospraying, centrifugation, and the use of a perfusion system.

Passive seeding, which involves pipetting a cell suspension directly onto the scaffold, is the simplest and most widely used method for attaching cells onto electrospun scaffolds. Electrospraying involves ejecting cell suspensions onto the scaffolds under an electric field, and it may be performed simultaneously with or after the synthesis of the scaffolds. Centrifugation or rotational seeding involves the deposition of the cells on the scaffolds under centrifugal force. Lastly, perfusion systems apply fluid flow to facilitate the delivery of cells into the scaffold's pores, resulting in more uniform cell distribution throughout the scaffold structure. These methods may produce variable effects on MSCs and must be optimized to maximize cell viability and function [22].

4. Molecular Interactions between MSCs and Electrospun Scaffolds

The survival and function of MSCs are significantly influenced by their surrounding microenvironment, which offers physical and biochemical cues crucial for cellular

homeostasis. Electrospun scaffolds, both synthetic and natural, transmit mechanical signals to modulate cellular behavior [23]. Consequently, these scaffolds are instrumental in controlling various intracellular signaling pathways, thereby affecting cell adhesion, proliferation, and function. Numerous studies have delved into the effects of the external environment on MSCs, laying the groundwork for our understanding of the interactions between MSCs and electrospun scaffolds [24].

4.1 MSC Adhesion

To engage with their surroundings, cells must establish physical connections either by attaching to the ECM or another cell. Adhesion is fundamental for cell communication and regulation, making it a pivotal aspect of biomaterial scaffold design. Effective adhesion facilitates crucial cellular behaviors such as spreading, proliferation, and performance of function [25]. Factors that affect adhesion include hydrophilicity, surface charge, and the presence of natural ligands.

The hydrophilicity of polymers plays a critical role in cell adhesion. The most commonly used synthetic polymers, such as PCL and PLA, are hydrophobic, and consequently, face challenges in initiating interactions with the hydrophilic surfaces of cell membranes. To overcome this problem, these synthetic polymers can be blended or functionalized with natural polymers that possess hydrophilic domains. These hydrophilic domains often take the form of polar functional groups (e.g., $-NH_2/-NH_3^+$, $-OH$, and $-COOH$) that can facilitate bond formation between the scaffold and cell surface receptors, thus promoting stable cell attachment and proliferation. For example, it has been shown that the addition of zein to electrospun PCL can increase the scaffold's hydrophilicity and enhance the attachment and growth of adipose tissue-derived MSCs (AMSCs) on the scaffold [26]. Similarly, fibrin has been shown to increase the hydrophilicity of PCL and improve the attachment and proliferation of BMSCs on the scaffold [27]. In another study, gelatin-blended PLGA scaffolds have also been shown to have higher hydrophilicity compared to PLGA alone, and this property translated to enhanced umbilical cord-derived MSCs (UMSC) attachment [28]. The surface charge of polymers can also affect cell adhesion. Synthetic polymers with cationic surfaces can associate with the negatively charged moieties found on the surface of cell membranes. For example, the integration of zein fibers rich in protonated amino groups into PCL has been shown to improve the scaffold's ability to interact with AMSCs' anionic cell membranes [26]. Conversely, polymers that are primarily anionic may have difficulties interacting with the cell membrane.

In their native microenvironment, cells constantly interact with adjacent cells and the ECM through protein ligands. CAMs, such as integrins and pattern recognition receptors, act as mechanobiological sensors that identify and

bind these ligands. When integrins bind to the ECM, they drive the assembly of focal adhesion complexes as well as the activation of integrin-associated protein kinases to initiate downstream signaling pathways for enhancing cell adhesion and survival [16,29,30]. Integrins bind ligands with lower affinity but at higher concentrations on cell surfaces, facilitating the formation of dense plaques upon activation, where multiple integrin molecules are anchored to cytoskeletal filaments to stabilize cell adhesion (Velcro principle) [31]. The most well-understood binding site for integrins is the arginine-glycine-aspartic acid (RGD) sequence, present in fibronectin and other ECM proteins. A study on the combination of gelatin and PLGA as scaffold material led to improved UMSC attachment due to the presence of RGD motifs, which play an essential role in the growth and migration of cells [28,32].

4.2 MSC Proliferation

Following attachment, cells can activate pathways that promote proliferation. The incorporation of biological polymers that can enhance cell adhesion onto electrospun scaffolds has been shown to also enhance the proliferation of MSCs [27,33,34]. The binding of integrin to ECM components activates the assembly of focal adhesion molecules, which not only facilitate stable adhesion but also trigger an intracellular cascade of signaling pathways that control cell behavior and survival [15]. In this “outside-in” signaling mechanism, integrins work with conventional signaling receptors to activate interrelated pathways that regulate cell proliferation, such as focal adhesion kinase/proto-oncogene *c-Src* (FAK/Src), mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), Wntless-related integration site/ β -catenin (Wnt/ β -catenin), and yes-associated protein/transcriptional coactivator with postsynaptic density-95/discs large/zona occludens-1 (PDZ)-binding motif (YAP/TAZ) pathways.

FAK is one of the most well-researched pathways of integrin signaling. Integrins clustering at cell-matrix contacts recruit FAK via intracellular adaptor proteins, and the clustered FAK molecules phosphorylate each other, forming a docking site for cytoplasmic tyrosine kinases from the Src family. Src and FAK reciprocally phosphorylate each other and other proteins within the junction, signaling the cell that it has adhered to a substrate suitable for growth and proliferation [31]. It has been shown that AMSCs seeded on decellularized ECM (dECM) had higher levels of phosphorylated FAK compared to those seeded on standard plates [35]. In another study, the addition of heparin and collagen led to increased phosphorylation of FAK in BMSCs [36].

The MAPK signaling pathway stimulates cells to proliferate or differentiate in response to tyrosine phosphorylation and the activation of Ras. The final kinase in the series is known as extracellular signal-regulated kinase (ERK) and is also a downstream target of FAK. The studies on MSCs seeded on ECM components that demonstrated increased

phosphorylation of FAK also showed increased phosphorylation of ERK [35,36]. In another study, it has been shown that collagen can enhance the adhesion, proliferation, and osteogenic differentiation of BMSCs through Ras homolog proteins [37].

The PI3K/Akt pathway can also be activated by FAK/Src proteins in response to integrin clustering [38,39]. In this pathway, PI3K generates phosphatidylinositol-3,4,5-trisphosphate, which in turn recruits Akt to the plasma membrane. Once activated, Akt inhibits apoptosis and promotes cell growth and proliferation through downstream signaling events [31]. Integrins play an important role in promoting the adhesion and survival of BMSCs onto ECM components through the activation of Akt [16,40]. It has also been shown that Akt plays an important role in mediating the proliferation of BMSCs after binding to glycoproteins through the CD44 receptor [41]. Cell growth regulation in the PI3K/Akt pathway relies in part on a protein kinase called mammalian target of rapamycin (mTOR), which promotes ribosome production and protein synthesis. The MAPK and PI3K/Akt pathways converge on mTOR because ERK can also activate this protein kinase.

