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Harnessing Dental Stem Cell Immunoregulation Using Cell-Laden Biomaterials

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Abstract

Successful tissue engineering therapies rely on the appropriate selection of the cell source, biomaterial, and regulatory factors. To be applied in a wide range of clinical applications, the ideal cell source needs to be easily accessible and abundant. Human orofacial tissues and teeth harbor several populations of mesenchymal stem cells (MSCs) with self-renewal and multilineage differentiation capabilities. The ease of access, relative abundance, and minimally invasive isolation procedures needed to harvest most types of the dental-derived MSCs render them a promising cell source for tissue engineering applications. A growing body of evidence has reported the profound immunoregulatory potential of dental-derived MSCs as compared with their bone marrow counterparts. Biomaterials can act as a physical barrier protecting the MSCs from the invasion of the immune system by hindering penetration of proinflammatory cells/cytokines, leading to higher viability of the encapsulated MSCs and improved tissue regeneration. Besides their protective capabilities, biomaterials can actively contribute to the immunoregulatory potential of the MSCs through their physical and chemical properties, including porosity and elasticity. However, despite recent advancement, the therapeutic capability of biomaterials to regulate the MSC–host immune system crosstalk and the mechanism underlying this immunoregulation has been poorly understood. It has been reported that biomaterials can regulate the viability and determine the fate of the encapsulated MSCs through modulation of the NF-κB pathway and the caspase-3 and caspase-8 proapoptotic cascades. Additionally, the physiomechanical properties of the encapsulating biomaterial have been shown to modulate clustering of TNF-α receptors on the encapsulated MSCs while regulating the production of anti-inflammatory factors such as indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE_2) through activation of the P38 MAPK pathway. In the current review, we sought to provide a thorough overview of the immunomodulatory functions of dental-derived MSCs and the role of biomaterials in their interplay with the host immune system.

Keywords: dental-derived mesenchymal cells, immunoregulation, tissue engineering, immune cell-stem cell crosstalk, biomedical materials, physiomechanical properties

Introduction

Mesenchymal stem cells (MSCs) are an attractive source of multipotent progenitor cells with low immunogenicity that can affect the proliferation and maturation of several major immune cells. Although clinical applications of bone marrow–derived MSCs (BMMSCs) have shown promising outcomes, difficulties in finding a donor-recipient match and the invasive surgical procedures for harvesting stem cells necessitate an easily accessible and patient-specific source of stem cells to overcome the existing dilemmas in regenerative medicine.

Another major issue limiting the clinical application of BMMSCs is that <0.01% of the cells residing in the bone marrow are the BMMSCs (Zhao and Liu 2016). Additionally, they start losing proliferation and multilineage differentiation capacity after the first passage as compared with the fresh cells (Banfi et al. 2000). The search for cells with characteristics comparable to BMMSCs led to the discovery of numerous types of MSCs harbored by mammalian teeth with self-renewal, colony formation ability, and potent immunoregulatory potential (Huang et al. 2009). The dental pulp is a valuable source for various MSCs, such as dental pulp stem cells and stem cells from human exfoliated deciduous teeth (SHED; Yamada et al. 2011). Regardless of whether they are harvested from third molars, incisors, or exfoliated deciduous teeth, the dental pulp–derived MSCs have multilineage differentiation

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potential and the capability to form various structures, including mineralized tissue and dentin.

Stem cells from apical papilla are highly proliferative multipotent stem cells and are easily obtainable from the soft tissue loosely attached to the apices of immature permanent teeth (Sonoyama et al. 2008). Periodontal ligament stem cells (PDLSCs) represent another population of multipotent cells with similar phenotypes to BMMSCs but superior growth potential (Moshaverinia et al. 2014). However, isolation of PDLSCs can be challenging due to the invasive nature of extraction by surgical interventions.

Dental follicle stem cells reside in the connective tissue loosely surrounding the developing tissue, and similar to the PDLSCs, their isolation necessitates tooth extraction (Bai et al. 2011). Gingival MSCs are easily accessible from healthy or inflamed gingiva and are readily found in discarded dental tissue samples (Ge et al. 2012). These neural crest–derived cells are capable of forming various tissues, such as bone and sensory components of the nervous system (Yamada et al. 2011; Pouraghaei et al. 2020).

