Immunomodulatory Properties of Stem Cells and Bioactive Molecules for Tissue Engineering

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Abstract

The immune system plays a crucial role in the success of tissue engineering strategies. Failure to consider the interactions between implantable scaffolds, usually containing cells and/or bioactive molecules, can result in rejection of the implant and devastating clinical consequences. However, recent research into mesenchymal stem cells, which are commonly used in many tissue engineering applications, indicates that they may play a beneficial role modulating the immune system. Likewise, direct delivery of bioactive molecules involved in the inflammatory process can promote the success of tissue engineering constructs. In this article, we will review the various mechanisms in which modulation of the immune system is achieved through delivered bioactive molecules and cells and contextualize this information for future strategies in tissue engineering.

Keywords

Mesenchymal stem cell, immunomodulation, cell delivery, bioactive molecules, tissue engineering, regenerative medicine

List of abbreviations

BMP-2	bone morphogenetic protein-2	MSCs	mesenchymal stem cells
CNS	central nervous system	MS	multiple sclerosis
CTLs	cytotoxic T lymphocytes	MI	myocardial infarction
DCs	dendritic cells	NK	natural killer cell
ECM	extracellular matrix	NO	nitric oxide
FBR	foreign body reaction	PBMC	peripheral blood mononuclear cell
FGF-2	fibroblast growth factor-2	PDGF	platelet derived growth factor
FGF-7	fibroblast growth factor-7	PEG	poly(ethylene glycol)
GvHD	graft-versus-host disease	PLGA	poly(lactic-co-glycolic acid)
HGF	hepatocyte growth factor	PMMA	polymethylmethacrylate
HLA	human leukocyte antigen	PGE2	prostaglandin E2
IR	infrared	SCI	spinal cord injury
ICAM-1	intercellular adhesion molecule-1	S1P	sphingosine-1-phosphate
IFN- γ	interferon- γ	Tregs	regulatory T-cells
IL	interleukin	TGF β	transforming growth factor β
ISCT	International Society for Cellular Therapy	TNF- α	tumor necrosis factor- α
IDO	indoleamine2,3-dioxygenase	TSG-6	TNF-stimulated gene 6 protein
MCP-1	monocyte chemoattractant protein-1	VCAM-1	vascular cell adhesion protein-1
MHC	major histocompatibility complex	VEGF	vascular endothelial growth factor

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1. Introduction

The canonical tissue engineering paradigm encompasses a combination of scaffold, cells, and bioactive factors to regenerate injured or diseased tissues. While many efforts have focused on the implantation of the particular tissue cell type in order to encourage tissue growth, delivery of progenitor cells has also become a common theme in tissue engineering strategies. More recently, as the importance of the local environment surrounding regenerating tissue has become increasingly recognized, attention has turned to how the immune system can be manipulated to serve the goals of implantable scaffolds, particularly with regards to suppressing the rejection of foreign bodies and enhancing the integration of scaffolds with native tissue. To this end, this review focuses on the potential implications of mesenchymal stem cell and immunological bioactive factor delivery on the success of tissue engineered constructs and emphasizes how they may be leveraged to move the field towards the goal of healing large tissue defects.

2. The Mesenchymal Stem Cell: Definition and Inherent Properties

The mesenchymal stem cell is defined broadly by the International Society for Cellular Therapy (ISCT) by minimal criteria: (1) the MSC must be plastic-adherent in standard culture, (2) the MSC must express CD73, CD90 and CD105 with absence of CD45 and CD34 among others, and (3) the MSC must be able to differentiate *in vitro* to osteoblasts, chondroblasts, and adipocytes [1].

There are numerous sources for the collection of MSCs including the bone marrow, adipose tissue, peripheral blood, dental pulp and various neonatal tissues each with slightly varying phenotype reviewed in Haas *et al.* 2011 [2].

Their usefulness in tissue regeneration is punctuated not only by their differentiation capacity but by their low immunogenicity. MSCs exhibit low levels of major histocompatibility complex (MHC) I and II, and are negative for surface markers CD40, CD80, and CD86, which are necessary for antigen presentation and co-stimulation of lymphocytes and allow these cells to generally evade host immune systems [3].

3. Mesenchymal Stem Cells as Potent Immunomodulators for Tissue Engineering

The manipulation and delivery of cells for tissue regeneration arguably marked the inception of the field and has become a common strategy employed in tissue engineering. Delivery of cells can be achieved through injection or implantation of devices composed of natural or synthetic materials. Delivered cells can be used to replace injured or damaged cells, to serve as progenitors to mature cells, or to secrete trophic factors that stimulate the local environment. More recently, the role of delivered cells in the modulation of the immune system has become a central point in the development of tissue regeneration strategies. Multiple studies have shown that mesenchymal stem cells (MSCs) can be used for both regeneration of tissues and modulation of immune response in diseases such as multiple sclerosis (MS), graft-versus-host disease (GvHD), and solid organ transplant rejection. There are currently 210 open and ongoing pre-clinical and clinical studies investigating the various roles of MSCs in potential therapies in immune therapy available on the National Institutes of Health registry. The immunomodulatory effects of MSCs, especially in concert with other immune cell types, hold incredible promise for the advancement of regenerative medicine and tissue engineering, and most studies on immunomodulation by delivered cells involve MSCs.

MSCs have long been studied for their regenerative potential in tissue engineering applications. More recently, MSCs have been examined for their low immunogenicity, immune masking properties, and immunomodulatory capabilities, including both immune stimulation and immunosuppressive effects, which have been the subject of extensive review [4–6]. In the realm of tissue engineering, their intrinsically low immunogenicity makes them attractive candidates for “off the shelf” products that incorporate allogenic cells for tissue engineering [3].

The immune system plays a critical role in the reorganization of tissues following injury. Delivered cells have aided in the repair of ischemic tissue through modulation of immune environments to facilitate not only repair of tissues, but also augmentation of vasculature remodeling through trophic effects [7–10]. In the majority of cases, such as those involving injury to the nervous system [11,12] or myocardial infarction (MI) [13,14], MSCs have been championed for their ability to reduce inflammation and tissue damage. In other cases, however, enhanced immune response in damaged tissue is beneficial for overall vasculature remodeling, a critical component of any tissue engineered graft [15,16].