The Wnt/ β -catenin signaling pathway is crucial for regulating various cellular processes, such as adhesion, proliferation, and differentiation. The canonical activation of this pathway involves the binding of Wnt ligands to Frizzled protein and lipoprotein receptor-related protein (LRP) co-receptor, leading to the stabilization and translocation of β -catenin into the nucleus, where it regulates gene expression associated with cell fate determination [31]. Among the genes activated is *c-Myc*, a transcription factor that drives cell proliferation. In one study, it has been shown that FAK phosphorylation can prevent β -catenin degradation and promote nuclear translocation in BMSCs seeded on ECM components [40]. However, in another study, it was observed that the AMSCs cultured on dECM had reduced nuclear β -catenin, potentially due to the increased recruitment of β -catenin for cell adhesion interactions [35]. Hence, further studies are needed to understand the β -catenin equilibrium in the context of MSC proliferation after ECM adhesion.

The YAP/TAZ pathway is also activated by integrin clustering and focal adhesion maturation. The cytoskeleton integrates mechanical strain arising from cell–ECM and cell–cell interactions. YAP/TAZ is activated through the release from inhibitors and modulation of nuclear–cytoplasmic shuttling. These transcriptional regulators also mediate the effects of Wnt signaling to promote cell proliferation and differentiation [42]. It has been shown that the attachment on dECM via integrins can enhance BMSCs’ expression of *YAPI* and proliferation [43].

4.3 MSC Secretome

Under optimal conditions, MSCs secrete a diverse array of soluble (e.g., immunomodulatory cytokines,

chemokines, and growth factors) and vesicular factors (e.g., extracellular vesicles), collectively referred to as the MSC secretome. The secretome's volume and composition are largely influenced by the MSCs' surrounding environment, prompting research into the effects of blended or functionalized electrospun scaffolds on MSC secretory functions.

The physical characteristics of electrospun scaffolds can enhance the ability of MSCs to secrete paracrine products. It has been shown that AMSCs cultured on electrospun PCL had significantly higher secretion of paracrine products, such as basic fibroblast growth factor (bFGF), hepatocyte growth factor, inducible nitric oxide synthase (iNOS), prostaglandin E₂ (PGE₂), transforming growth factor beta (TGF- β), and vascular endothelial growth factor, compared to those grown on standard culture plates. In turn, the AMSCs on electrospun scaffolds enhanced the anti-inflammatory secretion of macrophages, proliferation of vascular endothelial cells, and wound healing in rats with full-thickness skin defects [44]. It has also been observed that PCL with aligned fibers shows stronger effects in enhancing the secretion of paracrine products, such as iNOS and PGE₂, compared to scaffolds with randomly oriented fibers [44]. In another study, AMSCs on aligned PLGA fibrous scaffolds had a higher production of cyclooxygenase-2, PGE₂, and tumor necrosis factor alpha-induced protein 6 with the activation of FAK, ERK, and YAP/TAZ pathways [45].

Recent studies have shown that the addition of biological polymers can improve the ability to secrete regenerative factors. For instance, PCL nanofibers combined with platelet-rich plasma have been shown to boost MSC production of collagen, aiding bone tissue regeneration [46]. In the realm of skin tissue engineering, scaffolds composed of PCL and collagen have been effective in stimulating MSCs to release factors that promote wound healing, such as bFGF and angiopoietin 1 [47]. Additionally, incorporating RGD and TGF- β 1 into PCL fibers has resulted in enhanced extracellular matrix production by MSCs, which is beneficial for arterial regeneration [48].

5. Integration of Electrospun Scaffolds and Polysaccharides

Polysaccharides, which are polymers composed of monosaccharide units that are connected via glycosidic linkages, are the most abundant biological polymer in nature, and they may function as energy reservoirs, structural components, or mediators of intercellular signaling. Polysaccharides are an important component of the mammalian ECM's ground substance, which occupies the spaces between the cells and fibers. The ground substance is composed of GAGs, proteoglycans, and glycoproteins. GAGs are the most abundant heteropolysaccharide constituents of the ground substance, and these polymers are composed of repeating disaccharide units composed of a modified sugar (i.e., N-acetylgalactosamine or N-acetylglucosamine) and

a uronic acid (i.e., glucuronate or iduronate). GAGs are commonly linked to core proteins to form proteoglycans. Polysaccharides may also act as a component of glycoproteins [13]. Examples of GAGs that have been added to electrospun scaffolds for MSC delivery include chondroitin sulfate (CS) [49] and hyaluronic acid (HA) [50]. Apart from animal-derived polysaccharides, recent studies have also explored the use of other nature-derived polymers, such as alginate [51,52], cellulose [53], and chitosan [54].

5.1 Alginate

Alginate is a heteropolysaccharide extracted from several genera of brown algae, such as *Sargassum*, *Laminaria*, and *Macrocystis* [55]. It is comprised of two building blocks, namely guluronic acid (G-block) and mannuronic acid (M-block). It is generally regarded as non-toxic and non-immunogenic, making it an attractive material for tissue engineering, *in vitro* modeling, and drug delivery applications. In the presence of divalent cations, such as calcium (Ca²⁺), it forms a hydrogel through ionotropic crosslinking. Alginate hydrogels can be further modified through derivatization reactions involving their hydroxyl and carboxyl groups to modulate their polarity, solubility, and bioreactivity [56]. They can also be combined with other biological polymers to improve their porosity and biocompatibility [57]. In the context of generating scaffolds for MSC delivery, alginate has been combined with collagen to produce a composite hydrogel for the functionalization of electrospun PCL/gelatin scaffolds for the delivery of AMSCs to full-thickness wounds in rats. After 21 days, the wound closure for the MSC-loaded functionalized scaffold was 98% [51]. Alginate has also been combined with aloe vera powder to produce a composite hydrogel for the functionalization of electrospun PCL scaffolds for the subcutaneous delivery of EMSCs in mice. After 7 days, the MSC-loaded functionalized scaffold demonstrated lower M1 macrophage and higher M2 macrophage infiltration compared to the bare scaffold [52].

5.2 Cellulose

Cellulose, the most abundant naturally occurring polymer of glucose, is a homopolysaccharide composed of β -D-glucose monomers linked by β -1,4 glycosidic bonds. It is the main component of plant cell walls, but it may also be generated via fermentation by certain bacteria [58]. The hydroxyl groups can be used to facilitate the formation of hydrogels through physical crosslinking, but these groups can also be used to modify the polymer through a variety of reactions, such as esterification and etherification [59]. Cellulose acetate has been blended with PCL to produce an electrospun scaffold for the delivery of BMSCs into the injured Achilles tendons of rats. The scaffold was also functionalized with insulin, and the addition led to a significantly higher collagen deposition at 8 weeks [53].