Most dental/oral MSCs are easily accessible with less invasive procedures to provide enough cells at clinical scales. Additionally, they have shown superior growth profiles as compared with BMMSCs (Moshaverinia et al. 2014). These findings have resulted in the initiation of numerous clinical trials to examine the functionality of dental/oral MSCs for craniofacial repair or immune diseases (D'Aquino et al. 2009; Paz et al. 2018; Yamada et al. 2019).

Dental/oral MSCs exert profound immunoregulatory effects mainly through paracrine factors secreted upon activation by the inflammatory environment as well as cell-cell contact-dependent pathways. However, despite all the promising studies published to date, clinical applications of MSCs remain a major concern due to the various environmental stresses that cells encounter during transplantation and to the challenges mediated by inflammatory responses. Less than 1% of the transplanted stem cells can survive and engraft (Pittenger et al. 2019). Furthermore, the in vitro culture condition is different than the in vivo microenvironment in many ways, including the cell-matrix and cell-cell interaction.

Additionally, the ex vivo cultured MSCs lose their matrix and the necessary cell-cell interaction during transplantation, which will result in their poor engraftment and rapid clearance. Therefore, providing a stem cell–friendly biomimetic niche-like structure will enhance their ex vivo behavior and in vivo function. In this context, tissue engineering approaches composed of appropriate selection of scaffolding material, stem cells, and growth factors have recently witnessed enormous advancement in granting a safe environment for the MSCs to promote successful craniofacial tissue regeneration.

The cell delivery vehicle has a pivotal role in the functionality of stem cells in vivo. Biomaterials have been widely studied as an artificial niche mimicking the physiochemical properties of the extracellular microenvironment. Naturally, cells encounter a wide range of physicochemical signaling from the microenvironment surrounding them; however, how the cells respond to the physical and chemical properties of the artificial environment encapsulating them and how this cell-material encounter contributes to the immunoregulatory properties of the stem cells is largely unknown (Moshaverinia et al. 2015).

In this review article, we sought to provide an overview of the state of the art on emerging dental MSC-mediated immunoregulation approaches, the role of biomaterials in this immunoregulation, and the underlying mechanisms to pave the way for prospective clinical applications.

Immunomodulation by MSCs

The host immune system is known to affect MSC-mediated tissue regeneration. Contrary to the immune system invasion, MSC-mediated immunoregulatory pathways contribute to the maintenance of immune homeostasis by hindering excessive activation of inflammatory cells (innate and adaptive) and providing a tolerogenic environment during tissue regeneration (Prockop and Youn Oh 2012). It has been shown that the immunoregulatory potential of dental-derived MSCs is comparable to BMMSCs and is achieved by either binding to activated immune cells through cell-cell contact mechanisms or secreting soluble factors such as anti-inflammatory cytokines (Huang et al. 2009; Andrukhov et al. 2019).

Soluble factor–mediated mechanisms mainly include recruiting immune cells through secretion of different chemokines and then suppressing them via anti-inflammatory cytokines such as IL-10, transforming growth factor β (TGF-β), indoleamine 2,3- dioxygenase (IDO), nitric oxide, TNF-stimulated gene 6 (TSG-6), vascular endothelial growth factor, and prostaglandin E2 (PGE_2; Andrukhov et al. 2019).

PGE_2 is one of the most potent immunoregulatory elements produced by MSCs upon stimulation by inflammatory cytokines such as IFN-γ and tumor necrosis factor α (TNF-α). It can downregulate proliferation, differentiation, and maturation of immune cells (English et al. 2007). Cyclooxygenase 2 (COX-2) is a rate-limiting enzyme that controls the synthesis of PGE_2 in response to different physiologic conditions such as inflammation.