MSCs exert effects on both the innate and acquired immune systems with profound implications for not only monocyte differentiation and macrophage response but also in the behavior of T and B lymphocytes. Moreover, these immune cells in turn modulate the expression profiles and behavior of MSCs, painting a dynamic picture of complex crosstalk between cells of the immune system and MSCs with broad implications for the fields of regenerative medicine and tissue engineering. Figure 1 summarizes the effects of MSCs on the different cell types in both the innate and adaptive immunity. The majority of efforts in regenerative medicine have been in pursuit of hard tissue regeneration; accordingly, studies of immunomodulation in tissue engineering have largely been contextualized with hard tissues. As such, this review will focus on hard tissue engineering strategies primarily and will address some soft tissue applications.

Figure 1: Summary of effects on immune cell types in the innate (A) and acquired immune systems (B). Green denotes increased expression and red denotes decreased expression.

4. MSCs and the Innate Immune System

4.1. NK cells and MSCs

Natural Killer (NK) cells are a critical element of the innate immune system and make up about 10 to 15% of peripheral blood lymphocytes. They are principally involved in the destruction of viral and tumor afflicted tissues as they kill without MHC I restriction, thus giving them the name ‘natural killers’ [17].

MSCs can inhibit the proliferation of NK cells and alter their cytokine release profiles. *In vitro* evaluation of co-cultured MSCs and NK cells shows that while NK cells are able to kill allogenic MSCs, MSCs also inhibit the interleukin-2 (IL-2) mediated proliferation of NK cells [18]. The lethality of NK cells is substantially reduced if MSCs are primed by IFN- γ , as they would be in an inflammatory environment. MSCs express low levels of MHC I *in vivo*, allowing

them to avoid cytotoxic T lymphocytes (CTLs) [3]. However, in response to IFN- γ , MSCs downregulate NK receptors and upregulate MHC in order to avoid being killed by NK cells [17,19]. This phenomenon emphasized the need for MSCs to be activated in an inflammatory environment in order to begin modulating the immune system, a theme in many studies involving other aspects of the immune system [19–22].

The complex interactions between MSCs and NK cells highlight the importance of understanding the time dependent effects of MSC administration *in vivo*, as has been explored for hematopoietic stem cell transplantation [19]. MSCs strongly inhibit the proliferation of resting NK cells but only partially inhibit the proliferation of NK cells that have already been activated by IL-2. Activated NK cells have the ability to kill allogenic MSCs, which may reduce the efficacy of any MSC-centric treatment. This effect was found to be the result of three soluble factors secreted by MSCs: indoleamine2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2) and transforming growth factor- β (TGF- β) [19].

Together these studies present a complex dialogue between NK cells of the innate immune system and MSCs. MSCs have obvious modulation potential in altering NK response, but any tissue engineering approach will have to consider timing and the inflammatory milieu in order to adequately predict NK behavior in future approaches to tissue regeneration.

4.2. Dendritic Cells and MSCs

Dendritic Cells (DCs) are specialized, phagocytic antigen presenting cells that serve as a liaison between the innate and adaptive immunities in mammals by helping to activate T and B lymphocytes. They are derived from peripheral blood monocytes and take on specific characteristics upon activation that allow for the activation of acquired immune system responses [23].

MSCs exert their effects on DCs through secreted, soluble factors as well as cell-cell contact signaling, possibly involving Notch signaling [24–26]. *In vitro* experiments have shown that MSCs inhibit the differentiation of CD34+ and monocyte progenitors to DCs [27]. MSCs also reduce the expression of costimulatory molecules on immature DCs; MHCII, CD40, CD83 and CD86 are downregulated and upregulation of HLA-DR is attenuated [27,28]. These effects are mediated by IL-6 and granulocyte macrophage colony-stimulating factor (GM-CSF) which potently inhibit differentiation of DCs and, in the case of IL-6, can switch differentiation potential of monocytes toward the macrophage phenotype [29,30]. Another report suggests PGE2 and not IL-6 is the main soluble mediator in the arrest of DC maturation *in vitro* [24]. The addition of a PGE2 inhibitor to the culture restored DC maturation with no effect on IL-6 production [24]. These reports suggest that MSCs can have a profound effect on early stages of DC maturation through paracrine and contact effects.

Because DCs are not the main cell of the innate immune system responsible for phagocytosis, the effects of MSCs on DCs largely reflect the indirect effect of MSCs on T and B-cell stimulation [23]. Chronic solid organ rejection is due in large part to cell mediated immunity and the dendritic cell is a critical component of the activation of this response [31]. This attenuation of DC maturation and subsequent loss of DC stimulatory molecules including secreted factors emphasizes the role of the MSC in the abrogation of the link between innate and acquired immunities, which could be extremely beneficial for regenerative applications if exploited appropriately. In the future, these immunomodulatory effects could obviate the need for life long immunosuppression if tissue engineering constructs were to involve allogenic material, as has begun to be studied in the context of regulatory T-cell (Treg) and anergy induction through dendritic cells in islet transplantation [32].

4.3. Macrophages and MSCs

Macrophages are monocyte derived, phagocytic cells that function as part of the innate immune system and serve to facilitate clearance of damaged and diseased areas in tissue and also aid in the complex remodeling as these tissue begin to heal. The polarization paradigm between pro-inflammatory macrophages (M1) and wound healing macrophages (M2) highlights how macrophage response is a balance between two opposing phenotypes. This phenomenon is of great interest to the field of regenerative medicine and has been the subject of extensive reviews [33,34]. Macrophages are in large part responsible for the well-understood foreign body reaction (FBR), which is a substantial hurdle in tissue engineering and implantable medical devices in achieving biocompatibility [35,36]. Briefly, FBR is dependent on monocyte/macrophage chemotaxis, signaling through integrins, and ultimately the mannose receptor and soluble factors, including IL-4 and IL-13, to induce fusion into foreign body giant cells that mediate the inflammatory reaction, reviewed in Anderson *et al.* [35]. The foreign body is essentially sequestered by adherent macrophages and foreign body giant cells.

The FBR can be modulated by a wide variety of factors including biomaterial composition and surface topography of implanted devices [37]. Likewise, for MSCs, the morphology, stem cell character and, more recently, the immunomodulatory properties on macrophages can be affected by topography and material surface properties. MSCs co-cultured with THP-1 cells, a monocytic cell line, in porous ECM structures showed increased secretion of anti-inflammatory cytokines PGE2 and TSG-6 and decreased secretion of pro-inflammatory cytokines IL-6 and MCP-1 over two-dimensional adherent controls. Furthermore, the authors showed a reduction in TNF- α and increase in IL-10 secretion from monocytes which was accompanied by an attenuation of monocyte migration [38].