5.3 Chitosan

Chitosan is a polycationic heteropolysaccharide composed of N-acetyl-D-glucosamine and D-glucosamine units. It is produced from the N-deacetylation of chitin, the second most abundant natural polymer after cellulose and a major component of the cell wall of many fungi and the exoskeleton of insects and crustaceans [60]. The amino and hydroxyl groups on its surface aid in the binding of various substances. Specifically, the addition of carboxyl and methyl groups to chitosan can enhance its similarity to the GAGs found in the ECM. Carboxymethyl chitosan has been blended with PVA to produce an electrospun scaffold that can support the attachment and proliferation of placenta-derived MSCs (PMSCs) and facilitate complete closure of full-thickness wounds in rats after 14 days [54]. Chitosan has also been blended with PVA to produce an electrospun scaffold for the delivery of AMSCs in rat calvarial defects. The scaffold was also incorporated with platelet-rich plasma (PRP), and the addition led to significantly higher bone regeneration [61]. Chitosan nanoparticles have also been used to encapsulate brain-derived neurotrophic factor (BDNF) and to functionalize the surface of an electrospun PLGA scaffold for the delivery of AMSCs in a rat model of sciatic nerve injury. However, the MSC-loaded scaffolds had a significantly lower sciatic functional index (SFI) and gastrocnemius mass ratio than the autograft at 12 weeks [62].

5.4 Chondroitin Sulfate

CS is a GAG that is commonly found in cartilage, bone, and heart valves. It is composed of repeating disaccharide units of N-acetylgalactosamine sulfate and D-glucuronic acid. It is a fundamental component of aggrecan, the proteoglycan that confers shock-absorbing capacity to articular cartilage [13]. CS has been blended with PCL-PTHF to produce an electrospun scaffold for the delivery of BMSCs into full-thickness patellar cartilage defects in rats. After 8 weeks, the MSC-loaded CS-containing scaffold demonstrated superior repair of the cartilage defects compared to no treatment (International Cartilage Repair Society [ICRS] score of 10.67 versus 2.33) [49].

5.5 Hyaluronic Acid

HA is a GAG that is commonly found in synovial fluid, vitreous humor, and ECM of most connective tissues in the body. It is composed of repeating disaccharide units of N-acetylglucosamine sulfate and D-glucuronic acid. Unlike the other GAGs, HA is composed of a very long chain of sugars. Hence, HA can hold large amounts of water and can act as either a lubricant or shock absorber. It is also a major component of aggrecan [13]. Another important function of HA is to immobilize growth factors in the ECM. Indeed, HA was recently used to improve the integration of BDNF with PLA to generate an electrospun scaffold for the delivery of BMSCs into the hemisectioned spinal cords of rats.

The addition of HA did not change the scaffold's fiber diameter and stiffness. The surface of the scaffold was also functionalized with collagen, and the scaffold demonstrated a better improvement in motor function compared to the cell-free scaffold at 8 weeks [50].

6. Integration of Electrospun Scaffolds and Proteins

Proteins are the most functionally diverse polymers found in living organisms, and they play various important roles, such as structural components, contractile elements, transporters, receptors, enzymes, hormones, and antibodies. More importantly, proteins are an essential component of the ECM's structural fibers and ground substance (i.e., proteoglycans and glycoproteins). Proteoglycans are formed through the covalent attachment of GAGs to a core protein. The number of GAGs that can attach to the protein core can vary from 1–200, which gives rise to proteoglycans' remarkable diversity. On the other hand, glycoproteins represent a smaller group of proteins in the ECM. Nonetheless, multidomain glycoproteins play an important role in stabilizing the ECM by providing a structural framework as well as binding sites for cells and other substances in the ECM [13]. Examples of glycoproteins that have been integrated with electrospun scaffolds for MSC delivery include collagen [49–51,63–66], gelatin [51,67–69], laminin [62], and fibrinogen [70]. Apart from glycoproteins, growth factors, peptides, and plant-derived proteins have also been blended with synthetic polymers to optimize MSC delivery (Table 1, Ref. [49,63–69,71–73]).

6.1 Collagen

Collagen, the most abundant protein in the body and the most abundant structural component of the ECM is composed of three α chains that are intertwined to form a triple helix. Collagen molecules polymerize to form supramolecular aggregates, such as flexible fibrils with high tensile strength or networks [13]. Among the proteins that have been studied for tissue regeneration, collagen represents one of the most widely used substrates due to its excellent biocompatibility [74]. It has been integrated with synthetic scaffolds for MSC delivery in the context of repairing various tissues, such as bone [63], cartilage [49], heart [64], nervous tissue [50,65], skin [51], and trachea [66]. It has been shown that the addition of collagen increases the scaffold's hydrophilicity and swellability without affecting the scaffold's initial maximum tensile stress and porosity [64,66]. For bone regeneration, collagen has been blended with PLA to generate a scaffold for the treatment of calvarial defects in rats. The MSC-loaded scaffolds had significantly higher cell density and total callus formation compared to the bare scaffolds at 4 weeks [63]. Generation of bone adjacent connective tissues is also possible by adjusting the composition of biomaterials while maintaining the utilization of MSCs and collagen. A recent study on PCL-PTHF scaffolds dem-

Table 1. *In vitro* and *in vivo* studies on electrospun scaffolds with proteins for the delivery of MSCs therapies.

Protein	Synthetic polymer	Integration technique	MSC type	Seeding technique	<i>In vitro</i> results	Target tissue	Animal model	<i>In vivo</i> results	Ref
Collagen	PCL	Polymer blending, surface functionalization	AMSC	Direct seeding	After 21 days, the MSC-seeded blended scaffold showed around 1.2 times higher GAG content than the surface-functionalized scaffold.	Trachea	Rabbit	After 4 weeks, the blended scaffold showed a higher cell density in the regenerated tracheal tissue compared to the surface-functionalized scaffold.	[66]
Collagen	PCL-PTHF	Polymer blending	BMSC	Direct seeding	The blended scaffold showed around 1.4 times higher cell viability compared to the tissue culture plate after 21 days.	Cartilage	Rat	After 8 weeks, the MSC-loaded blended scaffold demonstrated better repair of full-thickness patellar cartilage defect compared to no treatment (ICRS 15.68 versus 2.33).	[49]
Collagen	PCT	Polymer blending	BMSC	Perfusion system	The MSCs maintained their stem cell properties following seeding on the blended scaffold.	Heart	Rat	Around 15% of the cells on the cardiac patch survived 5 weeks after transplantation. After 5 weeks, the blended scaffold significantly improved cardiac function compared to control (LVEF: 40.8% versus 28.9%).	[64]
Collagen	PLA	Polymer blending	BMSC	Electrospraying	The blended scaffold supported the proliferation of MSCs.	Bone	Rat	After 4 weeks, total cell count, total callus formation, and osteocalcin staining in the skull defect were around 1.6, 6, and 1.3 times higher in the cell-seeded scaffolds compared to cell-free scaffolds, respectively.	[63]
Collagen	PLCL, PANI	Polymer blending	AMSC	Direct seeding	The addition of collagen significantly increased the metabolic activity and neuronal differentiation of the seeded cells.	Nerve	Rat	After 6 months, there was no significant difference in the SFI and muscle mass ratio between the MSC-loaded and cell-free scaffolds. However, the MSC-loaded scaffold showed around 1.1 times higher nerve density compared to the cell-free scaffold.	[65]
CTGF	PCL	Surface functionalization	Not specified	Direct seeding	None reported.	Muscle	Rat	After 24 weeks, the cell-free scaffold had a significantly higher collagen deposition and abundant abscess formation. On the other hand, no complications were observed for the MSC scaffolds.	[72]
Gelatin	PCL	Surface functionalization	PLMSC	Direct seeding	The scaffolds supported the proliferation of MSCs.	Bone	Rat	After 6 weeks, the MSC scaffolds had greater collagen deposition and periostin expression in the periodontal defects compared to no treatment.	[69]
Gelatin	PLA	Surface functionalization	BMSC	Direct seeding	After 7 days, the functionalized scaffold showed a 1.2 times higher proliferation than the non-functionalized scaffold.	Bone	Rat	After 8 weeks, the MSC-loaded functionalized scaffold demonstrated around 1.7 and 1.4 times higher bone regeneration as seen on digital mammography and histological analysis, respectively, compared to the non-functionalized PLA scaffolds.	[67]