TGF-β is an important part of the immunoregulation exerted by MSCs and has potent antiproliferative effects on proinflammatory T cells by inhibiting the production of IL-2 and upregulation of cell cycle inhibitors in T cells. TGF-β has a substantial role in the development of regulatory T cells (Tregs) and inducing forkhead transcription factor 3 (Foxp3) expression at the mRNA and protein levels (Fantini et al. 2004). Therefore, an alternative approach for generating $CD4^+CD25^+$Tregs from nonregulatory $CD4^+CD25^-$T cells is to stimulate them with soluble factors secreted from MSCs, such as IL-10, TGF-β, and PGE_2, ex vivo (Rossetti et al. 2015). These de novo–generated Tregs share the characteristics of $CD4^+CD25^+$ Tregs, including expression of Foxp3, which is required for the development and function of Tregs and suppression of the proinflammatory T cells through direct cell-cell contact, secretion of IL-10 and TGF-β, or local competition for IL-2, depriving the necessary IL-2 for activation and maturation of the naïve T cells.

Besides the suppression of proinflammatory T cells, several studies have reported the capability of MSCs to inhibit the

activity and proliferation of natural killer cells, inhibit maturation of dendritic cells and impair T-cell priming by dendritic cells, and suppress the activity of proinflammatory macrophages (M1) while promoting polarization of the anti-inflammatory macrophage phenotype (M2; Chiesa et al. 2011; Song et al. 2020).

MSCs exert their paracrine functions, at least in part, through extracellular vehicles (EVs). EVs bear cargos of miRNA, mRNA, and proteins developed from the endosomal compartment of the cells or the plasma membrane, mirroring various functions of their parent cells, including intercellular communication and immune regulation (Rani et al. 2015). EVs carry advantages over their cells of origin, such as easier long-term storage capability, fewer safety concerns in terms of the lower probability of triggering innate and adaptive immune responses, and lower risk of tumorigenicity (Rani et al. 2015).

The FAS/FASL pathway is a well-known cell-cell contact pathway utilized by activated T cells to induce apoptosis in MSCs, thus negatively affecting MSC-mediated tissue regeneration (Akiyama et al. 2012). Interestingly, MSCs express Fas ligand (FASL) while activated T cells express higher levels of FAS. Consequently, the administered MSCs can utilize receptor FAS to control MCP-1 secretion for recruiting activated T cells, followed by inducing apoptosis through the ligand FASL. The apoptotic T cells can induce TGF-β production from macrophages, which will result in the production of Tregs and ameliorate the immune response.

Besides FASL, programmed death ligand 1 (PD-L1) is a transmembrane protein expressed by MSCs that can bind to the programmed death 1 (PD-1) receptor on lymphocytes and induce immunosuppression by suppressing inflammatory lymphocytes or inducing tolerogenic phenotypes (Augello et al. 2005).

Dental pulp stem cells are reported to suppress T cell–mediated immune responses by triggering FAS/FASL and PD-L1/PD-1 apoptotic pathways (Demircan et al. 2011; Zhao et al. 2012; De la Rosa-Ruiz et al. 2019).

EVs are known to play important roles in dental MSC-mediated immunoregulation by downregulating the secretion of proinflammatory cytokines and inducing regulatory phenotypes. For example, EVs liberated from SHED have positively contributed to recovery from brain trauma by reducing neuroinflammation and inducing M1-to-M2 polarization (Li et al. 2017).

Similarly, EVs derived from PDLSCs have been shown to alleviate inflammation in chronic periodontitis by altering the Th17/Treg balance through overexpression of microRNA-155-5p (Zheng et al. 2019). This overexpression has been shown to upregulate Tregs while downregulating Th17, resulting in an overall decrease in the Th17/Treg balance and alleviation of inflammation. Gingival MSC–derived EVs have shown anti-inflammatory potential through the production of a significant amount of interleukin 1 receptor antagonist (IL-1RA), which acts as an antagonist against the proinflammatory cytokine IL-1β and can downregulate TNF-α to mediate inflammation (Kou et al. 2018). These findings show that different physiologic and pathologic conditions are the determinants of the pathways recruited by MSCs to regulate immune responses.

Role of Biomaterial in MSC-Mediated Immunoregulation

The host immune system activation upon introduction of foreign material is inevitable. Immune response challenges, on one hand, and the importance of MSC immunoregulatory signals, on the other, highlight the need for a protective structure to ensure that MSCs survive after implantation and have enough time for adaption and activation to accomplish their immunoregulatory mission. However, the regulatory potential of the biomaterials to modulate the MSC–immune cell crosstalk is unknown.

