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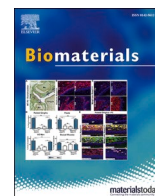
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Harnessing cell-material interactions to control stem cell secretion for osteoarthritis treatment

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ABSTRACT

Osteoarthritis (OA) is the most common debilitating joint disease, yet there is no curative treatment for OA to date. Delivering mesenchymal stromal cells (MSCs) as therapeutic cells to mitigate the inflammatory symptoms associated with OA is attracting increasing attention. In principle, MSCs could respond to the pro-inflammatory microenvironment of an OA joint by the secretion of anti-inflammatory, anti-apoptotic, immunomodulatory and pro-regenerative factors, therefore limiting pain, as well as the disease development. However, the microenvironment of MSCs is known to greatly affect their survival and bioactivity, and using tailored biomaterial scaffolds could be key to the success of intra-articular MSC-based therapies. The aim of this review is to identify and discuss essential characteristics of biomaterial scaffolds to best promote MSC secretory functions in the context of OA. First, a brief introduction to the OA pathophysiology is provided, followed by an overview of the MSC secretory functions, as well as the current limitations of MSC-based therapy. Then, we review the current knowledge on the effects of cell-material interactions on MSC secretion. These considerations allow us to define rational guidelines for next-generation biomaterial design to improve the MSC-based therapy of OA.

1. Introduction

Osteoarthritis (OA) is the most common debilitating joint disease, affecting 10–20% of the population worldwide [1]. It has important socio-economic repercussions with its high prevalence and disabling symptoms, resulting in a total healthcare cost currently estimated to be €400 billion per year in Europe [2]. Previously described as a consequence of aging, it is now established that many factors can induce OA, such as genetic predisposition, abnormal joint biomechanics, traumatic injury, load excess, repetitive joint use, overweight or abnormal bone density [3]. Current OA treatments depend on its severity. For low-to mild-stage OA, the administration of painkillers, nonsteroidal anti-inflammatory drugs (NSAIDs), as well as intra-articular injections of hyaluronic acid (HA) or corticosteroids, can help alleviate the pain. However, these short-lasting treatments do not prevent the progression of the disease. Regarding end-stage OA, the total joint arthroplasty is the gold standard treatment despite being an invasive and expensive surgical procedure [4]. To date, there is no curative treatment to OA, which inevitably progress toward the complete destruction of diseased joint

tissues.

Treating patients with cells that can respond to tissue alterations by secreting therapeutic factors is appealing. In addition to their differentiation capacities [5,6], mesenchymal stromal cells (MSCs) have attracted much attention as therapeutic cells for their capacity to respond to a pro-inflammatory microenvironment by the local release of anti-inflammatory, anti-apoptotic, immunomodulatory and pro-regenerative factors. It was shown that MSCs can mitigate the symptoms of a variety of inflammatory and inflammation-related disorders [7], including OA [8]. However, the conventional delivery of MSCs (in saline) leads to limited cell survival upon injection, cell leakage out of the target tissue/organ, and uncontrolled cell fate [9,10]. It is now commonly accepted that a microenvironment that supports the survival and bioactivity of transplanted cells is key to the therapeutic success. Thus, properly designing a biomaterial scaffold for the intra-articular delivery of cells is instrumental for the future translation of MSC-based OA therapy.

Hydrogels are often considered to be the most relevant class of biomaterials for cell encapsulation and delivery, as they can recapitulate

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most of the physicochemical features of a natural cell microenvironment [11,12]. Hydrogels can also provide cytoprotection and immunoisolation by creating a barrier against immune cells, with the hope of avoiding the use of long-term immunosuppressive therapy [13]. For the encapsulation and delivery of MSCs, practical parameters such as hydrogel precursor sterilizability, mixability with cells, and injectability need to be considered, along with several fundamental parameters including hydrogel cytocompatibility, permeability, and biodegradability. More importantly, it is now established that hydrogel physicochemical properties, especially hydrogel composition (e.g., polymer, adhesive peptides) and mechanical properties (e.g., stiffness, relaxation time), can alter cell functions. Advanced synthetic tools now allow to fine-tune these properties; and recent progress in the understanding of cell-matrix and cell-material interactions provide fundamental resources for the design of hydrogels able to guide the secretions of encapsulated MSCs, and potentially maximize their therapeutic effects. However, the idea of translating fundamental knowledge on cell-material interactions into successful therapeutic approaches is still in its infancy.