Activation of the pro-inflammatory M1 type through Toll-Like receptors assists in the activation of inflammatory cytokines in response to injury [39]. The temporal relationship between early predominance of inflammatory M1 macrophage response and the late predominance of wound healing M2 macrophage response is a delicate balance. The inflammatory state is required for clearance of debris and eradication of potential infections; conversely, the M2 response is required for ultimate resolution of the injury [40,41]. It is often the case that prolonged inflammation leads to excess and unnecessary tissue damage before the tissue is, often inadequately, regenerated. Biomaterial properties alone can affect macrophage polarization [33,41]. Taken together, choice of biomaterial and encapsulation with immunomodulators such as MSCs are of paramount importance when considering the modulation of the immune response effectively from pro-inflammatory to wound-healing.

Recent studies have shown the effects of MSCs in the modulation of macrophage phenotype and surface marker profile. Hydrogel scaffolds are attractive in tissue engineering applications since they are generally easily fabricated, can be made to have low immunogenicity, and can support delivered or native cell proliferation and differentiation. In one study, MSCs encapsulated within hyaluronic acid hydrogels exerted effects on CD14⁺ peripheral blood monocytes in a co-culture system causing them to adopt a less inflammatory macrophage profile with high CD206 and low CD16 and HLA-DR expression [42]. In a poly(ethylene glycol)-gelatin (PEG-Gelatin) hydrogel scaffold, it was shown that MSCs could increase IL-6 and IL-10 expression while down regulating TNF- α and IL-12 in macrophages which is favorable for wound resolution [43]. In PEG hydrogels encapsulating MSCs, co-culture with macrophages stimulated by lipopolysaccharide (LPS) caused the downregulation of pro-inflammatory cytokines IL-6 and TNF- α and an upregulation of the enzyme arginase. Arginase is required for

the conversion of arginine to ornithine and the subsequent production of collagen, and its expression within macrophages is indicative of a wound-healing phenotype [22]. This effect was likely mediated by PGE2 as treatment with a COX2 inhibitor partially recovered TNF- α expression in macrophages indicating a more inflammatory phenotype. *In vivo*, it was observed that the fibrotic response in FBR to the hydrogel constructs with encapsulated MSCs was partially attenuated, but this phenomenon was progressively lost as MSCs were further differentiated into an osteogenic lineage [22].

The effect of delivered stem cells on the local immune environment apart from their trophic effects has been studied within the context of myocardial infarction and vascular remodeling. Dayan *et al.* showed that MSCs were able to increase the activation of M2 macrophages while suppressing M1, pro-inflammatory macrophage activation possibly through an IL-10 mechanism in a mouse model for MI [13]. At 3 days, the proportion of CD206+ monocytes increased while overall levels of macrophage/monocyte derivative infiltration decreased strongly suggesting an anti-inflammatory effect of MSCs in the acute phase following MI. Another study showed that embolized MSCs in the lung following acute MI secreted anti-inflammatory cytokines that reduced the severity of MI in a mouse model [14].

MSCs have also been investigated for their immunomodulatory effects on macrophages following acute spinal injury. Nakajima *et al.* demonstrated that in an acute spinal cord injury (SCI) model in rats, injected MSCs did not differentiate but instead supported the generation of M2 macrophages over M1 macrophages via increases in IL-4 and IL-13 with concurrent decreases in TNF- α and IL-6. This modulation led to better functional outcomes and corresponded with better histological outcomes for myelin sparing, scar formation and axon preservation [11]. Barminko *et al.* sought to immobilize MSCs via encapsulation in alginate beads for increased cell viability following injection in the spinal fluid post SCI [12]. The investigators believed that suspension could increase cell viability, decrease the number of cells needed for therapeutic effect and increase the immunomodulatory potential by using 3D culture [44]. Indeed, the investigators used orders of magnitude fewer cells than studies not using encapsulation and also observed a similar upregulation in CD206+ macrophages [45].

The role of macrophage polarity is also of paramount importance in skin wound healing through the classical sequence of events of cutaneous repair, reviewed by Brown *et al.* [33]. Multiple studies involving MSCs derived from various sources have shown that enhanced cutaneous wound repair can be achieved with MSCs by polarizing macrophage response to M2. This effect was accompanied by an increase in IL-6 and IL-10 expression and a downregulation of TNF- α [46,47].

5. MSCs and acquired immunity

5.1. MSCs and T-cells

The role of MSCs in the immunomodulation of T-cell response of most subtypes of T-cells has been extensively documented [5,48]. In general, their effects can be seen as immunosuppressive as they modulate T-cell response from an inflammatory to a quiescent, regenerative mode for effector (Th1 versus Th2 paradigm) and Tregs. However, their exact effect on the differentiation and generation of T-cells are subject to debate with conflicting studies. This may be a result of heterogeneous populations used for *in vitro* assays [49].

Krampera *et al.* reported that MSCs cultured *in vitro* suppress proliferation of CD4+ and CD8+ cells as well as NK cells with no observable effect on B-cell proliferation [50]. Additionally, the investigators noted no production of FOXP3 transcription factor, indicating the generation of Treg cells, which is at odds with other studies [51–53].

The immunosuppressive effect of MSCs on T-cells likely includes soluble factors, cell-cell contact mediators, and cellular crosstalk between MSCs and T-cells [20,54,55]. Meisel *et al.* suggested that tryptophan depletion by IDO induced by priming MSCs with IFN- γ caused suppression of T-cell proliferation [54]. Another study suggested that nitric oxide (NO) produced by MSCs was involved in the suppression of STAT5 phosphorylation in T-cells, subsequently halting proliferation [54]. That same study found that MSCs from mice lacking NO synthase had no suppressive effect when cultured with T-cells; inhibitors to IDO and TGF- β had no restorative effect on T-cell proliferation whereas a PGE2 inhibitor did.[54,55] This may be due to interspecies variation for the roles of NO and IDO.[56]

In addition to trophic effects and angiogenic properties of MSCs on islet cells that could potentially be of use in treating type 1 diabetes, the immunosuppressive properties of MSCs in graft survival have also been evaluated [57–59]. One study showed that the immunosuppressive effects on T-cells were mediated by specific metalloproteinases that reduced surface expression of CD25 on T-cells. This attenuated T-cell proliferation *in vitro* and, in an *in vivo* model for islet cell transplantation, prolonged survival of fully allogenic grafts [60]. Another study concluded that co-administration of MSCs and sub-therapeutic cyclosporine A, but not either alone, induced an anti-inflammatory response largely mediated by IL-10 in a rat islet graft model [61]. Davis *et al.* encapsulated islet cells with MSCs in silk hydrogels with ECM and observed increased islet cell survival while attenuating the proliferation of splenocytes [58].