Tissue engineering approaches have emerged as a promising means to increase the efficiency of cell transplantation by harnessing the immunoregulatory potential of MSCs through designing protective structures. An ideal biomaterial must serve as a safe carrier to reduce shear stress during transplantation, avoid nutrient and oxygen deprivation, protect cells against immune cell attack, and allow enough time for adaption and activation (Hached et al. 2017).

The physical and chemical properties of a biomaterial—such as the composition, type, and degree of cross-linking, as well as the surface topography, porosity, and elasticity—are known to play important roles in paracrine functions of MSCs and in the regulation of a wide range of the immune cells, including the macrophages (Follin et al. 2015; Gonzalez-Pujana et al. 2020). Therefore, utilizing 3-dimensional (3D) systems provides us with a more reliable and realistic platform on which to perform stem cell studies in vitro, yielding better results in stem cell therapies in vivo.

Biomaterials as a Physical Barrier against the Inflammatory Microenvironment

Encapsulation of MSCs within an appropriately designed biomaterial could offer a desirable solution by acting as a physical barrier hindering penetration of proinflammatory cells while supporting their crosstalk through soluble factors, leading to higher viability of the encapsulated MSCs and improved tissue regeneration (Moshaverinia et al. 2015). It is noteworthy to consider that the MSCs are not constitutively regulatory; they need to be licensed by the inflammatory milieu to exert maximum suppressive effects (Lin et al. 2017).

Hydrogels can provide a safe and suitable environment for the encapsulated MSCs to be licensed to alleviate inflammation by secreting anti-inflammatory cytokines, inhibiting proliferation and activation of cytotoxic immune cells, and, more important, polarizing the inflammatory immune cells toward anti-inflammatory or regulatory phenotypes (Swartzlander et al. 2015; Hached et al. 2017).

Although encapsulating MSCs within hydrogels can prevent the penetration of inflammatory immune cells, hydrogels cannot effectively hinder the diffusion of small proinflammatory cytokines due to their highly porous structure. Increasing the degree of crosslinking might seem to be a solution to prevent the diffusion of cytotoxic molecules; however, increasing the crosslinking density might be detrimental for the encapsulated

cells due to impaired gas and nutrient exchange. TNF-α–antagonizing hydrogel has been developed by modifying PEG with a peptide mimicking the TNF-α recognition loop on TNF receptor 1 (TNFR1) that can bind to diffused TNF-α and inhibit binding to its receptor on the surface of the encapsulated cells (Lin et al. 2009).

Role of Surface Topography in MSC–Immune System Crosstalk

Besides the protective function of biomaterials, their surface topography is among the most important features; therefore, having a role in immunoregulation is not far from expectation. Fibrous scaffolds composed of mesh-like, random, or aligned fibers have been shown to induce secretion of anti-inflammatory cytokines from adipose-derived MSCs (ADMSCs) at significantly higher levels as compared with their secretion on tissue culture plates. Secretion of PGE_2 and TGF-β was found to be significantly higher in MSCs cultured on a fibrous scaffold with aligned fiber orientation as compared with 2 other orientations, but the secretion of IL-10 from macrophages was reported to be significantly higher and the secretion of TNF-α, significantly lower, in the group cultured with conditioned media obtained from the MSCs cultured on the scaffold (Su et al. 2017).

Role of 3D Microstructure in Immunoregulation

The 3D microstructure of a biomaterial is known as an important regulator of cellular behavior. The majority of studies have found 2-dimensional culture systems to be irrelevant or even misleading, since MSCs naturally reside in a 3D microenvironment where they can undergo cell-cell and cell-matrix interactions. The 3D culturing of MSCs in the form of a spheroid or within a biomaterial can manipulate the expression of surface markers on MSCs such as class I and II major histocompatibility complex, promote their sensitivity to the microenvironment, and increase paracrine secretion of immunoregulatory factors such as PGE_2, IDO, and TGF-β (Follin et al. 2016; Yang et al. 2017).