The aim of this review is to identify key parameters in biomaterial design to best promote and guide MSC paracrine functions for OA treatment. First, focusing on the pathophysiology of OA and the progress of MSC-based therapies, we will review the most recent literature on MSC secretion, and highlight several factors of particular interest for OA therapy. Then, we will explore the question of the influence of cell-material interactions on encapsulated MSC secretion, allowing us to identify specific mechanical and biochemical properties that promote MSC secretion. These considerations will allow us to define guidelines for the rational design of an injectable hydrogel to deliver and improve the therapeutic efficacy of MSCs for OA treatment.

2. Pathophysiology of OA

The most common sites of OA are the diarthrodial joints (e.g., knee, hip, digits). These joints are mainly composed of two adjacent bones, each covered by a layer of articular cartilage, together surrounded and maintained by a synovial membrane [14]. OA is mainly diagnosed by radiological imaging, and is characterized by articular cartilage degradation, cartilage space narrowing, synovial inflammation, subchondral bone sclerosis, and the formation of osteophytes (i.e., bone spurs), together leading to pain and loss of joint mobility [15–17]. Interestingly, magnetic resonance imaging (MRI) can also be used to reveal structural changes of the soft joint tissues (e.g., meniscus, ligament, synovial membrane) even before radiological OA evidences such as cartilage space narrowing, empathizing the fact that OA is a whole joint disease [18–21].

OA pathological processes have been extensively investigated [22–25]; yet, identifying all of the triggering factors and underlying mechanisms of the disease progression is still a challenge. As one of the main symptoms of OA, articular cartilage degradation has been deeply characterized. At the tissue level, a loss of glycosaminoglycans, proteoglycans, collagens and non-collagenous proteins is observed [26–29], together with an increase in water content resulting in cartilage matrix swelling. The expansion of the deep, calcified cartilage zone is observed, progressively taking over upper cartilage layers [30,31]. Fissures eventually appear, accompanied by exfoliation, leading to a loss of articular cartilage tissue. At the cellular level, chondrocytes, which are mature, non-proliferative cells responsible for cartilage matrix production, endure the disruption of their pericellular matrix, to which the cells are anchored via surface receptors such as integrins, annexins, CD44 or discoidin domain-containing receptor 2 (DDR2) [32,33]. This leads to the expression of proteinases such as serine protease HTRA1, matrix metalloproteinases (MMP-3 & -13) and aggrecanases (ADAMTS-4 and -5), that can actively degrade the matrix [34–37]. With the cartilage matrix degradation, the basal O₂ tension (<1%) in the avascular deep cartilage tends to increase, altering the protecting effect of hypoxia-inducible factor 1 α (HIF-1 α), which is a transcriptional

regulator that is known to promote cell secretion and survival [38,39]. As the cartilage matrix degradation progresses, degradation products such as damage-associated molecular patterns (DAMPs, or alarmins) are produced and can reach other chondrocytes or cells in the surrounding tissues (e.g., synovial membrane), further triggering and amplifying catabolic processes. In particular, synoviocytes can release pro-inflammatory cytokines and reactive oxygen species (ROS), maintaining via a feedback loop the chondrocytes in their catabolic state [14, 40,41]. Among the pro-inflammatory mediators, nuclear factor- κ B (NF- κ B) plays a central role in stress-induced pathways, enhancing the expression of HIF-2 α , which targets genes associated with cartilage destruction and hypertrophic phenotype, such as *MMP-13*, *COL10A1*, *VEGFA* or *RUNX2* [42–44]. Regarding secreted factors, numerous inflammatory cytokines are found at increased levels in the synovial fluid of OA patients, such as tumor necrosis factors (TNFs), interleukins (e.g., IL-1, -6, -8, -15), CC-chemokines ligand-5 and -19 (CCL-5, -19), which play a role in the promotion of abnormal cartilage degradation and in the alteration of the chondrocyte phenotype [45–48]. Moreover, the inflammatory state of OA favors the cellular infiltration of the synovial membrane by macrophages and T-cells, which can participate to the cartilage matrix degradation and chondrocyte apoptosis [49,50]. The inflammation level in OA is fluctuating, with alternating dormant (low level) and acute (high level) phases [51]. The causes of this phenomenon, called flare, remain unclear to date. In addition to the various biological pathways involved in OA, recent findings at the transcriptomic level indicate distinct molecular signatures of OA in cohorts of patients, suggesting a diversity of cellular responses to pathological triggers, and highlighting the complexity of the disease [52]. With complex biological processes, involving inflammation, mechanical and metabolic factors, OA is now commonly considered to be a multifaceted and multifactorial disease [53]. In this context, intra-articular cell therapy, using MSCs in particular, has emerged as one possible approach to mitigate OA and its flare-up, with the hope to ultimately treat the disease.