When primed by inflammatory markers such as IFN- γ , MSCs cultured *in vitro* with CD4+ peripheral blood mononuclear cells (PBMCs) showed the induction of an anti-inflammatory cytokine profile including IL-3, IL-10 and IL-13, which are indicative of a Th2 response. Furthermore, there was strong downregulation of Th1 pro-inflammatory cytokine profile including IFN- γ , IL-1a and b, TNF α , and TNF β [20]. In addition, MSCs were found to be able to inhibit IFN- γ release from Th1 cells and increase IL-4 production in Th2 cells *in vitro* [51]. These results provide some explanation for the promising results of Le Blanc *et al.* who utilized MSCs as a treatment for GvHD [62].

In addition to skewing differentiation of T-cell progenitors toward a Th2 phenotype, MSCs have been implicated in the production and recruitment of a subset of Tregs and in the induction of anergy [20,63–65]. It has been suggested that MSCs effect this modulation via direct contact and expression of MHC II in the absence of co-stimulatory molecules, as T-cell activation requires a two-signal process [20]. However, addition of costimulatory molecule B7 or anti-CD28 antibody to *in vitro* co-cultures of CD4+ T-cell progenitors and MSCs did not induce T-cell activation, suggesting that MSCs may be exerting their regulatory effect in a different manner [66]. Ultimately, T-cells generated under these conditions demonstrated a CD4⁺/CD25^{low}/CD69^{low}/FoxP3⁺ Treg profile. Soluble factors have also been implicated in modulation of this T-cell response; candidates include IL-10, hepatocyte growth factor (HGF), PGE2 and TGF- β [52,66]. However, conflicting reports exist in which suppression of antibody blocking of these factors *in vitro* did not result in the attenuation of the generation of the anti-inflammatory phenotype. The exosome of MSCs had been shown to induce the Treg phenotype as well [67].

While CD4+ cells have been shown to upregulate FoxP3 and be modulated into a Treg phenotype, MSCs secrete a soluble factor that inhibits the formation of CTLs; however, MSCs had no effect on CTL activity once they had entered the cytotoxic phase [68]. Furthermore, although MSCs express low levels of MHC I, they do not activate allogenic CTLs and do not induce TNF- α or IFN- γ expression. This result indicates that not only are MSCs immune

privileged in that they do not elicit CTL response but they also contribute to an immunosuppressed environment [69]. Prevosto and colleagues have demonstrated the generation of a regulatory CD8⁺ cell in response to co-culture with MSCs, although without upregulation of the traditional Treg marker FoxP3 [70]. Together, these studies indicate that MSCs are able to modulate the lineage and differentiation of CD8⁺ cells but probably do not play a role in suppression once they are active or in their effector state.

Another study demonstrated that MSCs primed with IFN- γ and TNF- α enhanced the expression of CD54 in MSCs, facilitating CCL20 binding to pro-inflammatory Th17 cells and inhibiting their effect. Cord blood cells briefly cultured in Th17 differentiation conditions or Th17 cells collected from patients with inflammatory disease were utilized in MSC co-culture assays where it was observed that MSCs inhibited the secretion of pro-inflammatory cytokines, IL-17, IL-22, IFN- γ , TNF- α , while upregulating IL-10. These effects are likely a result of soluble factors secreted by MSCs that are not differentiating; exosomes alone are enough to decrease T-cell proliferation and decrease IFN- γ production [22,71]. This result could have implications for tissue engineered devices that may not require MSCs for immune modulation but only the contents of their exosomes or secreted factors.

Additionally, FoxP3, which has been implicated in the specification of Treg differentiation, was upregulated in fully differentiated Th17 cells and accompanied immunosuppressive activity *in vitro*, suggesting that MSCs have an effect of making Th17 cells more quiescent even after lineage specification [52,72]. MSCs also have inhibitory effect on the differentiation of Th17 cells from naïve T-helper and on effector cells that is cell contact and prostaglandin dependent; downregulation of IL-17A and CD25 was observed in these cells cultured from CD4⁺ progenitors and from Th17 effector memory cells from a urinary obstruction model [73].

Aberrant T-cell responses have been implicated in many diseases such as GvHD, MS, rheumatic disease, and MSCs represent a promising avenue for therapy, extensively reviewed by Miguel *et al.* [74]. The modulation of T-cell response from pro-inflammatory Th17 and Th1 cells to a Th2 and Treg response could be invaluable for potential therapies in these diseases as well as for tissue engineering. While the majority of trials focusing on immunomodulation of T-cells have involved transplant rejection and systemic disease, these conclusions should be considered in the future of tissue engineering. Chronic rejection of foreign tissue is in large part mediated by T-cells, and long term grafts with allogenic material may be feasible in the future through immunomodulation with MSCs [31].

5.2. MSCs and B-cells

Studies regarding the effects of MSCs on B-cells have been limited, and none have been performed with tissue engineering constructs. There have been differing results in the effect of MSCs on B-cells. One study using T-cell depleted PBMCs cultured with MSCs indicated no effect on proliferation [50]. However, other studies indicate an arrest of B-cell proliferation accompanied by an inhibition of B-cell differentiation, as evidenced by decreased production of immunoglobulin and decrease in chemotaxis [75,76]. This difference can possibly be attributed to processing and isolation of B-cells from PBMCs as another study showed that the effect of MSCs on B-cells of both differentiation and inhibition of proliferation required the mediation of a CD3⁺ T-cell and that direct MSC to B-Cell contact was not necessary to cause the observed effect [49,77]. Indeed, direct co-culture of MSCs with CD19⁺ B-cells had no effect on their proliferation.[55]

6. MSCs as immune system activators: The other side of the coin

While MSCs are attractive for therapies because of their immunosuppressive capabilities, activation of the immune system by MSCs in low inflammatory states where TNF- α or IFN- γ signaling is suboptimal or when MSCs were administered with other immunosuppressants has been reported [4,78,79].