The porosity of the biomaterial is another important factor that regulates the stimulation of the encapsulated MSCs while inhibiting the infiltration of inflammatory cells (Fig. 1A). Hydrogels with microporous structure (80 to 120 µm) can passively promote cell-cell interactions, which increases paracrine secretion by the encapsulated cells and promotes their responsiveness to growth factors such as insulin-like growth factor 1 (IGF-1), while nanoporous hydrogels (5 to 20 nm) do not evoke similar responses (Qazi et al. 2020). Besides the pore size, the interconnectivity of the pores can significantly affect the cell behaviors since a biomaterial with a highly interconnected porous structure has a larger surface area for cell adhesion and gas and nutrient exchange, thus affecting the paracrine activity of MSCs (Dellacherie et al. 2019). However, the influence of

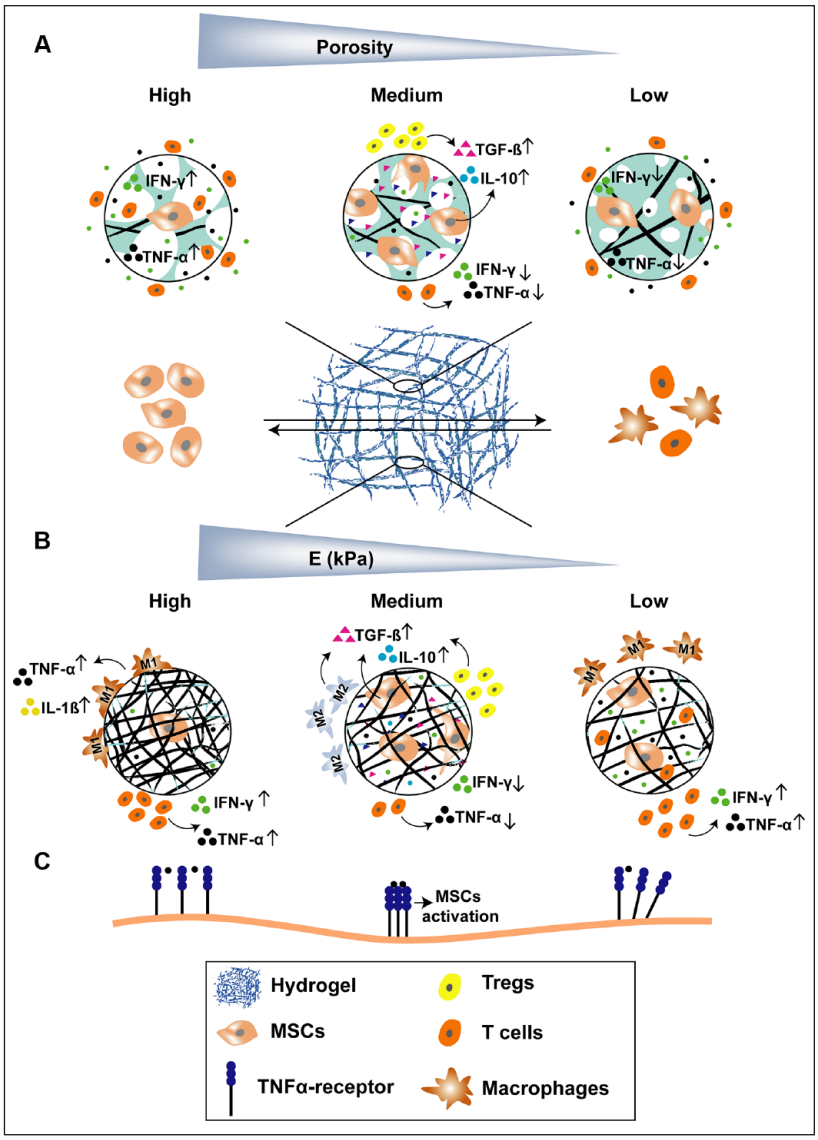

Figure 1. Role of biomaterials in mesenchymal stem cell (MSC)–mediated immunoregulation. (**A**) The porosity of the biomaterial can regulate the infiltration of inflammatory T cells and/or cytokines. The optimized pore size can hinder the infiltration of inflammatory T cells but allow slower diffusion of proinflammatory cytokines such as IFN-γ and TNF-α that are necessary for activation of MSCs to ameliorate inflammation by secretion of anti-inflammatory factors such as TGF-β and IL-10 and induction of Tregs formation. (**B**) The elasticity of the biomaterial regulates T-cell and proinflammatory cytokine infiltration, macrophage spreading and polarization, and activation of the encapsulated MSCs to downregulate the activity of the inflammatory cells and induce regulatory phenotypes such as Tregs and M2 macrophages. (**C**) Biomaterial elasticity can regulate clustering of the TNF-α receptors on the MSC surface.