3. MSC-based therapy for OA: progress and challenges

Adult MSCs have been identified in various tissues (e.g., adipose tissue, bone marrow, peripheral blood) where they were shown to participate to tissue homeostasis. In addition to their self-renewal and their differentiation capacities, adult MSCs can secrete a multitude of bioactive molecules, ranging from soluble cytokines to growth factors and hormones [54,55]. MSC secretion is regulated by MSC interactions with surrounding cells and the extracellular matrix (ECM). These interactions modulate signaling pathways and gene expression, and thus play a major role in cell behavior and immunomodulatory functions [56]. In an injured or inflammatory microenvironment, MSCs can adopt a pro-regenerative phenotype, secreting paracrine immunomodulatory and growth factors [57,58]. In response to pro-inflammatory cytokines (e.g., TNF- α , IL-1 β), MSCs can initiate a cross-talk with immune cells (e.g., T cells, B cells, monocytes/macrophages) via their secretome. Of particular interest for their immunomodulation property, prostaglandin E₂ (PGE₂), nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO) and TNF-inducible gene-6 (TSG-6), have been shown to be secreted by MSCs to regulate the activity of immune cells and the polarization of macrophages [59,60]. For instance, PGE₂ induces a shift from a pro-inflammatory to an anti-inflammatory and pro-regenerative macrophage phenotype, resulting in an increased secretion of IL-10 and an inhibition of various pro-inflammatory cytokines such as TNF- α [61]. MSCs are also able to secrete vascular endothelial growth factor (VEGF), glial-derived neurotrophic factor (GDNF) or hepatocyte growth factor (HGF), in order to stimulate resident cells and create a pro-regenerative microenvironment [55]. In addition to soluble factors, MSCs can exert their immunomodulatory ability through the secretion of extracellular vesicles (EVs) [62,63], which are small particles carrying numerous cellular components (e.g., proteins, nucleic acids,

lipids) with overall anti-fibrotic [64], anti-apoptotic [65], and anti-inflammatory [66] properties. The therapeutic potential of MSCs has led to the investigation of secretion-mediated MSC therapy in the context of a variety of conditions associated with inflammatory disorders, including wound healing [67], cardiovascular disease [68], and graft-versus-host disease [7]. Most of the completed clinical trials involving MSCs confirmed the feasibility and the safety of MSC-based therapies, encouraging further research especially for the treatment of inflammatory and degenerative diseases [7].

From an OA perspective, MSCs could respond to the diverse pathological stimuli of an osteoarthritic joint (Fig. 1). The secretion of immunomodulatory factors (e.g., PGE2, NO, IDO, TSG-6) seems to be highly relevant to regulate the infiltration of immune cells and the inflammation, in particular that of the synovial membrane. Moreover, MSCs could secrete HGF and basic fibroblast growth factor (bFGF), which have been identified as key mediators of the anti-fibrotic response of MSCs [69,70], and were shown to reduce OA chondrocyte dedifferentiation [69]. Furthermore, MSCs could prevent cartilage degradation through the secretion of thrombospondin-1 (THBS1) [71], transforming growth factor (TGF) β -induced protein [72], and EVs [73]. Finally, MSCs could, in theory, respond proportionally to the fluctuating inflammatory signals, over long periods of time, offering an ideal flare-responsive treatment to prevent the disease progression. In general, MSC secretome can exert anti-apoptotic, anti-fibrotic, anti-hypertrophic and immunomodulatory activities [74], which are all appealing properties to influence OA progression.