An important soluble factor for the modulation of T-cell proliferation is NO produced by MSCs. However, it has been shown that when cytokine signaling is inadequate for the production of sufficient levels of NO, MSCs enhance T-cell proliferation *in vitro* and increase the delayed-type hypersensitivity response in a mouse model [4]. In human MSCs, a knockdown of IDO causes similar immune cell proliferation as seen in mouse NO synthase inhibition. These results are consistent with the result of Renner *et al.* in which pretreatment with MSCs contributes to the acceleration of solid organ transplant rejection in mice [80].

Additionally, the immune privileged, low MHC I phenotype of MSCs is quickly lost as the cells differentiate. This represents a problem in tissue engineering implants with heterogeneous cells including MSCs that will rapidly increase MHC I expression upon differentiation. Indeed, many studies report that the immune privileged phenotype only persists for a week [22,42]. To combat that, some groups have begun to develop composite biomaterials for co-delivery of cells and immunosuppressants such as cyclosporine A loaded into PLGA microspheres within a polymeric framework [81].

Because MSCs play a dual role in enhancement or suppression of the immune system, further studies need to be performed to delineate the role of soluble factors and activation parameters for MSCs immunomodulation if MSCs are to be used in transplantation or tissue engineering applications. Though MSCs have strong potential in immunosuppression, recent clinical trial failures for GvHD and failure of T-cell suppression in a mouse model [82] accompanied by reports of worsening disease should give basic science and clinical investigators pause before attempting to leverage immune properties of MSCs before they are well defined [62].

7. Delivery of Bioactive Molecules

While the future of stem cells in tissue engineering looks bright, adequate control over stem cell differentiation and proliferation remains a major limitation in current stem cell based therapies. Biomaterials research has fostered the discovery of a variety of strategies that improve tissue regeneration. Many tissue engineering strategies involve the implantation of a biomaterial scaffold for delivery of bioactive substances to facilitate tissue regeneration. The implantation procedure compounded with foreign body reaction to the implanted scaffold cause local injury to vascularized tissues or organs and elicits an inflammatory response [83]. Historically, materials that are biologically inert were at the focal point of biomaterial research. However, by permitting and exploiting specific cellular responses to materials, a better integration and biomaterial performance can be achieved [83]. The immune system can be modulated by a wide variety of techniques such as surface modification of the biomaterial, generation of artificial extracellular matrix, and delivery of anti-inflammatory drugs from the biomaterial [83,84]. The effect of biomaterial type, shape, and surface chemistry has been the subject of extensive review elsewhere [85,86]. The following sections focus on the delivery of bioactive molecules with the goal of regulating the immune system to improve tissue regeneration. Table 1 summarizes the effects of various bioactive molecules from direct delivery and from systems involving MSCs.

Table 1. Table of Bioactive Molecules

Table of Bioactive Molecules			
Anti-inflammatory and upregulated by MSCs			
Bioactive Molecule	Delivery Methods or <i>In Vitro</i> System	Cellular and Tissue Response	Reference
Prostaglandin E2 (PGE2)	MSC co-culture	Inhibited NK cell cytotoxic activity and proliferation (requires IDO for both) Inhibited mature DC cells Inhibited DC differentiation Increased M2 macrophage generation Inhibited T-cell proliferation Inhibited Th17 differentiation	[19,22,24,54,73,87,88]
Indolamine 2,3 Dioxygenase (IDO)	MSC co-culture	Inhibited NK cell cytotoxic activity (requires PGE2 for synergism) and proliferation	[19]
Nitric Oxide (NO)	Co-culture	Decreased T-cell proliferation	[54]
IL-4	MSC transplantation into rat spinal contusion Co-culture	Increased M2 macrophage generation Increased Th2 response	[11,20]
IL-6 (MSC made)	MSC co-culture MSCs encapsulated in PEG-gelatin hydrogel co-culture Systemically infused MSCs for cutaneous wound repair Local injections of IL-6 with parathyroid hormone IL-6 deficiency	Inhibited DC Differentiation Decreased Th-17 proliferation Increased CD206+ M2 macrophage generation Homed to damaged tissue and increased M2 macrophage generation Increased mechanical properties of fracture callus and full resolution 5 weeks post-fracture Reduced cutaneous wound healing due to restriction in proliferation and remodeling	[28,43,46,89,90]
IL-10	MSC co-culture Systemic infusion in a mouse model for infrared-induced cutaneous fibrosis Transfection	Increased M2 macrophage generation Increased Th2 and Treg response Inhibited Th17 response Increased regulatory macrophage expression while decreasing macrophage levels overall 72 hours post injury revealed normal skin architecture without evidence of scar formation	[13,20,73,91-93]

	<p>induced overexpression in adult stromal cells</p> <p>Local delivery from PLGA scaffolds</p> <p>Gene delivery of IL-10 to culture of macrophages</p>	<p>Reduced leukocyte infiltration, upregulated macrophage derived IL-10, downregulated CD4+ and macrophage derived IFN-γ</p> <p>Favored the M2 phenotype and resisted the M1 phenotype when challenged with pro-inflammatory cytokines</p>	
IL-13	<p>MSC transplantation into rat spinal contusion</p> <p>Co-culture</p> <p>Local delivery of IL-4</p> <p>Influence of local IL-4 delivery on PMMA implant</p> <p>Incorporated into polysulfone conduits for nerve regeneration</p>	<p>Increased M2 macrophage generation</p> <p>Increased Th2 response</p> <p>Polarized macrophages towards the M2 phenotype</p> <p>Reduced local concentrations of IL-6 and TNF-α</p> <p>Increased level axonal regeneration and polarized macrophages towards the M2 lineage</p>	[11,20,94,95]
Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF)	MSC co-culture	Inhibited DC differentiation	[29]
Pro-inflammatory and downregulated by MSCs			
Tumor Necrosis Factor- α (TNF- α)	<p>MSC co-culture</p> <p>MSCs encapsulated in PEG-gelatin hydrogel co-culture</p> <p>MSC transplantation into rat spinal contusion</p> <p>Systemically infused MSC for cutaneous wound repair</p> <p>Direct delivery</p>	<p>Inhibited Th1 response</p> <p>Did not activate CTLs</p> <p>Inhibited M1 macrophage generation</p> <p>Inhibited M1 macrophage generation</p> <p>Homed to damaged tissue and increased M2 macrophage generation. Decreased Th-17 proliferation</p> <p>Stimulated the release of BMP-2 from osteoblasts and induces osteogenic differentiation</p> <p>Increased fracture callus mineralization</p> <p>Decreased bone mineralization at the growth plate</p>	[11,20,43,46,69,96–99]