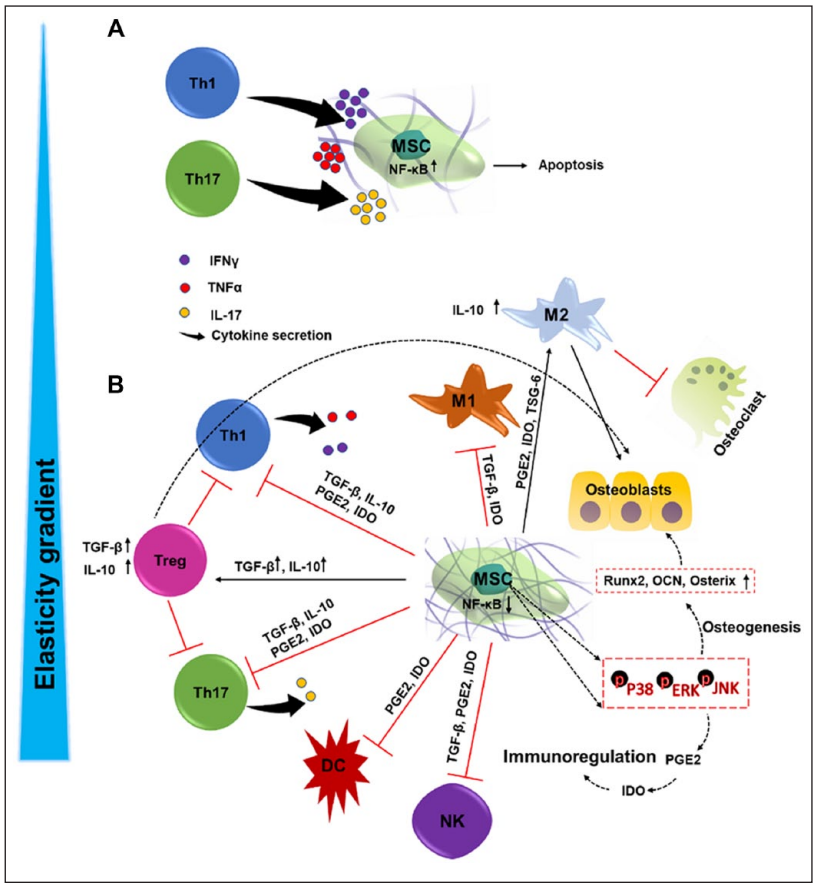

Figure 2. Molecular mechanisms involved in the biomaterial-assisted immunoregulation by mesenchymal stem cells (MSCs) and bone regeneration.

pore interconnectivity in the MSC-mediated immunoregulation has not been clearly understood.

The role of the encapsulating material on MSC–immune cell crosstalk has been studied by encapsulating MSCs in nanoporous hydrogels (e.g., hydrogels) in comparison with microporous scaffolds such as absorbable collagen sponge (Moshaverinia et al. 2015). Higher penetration of the proinflammatory T cells and cytotoxic cytokines have been shown in microporous scaffolds, which can induce apoptosis in the MSCs through caspase-3 and caspase-8 proapoptotic cascades, confirming the protective capability of the encapsulating material and its physiomechanical characteristics rather than its chemistry.

Role of Biomaterial Elasticity in Immunoregulation

It has been reported that the stiffness of a cell-laden hydrogel can affect the extent of associated macrophage activation, as it spreads more readily on a stiff substrate with better localized and denser F-actin and higher expression of proinflammatory cytokines including TNF-α and IL-1β in vitro; less macrophage accumulation can be seen on the surface of a soft hydrogel in vivo (Fig. 1B; Blakney et al. 2012).

It has been shown that matrix elasticity regulates the sensitivity of the MSCs to the inflammation in a manner that is dependent on actin polymerization and lipid rafts (Wong et al. 2020). Softer matrices support the clustering of TNF-α receptors on the MSC surface by facilitating actin polymerization, which can induce the production of monocyte-regulating chemokines such as CCL2 and CCL7 as well as anti-inflammatory factors such as TSG-6 in higher amounts as compared with a stiffer matrix in response to TNF-α stimulation (Fig. 1C).