While the field of joint regenerative medicine has been focused for decades on using MSCs as an alternative source of chondrocytes for cartilage repair [75–78], the recent observation that MSCs can exert a therapeutic effect through the secretion of soluble factors and/or EVs prompted the community to consider the intra-articular injection of MSCs as a new therapeutic approach. Several clinical trials have been performed to assess the regenerative and immunomodulatory potential of MSCs for OA treatment, and were listed in a recent review [8]. In general, pain relief and mobility improvement were observed. However, as reported in the context of other pathologies, the conventional intra-articular injection of MSCs in saline suffers from several limitations. Upon injection, MSCs endure shear forces that typically damage

cell membrane lowering cell viability [9]. MSCs tend also to migrate from the injection site toward the surrounding tissues [10,79], leading to fewer and fewer cells effectively exerting their therapeutic functions at the delivery site over time. Finally, following intra-articular injection, cell fate is no longer controlled, which could hinder clinical translation. In this context, biomaterial scaffolds are more and more considered as key components to improve MSC-based therapies owing to their ability to provide a more suitable microenvironment to the delivered cells [11, 80]. Beyond improving cell survival and limiting cell migration, the recent progress in materials design now allow to foresee the use of scaffolds tailored to maximize MSC therapeutic potential for OA treatment. Yet, developing material-assisted cell therapy requires to fully understand the effects of cell-material interactions on MSC secretion.

4. Cell-material interactions and MSC secretion

The field of material-assisted cell therapy for OA is yet in its infancy, and could greatly benefit from the most recent in vitro discoveries about cell-material interactions. It is now established that the architecture (e.g., porosity, fiber alignment), the mechanical properties (e.g., stiffness, relaxation time), and the composition (e.g., polymer, adhesive peptides) of materials can greatly affect the MSC biological functions, including secretion. In this section, we discuss the effects of cell-material interactions on factor secretion by MSCs (Table 1).

3.1. Biomaterial physical properties and MSC secretion profile

3.1.1. Materials allowing cell spreading and cell-cell interactions enhance MSC secretion

Recent progress in material design allow for tuning different physical characteristics of synthetic scaffolds, such as fiber orientation or porosity, thereby enabling the investigation of their relationship to MSC behavior and immunomodulatory properties [91,99,100]. For example, using electrospinning, fibrous polycaprolactone or polylactic acid scaffolds can be produced with either random or aligned fibers, allowing for the investigation of the effect of fiber alignment [81,82]. It was shown that the conditioned medium retrieved from MSCs seeded on aligned fibers contained higher concentrations of potentially therapeutic factors,