Table 1. Continued.

Protein	Synthetic polymer	Integration technique	MSC type	Seeding technique	<i>In vitro</i> results	Target tissue	Animal model	<i>In vivo</i> results	Ref
Gelatin	PLCL	Polymer blending	EMSC	Direct seeding	The addition of gelatin enhanced the hydrophilicity of the scaffold and the proliferation of MSCs on the scaffold.	Muscle	Mouse	After 6 weeks, the blended scaffold had around 1.6 times higher cell infiltration compared to the non-blended scaffold. The MSC-loaded blended scaffold also showed a high M2 macrophage and minimal M1 response.	[68]
Laminin	PLA	Surface functionalization	DPMSC	Direct seeding	The scaffolds supported the proliferation of MSCs.	Skin	Mouse	After 9 days, the MSC-loaded scaffolds stimulated the production of a complete epidermal layer in some of the animals. Functionalization with laminin did not appear to provide an advantage.	[71]
Soya protein isolate	PEO	Polymer blending	BMSC	Direct seeding	The blended scaffold supported the adhesion, proliferation, and preconditioning of MSCs.	Bone	Rat	After 2 weeks, the MSC-loaded scaffold showed enhanced new bone formation in the skull defect compared to the cell-free scaffold and no treatment.	[73]

Abbreviations: AMSC, adipose tissue-derived mesenchymal stem/stromal cell; BMSC, bone marrow-derived mesenchymal stem/stromal cell; CTGF, connective tissue growth factor; DPMSC, dental pulp-derived mesenchymal stem/stromal cell; EMSC, endometrial tissue-derived mesenchymal stem/stromal cell; ICRS, International Cartilage Repair Society; PANI, poly(aniline); PCL, poly(ϵ -caprolactone); PCT, poly(ϵ -caprolactone-co-TOSUO); PEO, poly(ethylene oxide); PLA, poly(lactic acid); PLCL, poly(lactide-co- ϵ -caprolactone); PLMSC, periodontal ligament-derived mesenchymal stem/stromal cell; PTHF, poly(tetrahydrofuran); SFI, sciatic function index.

Table 2. *In vitro* and *in vivo* studies on electrospun scaffolds with biological composites for the delivery of MSCs therapies.

Composite	Synthetic polymer	Functionalization techniques	MSC type	Seeding technique	<i>In vitro</i> results	Target tissue	Animal model	<i>In vivo</i> results	Ref
Alginate, aloe vera powder	PCL	Surface functionalization	EMSC	Direct seeding	The functionalized scaffold supported the proliferation of MSCs.	Muscle	Mouse	After 7 days, the MSC-loaded functionalized scaffold had significantly lower M1 macrophage and higher M2 macrophage infiltration than the bare scaffold.	[52]
Alginate, collagen, gelatin	PCL	Polymer blending, surface functionalization	AMSC	Direct seeding	The scaffold supported the attachment of MSCs.	Skin	Rat	After 21 days, the scaffold showed significantly higher wound closure (around 95%) compared to the control (around 65%).	[51]
BDNF, chitosan, fibrin, laminin,	PLGA	Surface functionalization	AMSC	Direct seeding	None reported.	Nerve	Rat	After 12 weeks, the MSC-loaded scaffolds had a significantly lower SFI and muscle mass ratio than the autograft. The MSC scaffolds with laminin and BDNF had a comparable mean fiber diameter and myelin sheath thickness to those of the autograft.	[62]

Table 2. Continued.

Composite	Synthetic polymer	Functionalization techniques	MSC type	Seeding technique	<i>In vitro</i> results	Target tissue	Animal model	<i>In vivo</i> results	Ref
BDNF, collagen, HA	PLA	Polymer blending, surface functionalization	BMSC	Direct seeding	The addition of collagen increased the scaffold's hydrophilicity and MSC adhesion.	Spinal cord	Rat	After 8 weeks, the MSC-loaded functionalized scaffold demonstrated a greater improvement in motor function compared to cell-free scaffolds (BBB score: around 14 versus 12).	[50]
Cellulose, insulin	PCL	Polymer blending, surface functionalization	BMSC	Rocking	None reported.	Tendon	Rat	After 8 weeks, the MSC-loaded insulin-functionalized scaffold had significantly higher collagen compared to the MSC scaffold without insulin.	[53]
Chitosan, PRP	PVA	Polymer blending	AMSC	Direct seeding	Blending improved the proliferation and osteogenic differentiation of MSCs.	Bone	Rat	After 8 weeks, the MSC-loaded PRP-blended scaffold had a higher area of bone regeneration in the calvarial defect as seen on CT imaging (around 80% versus 60%) and histology (around 65% versus 40%) compared to the MSC-loaded non-PRP-blended scaffold.	[61]
CTGF, fibrinogen	PCL	Surface functionalization	Not specified	Direct seeding	None reported.	Muscle	Rat	After 53 weeks, the MSC-loaded functionalized scaffold exhibited sufficient support, biocompatibility, and no mesh-related complications in a rat model of abdominal wall defect.	[70]
dECM	PCL, PGMA	Surface functionalization	AMSC	Centrifugation	The functionalized scaffold supported the proliferation and chondrogenic differentiation of MSCs.	Bone	Rat	After 12 weeks, there was no significant difference between the MSC-loaded dECM-functionalized scaffold and the MSC-loaded non-functionalized scaffold in terms of osteochondral regeneration.	[80]
Macrophage cell membrane	PLGA	Surface functionalization	BMSC	Direct seeding	The functionalized scaffold had a 4 times higher cell viability than the control.	Skin	Mouse	After 15 days, the functionalized scaffold showed significantly higher wound closure (97.06%) than the non-functionalized scaffold (86.91%).	[82]
PRP	PCL	Surface functionalization	AFMSC	Direct seeding	The scaffold had a lower cell viability compared to the tissue culture plate.	Bone	Rat	After 8 weeks, all the cranial defects with the MSC-loaded functionalized scaffold had blood vessels and improved collagen deposition compared to the non-functionalized scaffold.	[81]
RGD, TGF- β 1	PCL	Surface functionalization	Not specified	Direct seeding	RGD improved cell adhesion and proliferation, while TGF- β 1 promoted hMSC differentiation into vascular smooth muscle cells.	Vascular tissue	Rat	After 1 week, the MSC-loaded peptide-functionalized grafts had significantly higher cell infiltration and ECM production. Compared to MSC-loaded RGD grafts, the MSC-loaded RGD/TGF- β 1 grafts had significantly thicker smooth muscle cell layers.	[48]