These findings point to an active role of the biomaterial microstructure and elasticity in inducing secretion of anti-inflammatory factors such as TGF-β, IDO, and PGE_2 from encapsulated MSCs to downregulate the activity of inflammatory cells and the formation of regulatory phenotypes. The elasticity of the substrate can also regulate the fate of surrounding T cells and modulate their key functions, including migration, differentiation, and cytokine secretion, by triggering mechanosensory mechanisms (Saitakis et al. 2017). Therefore, elasticity can be tailored to induce Treg formation from naïve T cells. Treg induction from conventional naïve mouse $CD4^+$ T cells was observed at significantly higher levels on a substrate with relatively low stiffness (100 kPa) versus a stiffer one (3 MPA; Nataraj et al. 2018).

We recently demonstrated that higher elasticity and lower porosity of the hydrogel biomaterial produced significantly better bone regeneration in wild-type mice as well as immunocompromised mice supplemented with pan T cells. This was achieved by hindering the penetration of proinflammatory cells/cytokines, resulting in lower apoptosis by downregulation of NF-κB p65 expression in encapsulated SHED (Ansari et al. 2017).

Molecular Mechanisms Involved in Biomaterial-Assisted Immunoregulation

Studies have shown that environmental signaling from mechanical forces, biological stimuli, and physical material properties can affect various cellular behaviors, including spreading, assembly of focal adhesions and actin stress fiber formation, and differentiation (Bissell et al. 1982; Pouraghaei et al. 2020). It has been shown that matrix stiffness controls the immunoregulatory potential of MSCs by changing the cell morphology and cytoskeletal polymerization in a way that decreasing the matrix stiffness enhances the expression of immunosuppressive genes such as COX-2, TSG-6, and IDO (Ji et al. 2019).

In contrast, it has been shown that matrix stiffness not only affects the immunoregulatory potential of MSCs encapsulated within an alginate hydrogel but also regulates their response to

cytokine stimulation through a stiffness-dependent NF-κB pathway by differential expression of IDO1, OPN, and COX-2 by the encapsulated MSCs in response to cytokine stimulation showing a significant upregulation by increasing the matrix stiffness (Darnell et al. 2018). This controversy in the 2 reports mentioned earlier can stem from the different range of the studied stiffness and the material selection.

Diaz et al. (2017) reported that vascular wall shear stress forces applied to MSCs can trigger the NF-κB-COX2-PGE_2 signaling pathway to suppress the secretion of TNF-α from the activated immune cells. In addition to suppression of TNF-α, the localized secretion of PGE_2 from MSCs is known to downregulate the activity of T and natural killer cells and induce polarization of M1-to-M2 macrophages (Németh et al. 2009).

The upregulation of COX-2 results in increased expression of PGE_2 and strengthens the immunoregulatory potential of MSCs, while inhibition of COX-2 diminishes the PGE_2-mediated immunosuppression (Li et al. 2015). Mitogen-activated protein kinases (MAPKs) convert extracellular stimuli such as growth factors, cytokines, and matrix elasticity into a variety of cellular responses. P38 MAPK is an intracellular signaling pathway that plays an important role in MSC immunoregulation through COX2/PGE_2 synthesis and can be activated by a trigger such as matrix elasticity (Meyer-ter-Vehn et al. 2011). The COX2 mRNA expression is regulated posttranscriptionally by phosphorylation of P38 MAPK. Interestingly, the interplay among mechanical stress, COX/E-prostaglandin receptor (EP) expression, and the actin cytoskeleton has been identified to act in such a way that increasing mechanical stress results in an increase in expression of COX2/EP4, followed by an increase in production of PGE_2 (Martineau et al. 2004). It seems that matrix elasticity upregulates the activity of P38 MAPK, which in turn upregulates the COX2/PGE_2 pathway. Additionally, IL-17A enhances the Treg induction ability of MSCs through the COX2/PGE_2 pathway since PGE_2 can bind to EP4 on $CD4^+$ T cells and inhibit differentiation of Th17, altering the balance between Th17 and Treg cells (Bai et al. 2018).