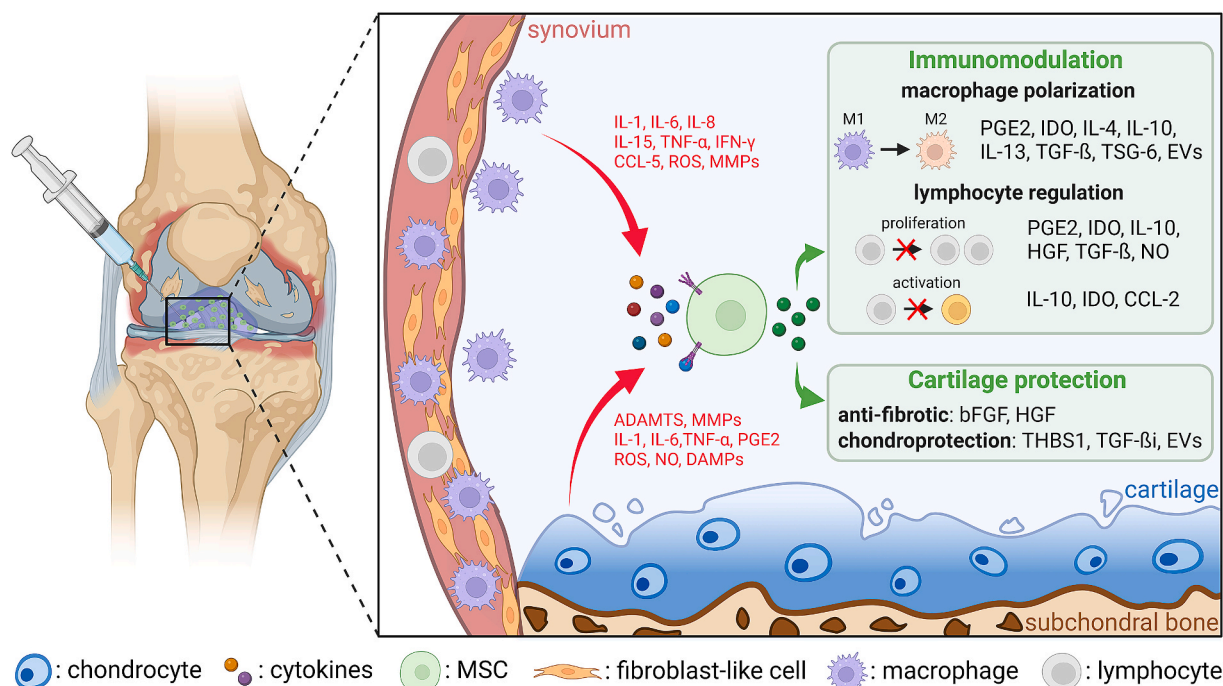


Fig. 1. MSC secretion as a promising approach to prevent OA.

Table 1
Effects of cell-material interactions on factor secretion by MSCs.

Investigated characteristics	Materials	Cell types	Results		Ref
			In vitro	In vivo	
Structure	Random vs aligned electrospun PCL ^a fibers in 2D	Rat ASCs ^b	- More immunomodulatory factors secreted on aligned fibers - Promotion of macrophage polarization and endothelial cell proliferation by conditioned medium from MSCs on aligned fibers	- The conditioned medium retrieved from MSCs seeded on aligned fibers accelerated wound healing in a rat model.	[81]
	Random vs aligned electrospun PLA ^c fibers in 2D	Human ASCs	- More immunomodulatory factors secreted on aligned fibers - Correlation with increased FAK ^d activation and YAP/TAZ ^e signaling		[82]
	Nanoporous (10 nm) vs microporous (125 μm) alginate hydrogels in 3D	Rat BMSCs ^f	- Secretion and cell-cell interactions promoted on microporous scaffolds - Involvement of N-cadherin in the heightened secretion		[83, 84]
	Porous PEG ^g microgels in 3D (13 μm vs 110 μm vs 190 μm)	Human BMSCs	- Higher cell-cell interactions and growth factors secretion with increased porosity		[85]
	Gelatin scaffolds in 3D (400 μm vs 500 μm vs 600 μm)	Human BMSCs	- Cell-cell interactions and secretion maximized by medium pore size (≈500 μm)		[86]
Stiffness	PAAm ^h hydrogels in 2D 0.5 kPa vs 200 kPa	Human MSCs	- More immunomodulatory factors and growth factors secreted on soft hydrogels - Involvement of cytoskeletal rearrangement		[87]
	PAAm hydrogels in 2D 0.15 kPa vs 10 kPa	Human ASCs	- More angiogenic factors secreted on soft hydrogels - Correlation between matrix stiffness, secretion and intracellular ROS ⁱ level		[88]
	Fibrin hydrogels in 3D 2 kPa vs 45 kPa	Human BMSCs (spheroids)	- VEGF ^j secretion promoted by stiff hydrogels - PGE2 ^m secretion promoted by soft hydrogels		[89]
	Alginate hydrogels in 3D 2 kPa vs 35 kPa	Human BMSCs	- More immunomodulatory factors secreted by TNF-α ⁿ -stimulated MSCs in soft hydrogels - Correlation with TNF-α receptors clustering	- Production and chemotaxis of monocytes from MSCs enhanced by soft matrix, in a mouse model.	[90]
Viscoelasticity	Purely elastic vs viscoelastic alginate hydrogels in 3D	Human BMSCs	- Elevated VEGF and endothelial cell proliferation with conditioned medium from MSCs in viscoelastic hydrogels - Elevated COX-2 and TSG-6 gene expression and PGE2 secretion in viscoelastic hydrogels	- Bone regeneration promoted by MSCs in viscoelastic hydrogels, in a rat calvarial defect model	[91]
					[92]
Biochemical cues	HPMC ^o vs alginate hydrogels in 3D	Human ASCs	- Elevated IDO ^p , PGE2, HGF ^q secretion in HPMC vs alginate		[93]
	Collagen VS gelatin hydrogels with or without laminin in 3D	Human BMSCs	- Upregulated secretion of more than 20 growth factors in collagen hydrogels (e.g., VEGF, HGF, TGF-β1) - Further upregulation with laminin inside collagen hydrogel		[94]
	Collagen scaffold, with or without chondroitin sulfate in 3D	Mice BMSCs	- Elevated COX-2, iNOS ^r and TGF-β gene expression with chondroitin sulfate	- Reduced leukocytes infiltration by MSCs encapsulated in collagen and chondroitin sulfate, when subcutaneously implanted in a rat model	[95]
	QK peptide in alginate hydrogels in 3D	Human BMSCs	- Endothelial cell proliferation promoted by conditioned medium from MSCs in QK-modified hydrogels		[91]
	RGD peptide in alginate hydrogels in 3D	Human BMSCs (spheroids)	- VEGF secretion and cell proliferation promoted by RGD-modified hydrogels		[96]
	RGD vs GFOGER peptide in PEG hydrogels in 3D	Human BMSCs	- Elevated VEGF, IL-6 and IL-8 secretion in GFOGER-modified hydrogels compared to RGD	- Greater bone repair with MSCs in GFOGER-modified hydrogels, in a mice radial defect model compared to RGD	[97]
	RGD vs RRETAWA peptide in PEG hydrogels in 3D	Human BMSCs	- Elevated BMP-2 ^s secretion in RRETAWA-modified hydrogels - Greater proliferation in RGD-modified hydrogels		[98]
	HAVDI peptide	Human BMSCs	- Upregulated secretion of more than 70 growth factors for MSCs in HAVDI-modified hydrogels showed		[85]
	Rat BMSCs	- Upregulation of several growth factors (e.g. VEGF, IL-10) for IGF ^t -stimulated MSCs in HAVDI-modified hydrogels - Myoblast migration promoted conditioned medium from IGF-stimulated MSCs in HAVDI-modified hydrogels		[84]	