Abbreviations: AFMSC, amniotic fluid-derived mesenchymal stem/stromal cell; AMSC, adipose tissue-derived mesenchymal stem/stromal cell; BBB, Bresnahan, Beattie, and Basso; BDNF, brain-derived neurotrophic factor; BMSC, bone marrow-derived mesenchymal stem/stromal cell; CTGF, connective tissue growth factor; dECM, decellularized extracellular matrix; EMSC, endometrial tissue-derived mesenchymal stem/stromal cell; HA, hyaluronic acid; PCL, poly(ϵ -caprolactone); PGMA, poly(glycidyl methacrylate); PLA, poly(lactic acid); PLGA, poly(lactide-co-glycolic acid); PRP, platelet-rich plasma; PVA, poly(vinyl alcohol); RGD, arginine-glycine-aspartic acid; TGF- β 1, transforming growth factor beta 1.

-onstrated the ability of collagen to induce chondrogenesis of seeded MSCs *in vivo* and accelerate the restoration of full-thickness knee cartilage defects in rats. The MSC-loaded collagen-blended scaffold demonstrated excellent repair of the defects at 8 weeks (ICRS: 15.68) [49]. A rat model aimed at myocardial tissue regeneration in the setting of infarction showed a similar ability of MSC-seeded collagen-blended patches to induce appropriate cellular repair, promote angiogenesis, and ultimately improve cardiac function after 5 weeks [64]. For nerve repair, the addition of collagen to the PLCL/PANI scaffold significantly increased the metabolic activity and neuronal differentiation of the seeded AMSCs *in vitro*. Although there was no significant difference in the SFI and gastrocnemius mass ratio between the MSC-loaded and cell-free scaffolds, the MSC-loaded scaffold generated a significantly higher nerve density at 6 months [65]. Lastly, as previously mentioned, collagen has also been combined with polysaccharides, such as alginate [51] and hyaluronic acid [50], to optimize MSC delivery in the context of skin regeneration and spinal cord repair, respectively.

6.2 Gelatin

Gelatin is derived from the denaturation of collagen. Although gelatin does not possess the triple helical structure of collagen, certain functional attributes, such as biocompatibility and hydrogel-forming capability, are retained in gelatin. Recent investigations have demonstrated the applications of MSC-seeded gelatin-containing scaffolds for tissue regeneration. Common tissue types that have been regenerated utilizing MSCs in gelatin-containing scaffolds include musculoskeletal and integumentary tissues [51,67,69]. It has been shown that the addition of gelatin increases the scaffold's hydrophilicity [28,68]. It may also increase the scaffold's fiber diameter and pore size [68]. In the context of bone regeneration, a recent study reported MSC-seeded gelatin-functionalized scaffolds that facilitated higher levels of BMSC proliferation *in vitro* and superior bone regeneration *in vivo* when used to repair rat calvarial defects compared to MSC-seeded non-functionalized scaffolds [67]. Similarly, the repair of periodontal fenestration defects was also demonstrated in a rat model using an MSC-seeded gelatin-functionalized scaffold. The scaffolds generated greater collagen deposition and periostin expression in the periodontal defects compared to no treatment at 6 weeks [69]. Another study examined the potential of gelatin for pelvic muscle repair and found that the addition of gelatin can enhance the hydrophilicity of the scaffold and the proliferation of EMSCs on the scaffold. After 6 weeks, the MSC-loaded gelatin-blended scaffold had a significantly higher cell infiltration compared to the MSC-loaded but non-blended scaffold (Fig. 2, Ref. [68]). The gelatin-blended scaffold also showed high M2 macrophage and minimal M1 macrophage responses [68].

6.3 Laminin

Laminin is a glycoprotein found in the basal lamina, and it possesses binding sites for various ECM fibers and ground substance components. It plays an important role in supporting the adhesion, migration, growth, and differentiation of various cell types. Laminin has been blended with PLGA to produce an electrospun scaffold for the delivery of AMSCs in a rat model of sciatic nerve injury. Although the scaffolds had a significantly lower SFI and gastrocnemius mass ratio than the autograft at 12 weeks, they produced a comparable mean fiber diameter and myelin sheath thickness to those of the autograft [62]. Laminin has also been used to functionalize PLA scaffolds for the delivery of DPMSCs to full-thickness skin defects in mice. The delivery of MSCs stimulated the production of a complete epidermal layer in some of the animals. However, functionalization with laminin did not appear to enhance the efficacy of delivered MSCs [71].

6.4 Fibrinogen

Fibrinogen is the largest plasma protein, and it acts as an important component of the coagulation cascade. The cleavage of fibrinogen gives rise to fibrin monomers that rapidly polymerize to form an impermeable mesh, which binds red blood cells and platelets at the site of vascular injury [13]. It has been shown that the addition of fibrin increases the scaffold's hydrophilicity [27]. Fibrinogen has been used to functionalize the surface of PCL for the delivery of MSCs into abdominal wall defects in rats. It has been shown that the MSC-loaded functionalized scaffold can provide adequate support without major complications for up to 53 weeks [70]. Fibrin has also been used for the delivery of MSCs into the injured sciatic nerves of rats. However, the fibrin-containing scaffold showed the lowest SFI and gastrocnemius mass ratio of all the experimental groups, which suggests that the fibrin matrix could interact with critical regenerative factors and reduce the overall efficacy of MSC-loaded scaffolds [62]. Indeed, it has been shown that fibrinogen may promote cell adhesion at low densities, but it may also prevent cell adhesion at high loading densities [75], which could limit the ability of MSC to exert regenerative effects at the site of injury.