	Use of inhibitor		
Interferon- γ (IFN- γ)	MSC co-culture	Inhibited Th1 and Th17 response Did not activate CTLs	[20,69]
IL-1 α/β	Direct stimulation PLGA loaded with IL-1 antagonist MSC co-culture Systemic infusion in a mouse model for IR-induced cutaneous fibrosis	Promoted osteoblastic proliferation, activated osteoclastic dependent bone resorption, and inhibited differentiation of chondrocytes Induced keratinocyte migration and proliferation Increased synthesis of FGF-7 Attenuated IL-1 β dependent intervertebral degeneration Inhibited Th1 response Increased regulatory macrophage expression while decreasing macrophage levels overall	[20,47,100–103]
IL-6 (macrophage made)	MSCs encapsulated in PEG hydrogel MSC transplantation into rat spinal contusion	Expression abrogated in macrophages upon co-culture increasing M2 generation Inhibited M1 macrophage generation	[11,22]
IL-12	MSCs encapsulated in PEG-gelatin hydrogel co-culture MSC co-culture	Inhibited M1 macrophage generation Inhibited Th1 response	[20,43]
IL-17	MSC co-culture	Inhibited Th17 response	[73]
Growth Factors			
Transforming Growth Factor-TGF- β 1	Local delivery of TGF- β 1 Co-delivery of TGF- β 1 and PDGF	Increased inflammatory cell migration Identified as chemoattractant for macrophages	[83,104]
Small bioactive molecules			
Sphingosine-1-phosphate (S1P)	PLGA loaded with S1P <i>in vivo</i> Local delivery of S1P <i>in vivo</i>	Induced migration of pre-osteoblast, pre-osteoclasts, and lymphocytes Observed luminal expansion of arterioles and de novo bone formation Promoted M2 macrophages and anti-inflammatory monocytes	[40,105,106]

7.1. Delivery of Pro-inflammatory Cytokines

As previously discussed, pro-inflammatory cytokines have been shown to be intricately linked with the pathophysiology of several disease states such as osteoarthritis and MS [107,108]. In the field of biomaterials, several pro-inflammatory molecules are well established mediators of the foreign body reaction [109]. However, the pathological consequences seen in

these conditions are largely due to unregulated chronic exposure to inflammatory mediators. An acute inflammatory response results in the immediate recruitment of neutrophils and macrophages, which release a temporally and spatially varying “cocktail” of pro-inflammatory cytokines and growth factors. As discussed previously, local elevation of several cytokines, including IL-1, IL-6, IL-11, IL-18 and TNF- α , are required to remove damaged or necrotic tissue, break down extracellular matrix proteins and stimulate angiogenesis [110,111]. Again, it is critical that anti-inflammatory mediators follow these pro-inflammatory signals in order to prevent excessive inflammation and chronic inflammation [10]. Prolonged exposure to an environment rich in pro-inflammatory mediators leads to the formation of fibrotic scars, granulation tissue and increased formation of type I and III collagen [35]. It has been shown that a quick resolution (less than two weeks) to inflammation favors tissue regeneration and implant acceptance, while an unresolved inflammatory phase indicates therapeutic failure [112]. A thorough understanding of these bioactive molecules is critical for constructive tissue remodeling and regeneration [40]. Thus, the direct modulation of pro-inflammatory cytokines offers a new strategy that can further enhance tissue regeneration. Although the delivery of pro-inflammatory molecules is largely limited to the bench top due to the risk of systemic inflammation, these studies have revealed the importance of the modulating the initial pro-inflammatory response rather than circumventing the immune system.

7.1.1. Tumor Necrosis Factor- α

The TNF- α superfamily is composed of 19 different ligands that are critical for mediating a wide range of processes such as regulation of the immune system, protection against microbial infection, and immune surveillance [113]. In addition, TNF- α can promote apoptosis or cell survival, depending on the specific cell surface receptor activated [110]. During the initial acute inflammatory response, high levels of TNF- α are secreted, along with other pro-inflammatory cytokines such as IL-1 and IL-6, resulting in the infiltration of other inflammatory cells and initiating the process of angiogenesis [96,114,115]. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion protein-1 (VCAM-1) are upregulated in order to enhance the recruitment of mesenchymal cells from the surrounding tissues [97]. Notably, recent studies have revealed that in the complete absence of TNF- α , osteogenic stem cell differentiation is delayed [116]. This is likely due to TNF- α stimulating the release of BMP-2 from neighboring osteoblasts and the activation of two stem cell receptors tumor necrosis factor receptors 1 and 2 [96,97]. Within the initial 24 hours after injury, the local concentration of TNF- α reaches a maximum and returns to baseline at 72 hours [110]. During this interval, macrophages are a major producer of this pro-inflammatory cytokine and this temporal pattern is believed to induce the release of other bioactive molecules that are critical for bone regeneration [110]. Zhou *et al.* revealed that blocking TNF- α signaling does not alter the acute inflammatory response; rather, it decreases mesenchymal and osteoprogenitor cell infiltration [99]. At post-injury day eight, daily treatment with a TNF- α antagonist resulted in a significantly decreased angiogenic response and production of FGF-2 compared to saline controls, yet it induced an increase in osteoblastic differentiation at the growth plate [99]. Conversely, a significantly increased fracture callus mineralization at 4 weeks was noted when daily local injections of TNF- α were administered during the first couple of days post-fracture [98]. These studies highlight the diverse actions of TNF- α that are dependent upon the microenvironment and regenerative mechanisms. Though few *in vivo* studies have been published it appears that TNF- α is a critical regulator of *de novo* bone formation and should be considered in future of bone tissue engineering and repair [117].