PGE_2 triggers protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) signaling, which in turn leads to IDO transcription (Hennequart et al. 2017). Based on the fact that PGE_2 production results from the constitutive expression of COX2 triggered by MAPK signaling, the mechanical properties of the encapsulating material seem to affect the expression of IDO in MSCs.

In addition to the role of MAPKs in immunoregulation, 3 members of the MAPK superfamily—namely, Jun amino-terminal kinases (JNKs), P38, and extracellular signal–related kinases (ERKs)—are involved in MSC-mediated osteogenesis through the upregulation of osteogenic markers such as RUNX2, OCN, and osterix (Greenblatt et al. 2013). Figure 2 summarizes the molecular mechanism underlying the MSC-mediated immunoregulation involved in bone regeneration and the role of biomaterial elasticity in this context.

In the natural tissue microenvironment, cells reside in a mechanically dynamic extracellular matrix, which plays an important role in governing cellular behavior, including paracrine secretion. It has been reported that an artificial extracellular matrix with dynamic stiffness can promote the paracrine secretion of the encapsulated MSCs better than a static system through activation of Yes-associated protein (YAP) resulting from the polymerization of F-actin (Lin et al. 2020).

Surface topography is another physical cue triggering the immunoregulatory function of MSCs. It has been shown that ADMSCs cultured on aligned fibrous scaffolds produce elevated levels of immunoregulatory factors as compared with ADMSCs cultured on a scaffold with randomly oriented fibers. This effect operates through focal adhesion kinase (FAK)–dependent mechanisms as it is directly linked to COX-2 transcriptional regulation. The elevated immunoregulation observed with the aligned fibrous scaffold is associated with the YAP/TAZ signaling pathway, as evidenced by higher expression levels of COX-2, TSG-6, and IL-1ra mRNA (Wan et al. 2018). Elevated levels of phosphorylated FAK have been observed in PDLSCs under compressive stimulation, followed by higher PGE_2 production (Kang et al. 2010).

T cells are mechanosensitive cells with the ability to discriminate a variety of stiffness levels and respond accordingly. The molecular mechanism underlying this mechanosensing can be induced, in part, through TCR triggering (Saitakis et al. 2017). TCR/CD3-induced cytokine secretion from effector and memory $CD4^+$ T cells can be enhanced by increasing the stiffness of a TCR ligand–bearing substrate within the physiologic stiffness range (0.5 to 100 kPa). An increase in stiffness levels up to 100 kPa is associated with an increase in the TCR-triggered induction of T-cell glycolytic switch, metabolism, and cell cycle progression. The advancements in understanding the role of biomaterials in MSC–immune cell crosstalk could pave the way for more functional and rational translation of stem cell–mediated therapeutic approaches.

Conclusion and Prospects

Many types of dental tissue–derived MSCs have gained extensive attention due to their ease of accessibility and availability, which could enable their allogeneic use. However, the invasion of host immune cells can hamper the immunoregulatory functions of the MSCs. In this context, tissue engineering approaches have emerged to provide protective structures against the infiltration of proinflammatory cells/cytokines, securing cell survival and enhancing tissue regeneration. Besides protection, biomaterials could actively contribute to immunoregulation by balancing pro- and anti-inflammatory responses while providing a 3D environment to enhance the immunoregulatory potential of the encapsulated MSCs. The physiomechanical properties of the biomaterial (e.g., porosity and elasticity) can be tailored to trigger immunoregulatory cascades in the MSCs to boost immunoregulation. Biomaterial physical cues can induce polarization of inflammatory cells toward regulatory phenotypes such as M2 macrophages and Tregs. Alternatively, biomaterials could be used to directly deliver MSC-derived extracellular vesicles to exert immunoregulation with a lower probability of triggering innate and adaptive immune responses.

In summary, an advanced understanding of the interactions among MSCs, immune cells, and biomaterials that modulate

immunoregulation and response to inflammation could be translated into the development of advanced biomaterials and change the way that experts approach tissue regeneration. Such advancements can open new horizons toward more efficient personalized therapies and optimized clinical outcomes.

Author Contributions

S. Pouraghaei Sevari, A. Moshaverinia, contributed to conception and design, drafted the manuscript; S. Ansari, C. Chen, contributed to conception and design, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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