6.5 Peptides

Peptides are short sequences of amino acids that are easily synthesized or produced via recombinant protein expression, making them attractive agents for the fabrication of biomaterials. The RGD peptide was initially discovered as the essential structure recognized by cells in fibronectin, the most abundant glycoprotein in connective tissues [76]. Significant advancements have been achieved in comprehending the molecular interactions responsible for cell adhesion, and it is now established that this peptide serves as a cell recognition site for several proteins in the ECM and the bloodstream, such as collagen, fibrinogen, laminin,

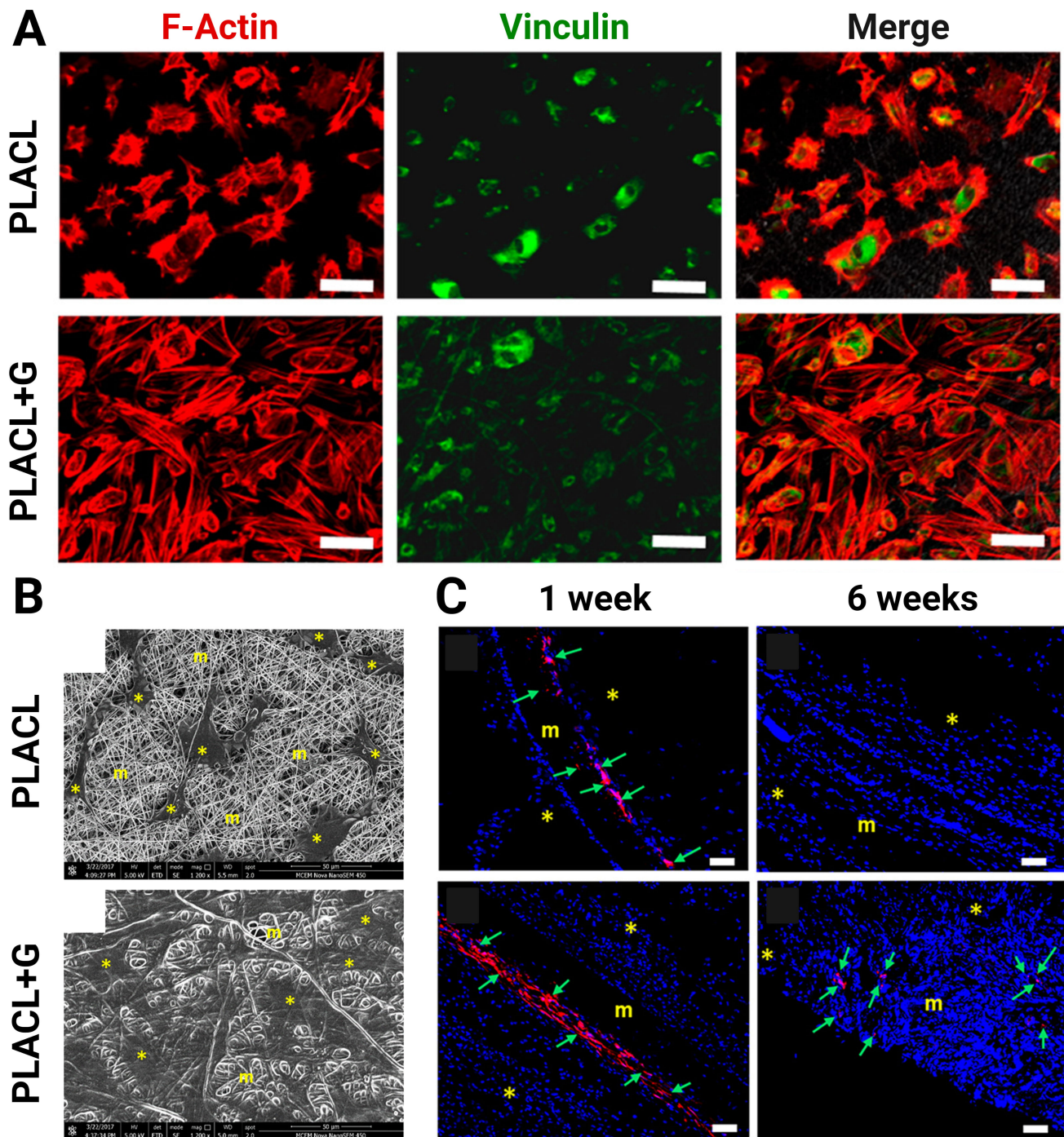


Fig. 2. Integration of electrospun mesh with gelatin. (A) The endometrial mesenchymal stem cells (EMSCs) on the gelatin-blended scaffold (PLACL+G) showed continuous spreading of filamentous F-actin and vinculin over the mesh surface (bar = 50 μ m). (B) PLACL+G allowed more growth and penetration into the scaffold as shown on scanning electron microscopy images compared to PLACL. (*, EMSC; m, mesh). (bar = 50 μ m). (C) PLACL+G also had higher *in vivo* retention of EMSCs than PLACL (*, host tissue; m, mesh; green arrow, EMSCs; bar = 100 μ m). Reproduced with permission from Mukherjee S *et al.* [68], Biomacromolecules; published by American Chemical Society, 2019.

osteopontin, thrombospondin, vitronectin, and von Willebrand factor. The RGD sequences in these proteins are recognized by integrins. RGD-functionalized scaffolds were recently utilized to reconstitute the mechanical and cellular profile required for vascular tissue reconstruction. The ad-

dition of RGD improved the adhesion and proliferation of seeded MSCs *in vitro* (Fig. 3, Ref. [48]). The MSC-loaded RGD-functionalized grafts had significantly higher cell infiltration and ECM production compared to the bare grafts 1 week after implantation in rats [48].