7.1.2. IL-1

Both TNF- α and IL-1 are best known as acute phase mediators. The family of IL-1 cytokines are intimately involved in innate and adaptive immune responses [118]. The effects of IL-1 on bone regeneration are largely similar to that of TNF- α . It is secreted in the same temporal manner initially. However, there is a second peak in local levels around post injury week three [110]. *In vitro* studies have shown IL-1 to promote osteoblastic proliferation, activate osteoclastic dependent bone resorption and inhibits differentiation of chondrocytes [100,101]. While an *in vivo* study by Lange *et al.* revealed little influence of IL-1 on fracture healing [101]. Despite little influence on bone regeneration *in vivo*, in skin wounds IL-1 works in an autocrine manner to encourage keratinocyte migration and proliferation and activates fibroblasts to increase FGF-7 secretion [102]. Thus it helps to indirectly catalyze the re-epithelialization. However, high local levels of IL-1 for an extended time has an injurious effect on wound healing [102]. Additionally, Gorth *et al.* showed that the *in vivo* delivery of poly(lactic-co-glycolic acid) (PLGA) microspheres loaded with IL-1 antagonist attenuated IL-1 β dependent intervertebral degeneration [103]. Although further investigation into the efficacy of cytokine-based therapy is needed, this underscores the future potential of modulating cytokine levels for the regeneration of damaged tissue.

7.1.3. IL-6

Although IL-1 and TNF- α are known to be potent stimulators of the acute phase reaction, neither cytokine is able to induce the full spectrum of acute phase proteins alone [119]. It has been shown that IL-6 is required in order to generate the full acute phase spectrum [100]. Despite IL-6 being a powerful inducer of acute phase proteins, it has several anti-inflammatory properties and pro-regenerative properties [120]. Local levels of IL-6 influence the differentiation of osteoblast and osteoclasts, trigger secretion of vascular endothelial growth factor and thus trigger angiogenesis, and play both a neuroprotective and neurotrophic role in the central nervous system (CNS) after traumatic injury [121,122]. Rozen *et al.* injected IL-6 and parathyroid hormone fragments *in vivo* for two weeks following bone fracture [89]. It was shown that the delivery of these factors significantly increased the mechanical properties of the fracture callus, and full healing was achieved 5 weeks post-fracture [89]. In the area of skin wound healing, IL-6 deficient mice have been shown to have impairment in wound healing as compared to wild type controls [90]. While Lin *et al.* could not rule out that the reduction in inflammatory response was responsible for this finding, it is more probable that IL-6 mediates crucial aspects of proliferation and remodeling [90]. Even though there are a limited number of *in vitro* and *in vivo* studies into the local delivery of pro-inflammatory cytokines there is an increasing appreciation to the role these important mediators play on tissue regeneration.

7.2. Delivery of Anti-inflammatory Cytokines

Because the immune system has the potential to cause extensive and systemic secondary damage, biomaterials have been designed to evade or modulate the host's immune response. These methodologies have been largely focused around limiting protein deposition by using materials that are immune-inert, altering the surface topology or by using surface coatings [84,112]. Additionally, the use of systemic anti-inflammatory cytokines that modify the immune response have been utilized in order to dampen the inflammatory response. Clinical studies have previously investigated the inhibition of specific pro-inflammatory cytokines with biologics

[123,124]. However, this strategy leaves the host vulnerable to infection [112]. By using localized controlled delivery of soluble factors that can either create an anti-inflammatory microenvironment or influence the infiltrating cell type, modulation of the local immune response can be tuned for more favorable tissue regeneration.

7.2.1. Interleukin-10

IL-10 is a family of Type II anti-inflammatory cytokines that includes IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29 [125]. IL-10 was originally discovered as a bioactive molecule secreted from Th2 cells that blocks the activation and cytokine production from Th1 cells [126]. Further investigation has shown that IL-10 influences several important functions of macrophages and monocytes, inhibits the production of IL-1 β and TNF- α , and downregulates the expression of MHC class II and co-stimulatory molecules [127]. Additionally, IL-10 has been shown to play an integral role in scarless wound healing [128]. Peranteau *et al.* investigated the effect of transfecting adult murine stromal cells to overexpress IL-10 on scar healing. It was shown that at 72 hours post-injury, the stromal cells that overexpressed IL-10 failed to display any histological elements of scar formation and regained normal dermal architecture [91]. Recently, the localized delivery of IL-10 from PLGA scaffolds has been shown to modulate the cytokine production from leukocytes [92]. Gower *et al.* examined whether PLGA scaffolds seeded with an IL-10 encoding vector and implanted within the intraperitoneal fat of a murine model was effective at modulating the inflammatory response [92]. The study revealed that IL-10 delivery significantly decreased the number of leukocytes present within the scaffold. In addition, macrophages upregulated IL-10 secretion while dendritic cells, CD4+ T-cells and macrophages down regulated IFN- γ [92].

As discussed previously, macrophages possess the unique ability to switch phenotype based upon cues present in the microenvironment (the M1 versus M2 paradigm) [34,129]. IL-10 is a powerful stimulus for the induction of the M2c macrophage phenotype, which plays a major role in matrix deposition and tissue remodeling [130]. Additionally, M2c macrophages secrete soluble bioactive molecules such as TGF- β and platelet derived growth factor (PDGF) that are critical for the proper regeneration of native tissue [131]. In fact, PDGF derived from macrophages may be a critical source of this growth factor during tissue repair and regeneration [132]. Expanding upon this knowledge, Boehler *et al.* hypothesized that a sustained anti-inflammatory microenvironment would induce, retain and maintain an M2 macrophage response even when challenged with pro-inflammatory cytokines [93]. The study revealed that gene delivery of IL-10 stimulated the transition to an M2 phenotype [93]. Moreover, when IL-10 transfected cells were challenged with pro-inflammatory cytokines they maintained their M2 phenotype while macrophages treated with recombinant IL-10 protein quickly reverted to a pro-inflammatory state [93]. This was characterized by significantly reduced levels of TNF- α at day 6 in the lentiviral treated group when compared to the recombinant IL-10 treated group. This study demonstrated that genetic delivery of IL-10 could compete with pro-inflammatory signals present within the microenvironment to favor an M2 phenotype and thus help to resolve inflammation and further enhance regeneration.

7.2.2. Interleukin-4

IL-4 is an anti-inflammatory mediator that is part of the type I cytokine family. It is secreted by basophils, mast cells, certain phenotypes of CD4+ T-cells, and Th2 cells [133], and it is an important modulator of a wide variety of cell types including T-cells, B-cells, monocytes, macrophages, fibroblasts, hematopoietic progenitors, and endothelial cells [134]. Investigation

into IL-4 has revealed a host of functions such as inducing the differentiation of naïve T-cells, stimulating the production of other cytokines such as IL-5, IL-10 and IL-13, discouraging the migration of pro-inflammatory CD4⁺ IFN- γ secreting cells, and preventing the apoptosis of hematopoietic stem cells [133].