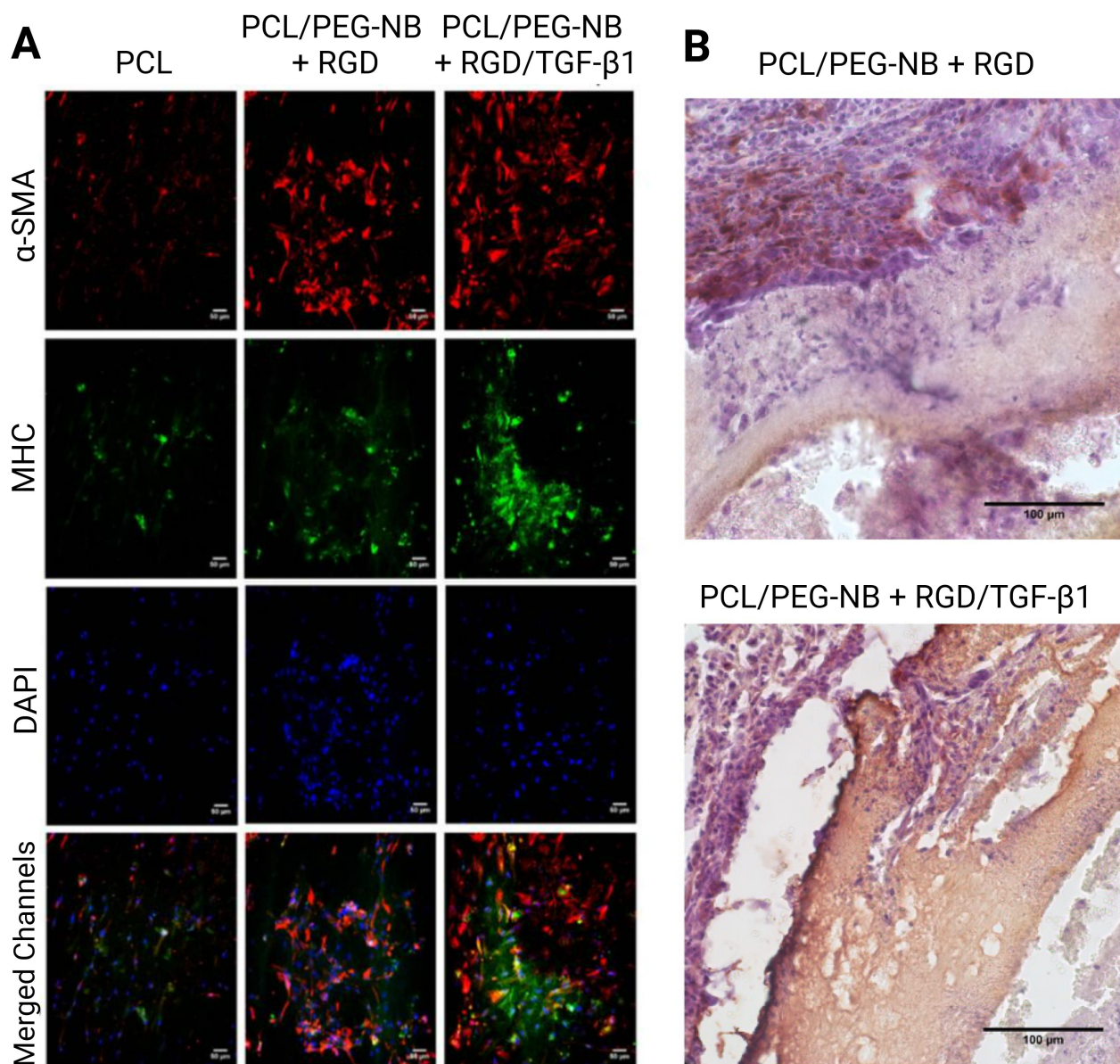


Fig. 3. Functionalization of electrospun vascular grafts with RGD and TGF- β 1. (A) The addition of RGD and TGF- β 1 enhanced the proliferation and differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) (α -SMA, alpha smooth muscle actin; MHC, myosin heavy chain; bar = 50 μ m). (B) The addition of TGF- β 1 led to a significantly thicker smooth muscle cell layer (brown layer, α -SMA; bar = 100 μ m). Reproduced with permission from Iglesias-Echevarria M *et al.* [48], ACS applied bio materials; published by American Chemical Society, 2021. RGD, arginine-glycine-aspartic acid; TGF- β 1, transforming growth factor-beta 1.

6.6 Growth Factors

Growth factors are biologically active molecules that can affect the growth and differentiation of cells. Many growth factors are proteins, and these types of growth factors typically bind with a high affinity to a specific plasma membrane-bound receptor to exert their effect [77]. Methods that utilize this class of molecules to functionalize scaffolds capitalize on the extensively researched impacts of these molecules on the maintenance of homeostasis in native tissues. Examples of growth factors that have been used to functionalize electrospun scaffolds to enhance MSC

delivery include BDNF [50,62], connective tissue growth factor (CTGF) [70,72], insulin, and transforming growth factor-beta 1 (TGF- β 1). BDNF is a growth factor that has been shown to contribute to the formation of neurites, axonal outgrowth, and prevention of apoptosis after nerve damage [78]. Hence, it has been used to functionalize synthetic scaffolds for the delivery of MSCs in the context of nervous tissue repair [50,62]. CTGF is a multifunctional protein that was originally found to promote fibroblast proliferation and ECM formation, but it has been shown to regulate the adhesion, proliferation, and differentiation of

other cells such as chondrocytes, epithelial cells, neuronal cells, and smooth muscle cells [79]. It has been recently utilized to functionalize synthetic scaffolds for the delivery of MSCs for the repair of abdominal wall and pelvic floor defects [70,72]. Insulin, the pancreatic hormone that is responsible for regulating blood glucose levels, has been shown to induce the proliferation of various cell types, including MSCs. Hence, it has been used to functionalize synthetic scaffolds for the delivery of BMSCs into rats with Achilles tendon injuries [53]. TGF- β 1 is a growth factor that promotes the proliferation of myofibroblasts and is one of the most potent fibrogenic cytokines. This property was recently used to functionalize MSC-loaded electrospun grafts for vascular tissue repair (Fig. 3). The addition of TGF- β 1 led to significantly thicker smooth muscle cell layers [48].

7. Functionalization with Composites

Biological polymers can be combined to form artificial composites with improved physicochemical properties and higher biocompatibility. Artificial composites can be formed by combining polymers from similar (e.g., protein-protein) or different classes (e.g., polysaccharide-protein). Examples of protein combinations that have been used to functionalize electrospun scaffolds for the delivery of MSCs include the following: CTGF and fibrinogen [70]; RGD and TGF- β 1 [48]. Examples of polysaccharide-protein combinations that have been used to functionalize electrospun scaffolds for the delivery of MSCs include the following: alginate, collagen, and gelatin [51]; BDNF, chitosan, laminin, and fibrin [62]; BDNF, collagen, and HA [50]; cellulose and insulin [53] (Table 2, Ref. [48,50–53,61,62,70,80–82]). Natural composites, which innately contain a variety of polymers and bioactive molecules, can also be used to functionalize synthetic scaffolds. Examples of natural composites include decellularized ECM (dECM) and PRP. One of the advantages of dECM is that it may maintain the 3D architecture of the original tissue. It may also retain several cell growth factors, which can enhance the proliferation or differentiation of seeded cells [83]. It has been used to functionalize synthetic electrospun scaffolds for the delivery of BMSCs in osteochondral defects in mice. However, no significant difference was observed between the dECM-functionalized and the non-functionalized scaffolds in terms of *in vivo* osteochondral regeneration [80]. PRP is produced via centrifugation of citrated blood and is valued for its abundance of various growth factors and cytokines. It has been used to functionalize scaffolds for the delivery of MSCs in the context of bone regeneration. A study involving rat calvarial defects showed that PRP functionalization can increase vascularization and collagen deposition after 8 weeks [81]. Another study on rat calvarial defects demonstrated that the addition of PRP can enhance bone regeneration without significantly affecting fiber morphology [61].

8. Future Directions

Moving forward, future investigations in the field of scaffold-based MSC delivery should focus on addressing crucial issues regarding the scaffold materials, MSCs, and clinical translation (Fig. 4).

Firstly, the combination of different scaffold materials should be optimized to address the challenges of accelerated degradation, mechanical limitations, and physicochemical incompatibility. Although recent studies have shown that the addition of biological polymers does not affect the initial mechanical properties of electrospun scaffolds, the rapid *in vivo* degradation of biological polymers can compromise both scaffold stability and the sustained delivery of MSCs and secreted factors. Hence, optimization studies must be performed to balance *in vivo* degradation and mechanical stability. Furthermore, the hydrophilic nature of most biological polymers makes blending with more mechanically stable hydrophobic polymers challenging. To overcome this, novel materials and innovative fabrication techniques, such as 3D printing, microfluidics, and click chemistry, should be explored to enable the integration of polymers with diverse physicochemical properties and the creation of more complex and functional scaffolds.

Secondly, further research is needed to address our limited understanding of stem cell niches, cell-scaffold interactions, and variability among MSC types. A fundamental step is to investigate the specific matrix composition and architecture that best preserves the beneficial properties of different MSC types. Mechanistic studies should also be conducted to delve more deeply into the complex interactions between various MSC types and polymers. The results of these studies can guide scaffold design for enhancing material biocompatibility and cell-to-matrix interactions, providing a more conducive microenvironment for MSC growth and release of paracrine signals. Additionally, integrating emerging stem cell technologies, such as priming and genetic modification, can help prevent premature cell death and further enhance therapeutic outcomes. Finally, to standardize therapeutic outcomes, comprehensive molecular profiling of various MSC types should be performed to identify key markers for selecting optimal cell types for specific applications.

Thirdly, several critical issues must be tackled to facilitate the clinical translation of MSC-loaded electrospun scaffolds. Although electrospinning in itself has been shown to be scalable, with several products incorporating electrospun fibers advancing into human use or testing, some blending and functionalization methods remain challenging to scale for mass production. Establishing collaborations between academia and industry can help overcome this hurdle, facilitating the translation of promising technologies into commercially viable products. In addition, preclinical studies in large animal models are crucial for assessing safety and efficacy in human trials. Therefore, immediate actions must be taken to initiate large-scale ani-

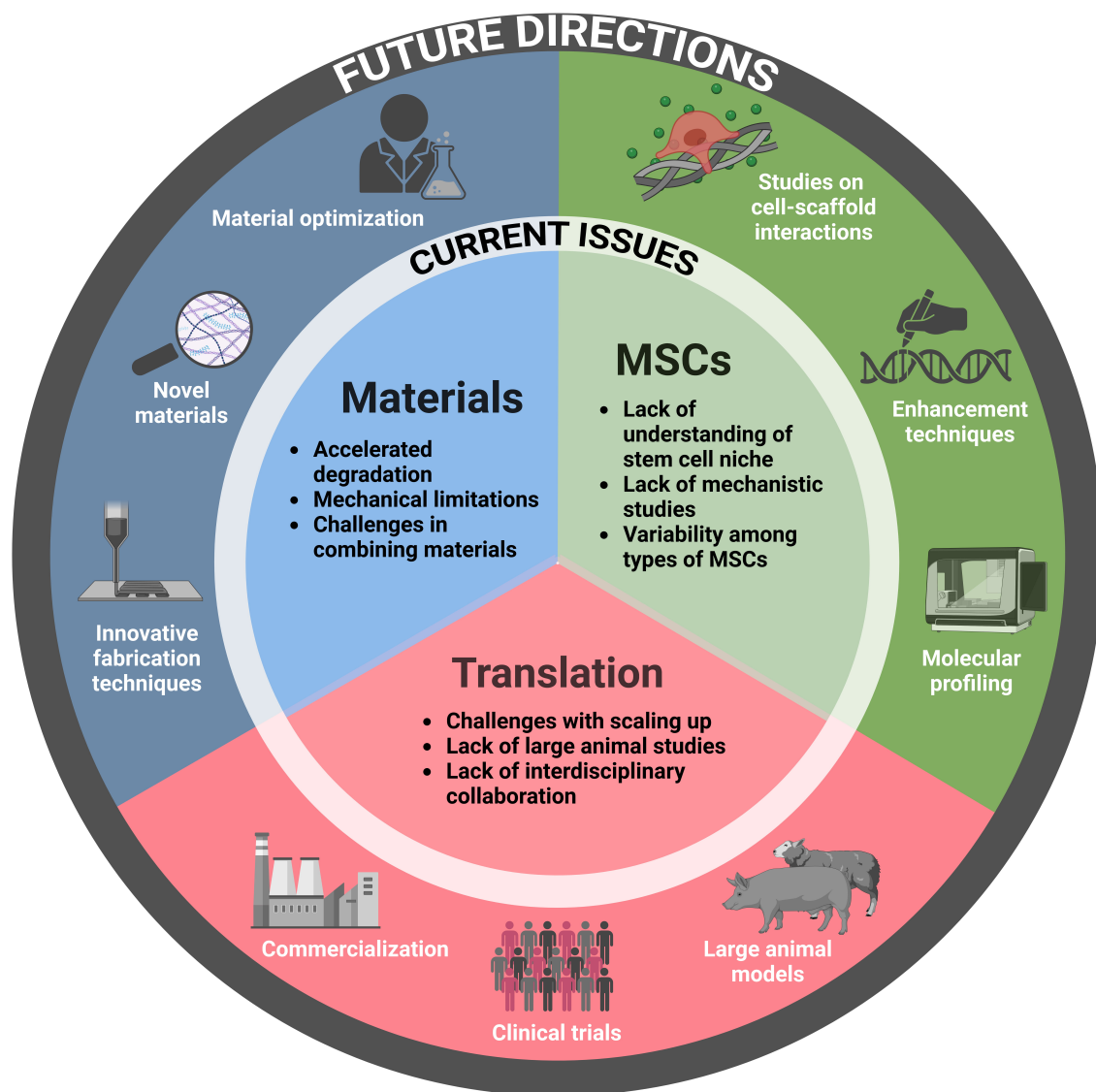


Fig. 4. Current issues in the integration of electrospun scaffolds and biological polymers and the future research directions to improve its utility for MSC therapies. The figure has been created using <https://www.biorender.com/>.

mal investigations. Nonetheless, rigorous clinical trials are needed to evaluate the safety and efficacy of MSC-based therapies, and successful conduct of human studies requires close collaboration among materials scientists, stem cell biologists, clinicians, and regulatory experts.

By addressing these challenges and leveraging interdisciplinary expertise, scaffold-based MSC delivery holds great promise for revolutionizing regenerative medicine and improving patient outcomes.

9. Conclusion

In conclusion, the integration of electrospun scaffolds and biological polymers represents a promising strategy for enhancing the delivery and efficacy of MSC therapies. By leveraging the advantages of both synthetic and natural polymers, researchers have developed techniques to optimize scaffold properties, thereby creating a conducive mi-

croenvironment for MSC adhesion, proliferation, and function. Through polymer blending, surface functionalization, and the development of composite materials, scaffold-based delivery systems offer an ability to control the presentation of bioactive cues to MSCs, enhancing cell-scaffold interactions and therapeutic outcomes. Moreover, investigations on the molecular interactions between MSCs and integrated scaffolds have provided valuable mechanistic insights, guiding the design of next-generation scaffolds. Future endeavors should focus on integrating emerging technologies in biofabrication and stem cell therapy to further elevate the potential of MSC therapies in regenerative medicine.

Author Contributions

Conceptualization, AJRB, AM, DKMB, BMM, and MPM; data curation, AJRB, AM, DKMB, and BMM;

writing—original draft preparation, AJRB, AM, DKMB, and BMM; writing—review and editing, AJRB, AM, DKMB, BMM, and MPM; supervision, MPM; funding acquisition, MPM; All authors have read and agreed to the published version of the manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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