In patients with end-stage arthritis of the hip or lower extremities, total joint replacement is a very successful treatment strategy to alleviate pain and improve daily function [135]. Following implantation all prosthetic surfaces undergo wear-associated damage. This results in particles being generated that can remain in the joint, spread to the regional lymph nodes, or even worse, be released systemically [136]. These particles elicit a chronic inflammatory response, leading to the destruction of bone within the implant bed. Additionally, patients may require additional surgical procedures or suffer from pathological fractures due to implant failure or degradation [40,135]. Rao *et al.* found there was a higher expression of M1 macrophages in the tissue of revision surgeries, compared to synovia from patients undergoing primary joint replacement [137]. Thus, the local delivery of cytokines that influences macrophage plasticity towards the M2 phenotype could suppress the inflammatory reaction and prevent implant failure. To this end, several studies have investigated the delivery of IL-4 to modulate the polarization of macrophages and synthesized bioactive factors [94,134,137–140]. One such study investigating the effects of local delivered IL-4 on human monocytes following *in vitro* challenge to polymethylmethacrylate (PMMA) revealed that IL-4 inhibits the expression of GM-CSF, IL-6, and TNF- α at 48 hours post exposure [134]. These studies strongly suggest that strategies that utilize IL-4 to influence macrophage phenotype may be a viable strategy to reduce the resorption of periprosthetic bone. As previously discussed, not only do M2 macrophages encourage an anti-inflammatory microenvironment but they play an important role in tissue regeneration.

Mokarram *et al.* demonstrated that incorporation of recombinant IL-4 inside polysulfone conduits for peripheral nerve repair polarizes macrophages towards an M2 regenerative phenotype, significantly increases the number of axons by about 20 times, and improves the rate of axon growth when compared to non-cytokine treated and IFN- γ treated groups [95]. The use of anti-inflammatory cytokines to enhance or accelerate tissue regeneration has the potential to enhance the current treatment of several pathological conditions. Although our current knowledge of the critical interaction between tissue regeneration and the immune system for tissue engineering is rather unsophisticated, several *in vivo* studies have exemplified the utility of modulating the inflammatory response by anti-inflammatory cytokines.

7.3. Growth factors

Growth factors can facilitate cellular communication by binding specialized cellular receptors located on target T-cells. After growth factor binding to the target receptor, the cellular response depends upon the specific growth factor, the target T-cell, cell density, receptor type, and other signals present within the microenvironment [141]. Research has revealed that wound healing involves a complex and intricate coordination between the immune system and the tissue cells [83]. Several growth factors such as vascular endothelial growth factor (VEGF), TGF- β and PDGF are responsible for orchestrating wound healing and has been the subject of other reviews [142,143]. TGF- β 1, for example, has been shown to play a critical role in the recruitment of inflammatory cells [104]. In the presence of IL-6, TGF- β 1 stimulates the differentiation of Th17 cells [144]. Additionally, it induces monocytes to increase production of pro-inflammatory cytokines. Given the previously presented material, one might think that TGF- β 1 only acts solely in a pro-inflammatory manner. However, while TGF- β 1 stimulates the

production of IL-1 from monocytes it suppresses the ability of IL-1 to induce lymphocytic proliferation [104]. Additionally, in fracture healing TGF- β 1 secreted from platelets localized to the initial hematoma has been shown to increase the mechanical properties and induce callus formation [97]. Thus, even though TGF- β 1 influences several pro-inflammatory processes it may ultimately protect the host from a prolonged inflammatory state. Furthermore, PDGF in combination with TGF- β 1 has been shown to direct macrophage migration towards the injury site [83]. While biomaterials loaded with various growth factors has been extensively investigated for their *in vivo* potential at regenerating numerous types of tissue, strategies to exploit their immunomodulatory properties are still young.

7.4. Small Bioactive Molecules

Due to several drawbacks associated with exogenous delivery of proteins, such as high production cost, off target protein activity, and purification concerns, the utilization of small immunoregulatory molecules has emerged. The term small molecule refers to a non-protein based bioactive compound that is typically less than 1000 Da [145]. A review by Laurencin *et al.* has examined several *in vitro* and *in vivo* studies on the effects of several small bioactive molecules in the field of bone tissue engineering [146]. This systematic review identified fifteen small molecules that have been investigated for their pre-clinical efficiency to regenerate bone. Sphingosine-1-phosphate (S1P) activates several G-couple protein receptors that are responsible for endothelial cell migration and the recruitment of lymphocytes [105]. Additionally, it has been shown that S1P facilitates the migration of pre-osteoblasts and pre-osteoclasts [105]. In a study by Sefcik *et al.*, S1P was encapsulated in PLGA microspheres and used to study their effect on bone regeneration in a critical-size rat cranial defect [106]. The local delivery of S1P promoted luminal expansion of arterioles and significantly enhanced new bone formation [106]. Other studies have suggested that this effect is due to S1P promoting M2 macrophage phenotype and encourages the recruitment of anti-inflammatory monocytes [40]. The potential utility of small molecule based immunomodulation for tissue engineering is vast and has shown great promise in the field of musculoskeletal tissue engineering. Several reviews have focused on the influence these compounds have on regeneration [145–149]. However, the interaction between these small bioactive molecules and the immune system is only beginning to be understood.

8. Conclusions

As the field of tissue engineering matures, sophisticated biomaterials and delivery strategies have emerged to leverage cells and bioactive molecules for regeneration. The immune system plays a significant role in the normal growth and repair of tissues, and given this knowledge, it is unsurprising that successful tissue regeneration would require optimal modulation of local inflammation. Future directions for immunomodulatory tissue engineering strategies are diverse at this point. While direct controlled delivery of proteins and cells can be challenging, there are an abundance of materials that show promise as carriers. In addition, the search continues for small molecule drugs capable of mediating cell responses *in vivo* and that present fewer challenges for local delivery. Incorporation of these small molecules directly into biodegradable tissue engineering scaffolds, either with or without MSCs, may enhance scaffold-host tissue integration, improve regeneration, and reduce foreign body response. Significant challenges to this ideal remain to be addressed, such as the optimal kinetics of immunomodulatory effector delivery, the appropriate balance of pro- and anti-inflammatory mediators, and further elucidation of the effects of exogenous MSC or other immune cell delivery *in vivo*. The

increasing recognition of the immune system as a major player in tissue regeneration heralds a promising direction for the field, one that recognizes that modulation of the immune system is beneficial not only for growth of new tissue but for practical translation of scaffolds into clinical use.

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