^jCOX-2, cyclooxygenase-2.

^kTSG-6, TNF-inducible gene-6.

^a PCL, polycaprolactone.

^b ASCs, adipose tissue-derived MSCs.

- ^c PLA, polylactic acid.
- ^d FAK, focal adhesion kinase.
- ^e YAP/TAZ, yes-associated protein/transcriptional coactivator with PDZ-binding motif.
- ^f BMSCs, bone marrow-derived MSCs.
- ^g PEG, polyethylene glycol.
- ^h PAAm, polyacrylamide.
- ⁱ ROS, reactive oxygen species.
- ^l VEGF, vascular endothelial growth factor.
- ^m PGE2 prostaglandin E2.
- ⁿ TNF- α , tumor necrosis factor- α .
- ^o HPMC, hydroxypropylmethylcellulose.
- ^p IDO, indoleamine 2,3-dioxygenase.
- ^q HGF, hepatocyte growth factor.
- ^r iNOS, nitric oxide synthase.
- ^s IL, interleukin.
- ^t BMP-2, bone morphogenic protein-2.
- ^u IGF, insulin-like growth factor.

such as PGE2, NO or HGF, and was able to direct macrophages toward an anti-inflammatory phenotype [81]. The observed higher immunomodulatory functions of MSCs correlated with the activation of focal adhesion kinase (FAK) and the yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) pathways [82,101]. On the contrary, the elongation of MSCs cultured on random fibers is restricted, preventing FAK activation and hampering MSC secretion. Together, these results indicate that MSCs seeded on aligned fibers can adopt a more stretched conformation, resulting in the activation of FAK signaling pathway and the nuclear translocation of YAP/TAZ, which in turn triggers the upregulation of various anti-inflammatory and growth factors.

While pore size plays an obvious role in the diffusion of molecules, with small pores potentially limiting the diffusion of soluble factors [102], several studies have also shown an impact of porosity on cell mechanotransduction and behavior. In this context, new techniques, such as freeze-drying and 3D printing, have been developed to tune the porosity of biomaterials [103–105]. Using a freeze-drying approach, Qazi et al. designed two distinct alginate hydrogels with similar stiffness (20 kPa), having either nanometric (5 nm) or micrometric (120 μ m) pores [83]. After having confirmed that soluble factors were not entrapped in the nanoporous scaffold, the authors observed that MSCs embedded in the microporous scaffold displayed a higher secretion of almost all of the investigated growth factors (e.g., VEGF, HGF, IL-10). The authors further highlighted the role of N-cadherin, a cell-cell adhesion protein, in the heightened secretion activity. MSCs encapsulated in nanoporous hydrogels expressed less N-cadherin than those encapsulated in microporous scaffolds, which resulted in an overall decrease of growth factors secretion. Later on, the authors showed that stimulating MSCs with insulin-like growth factor-1 (IGF-1), which is known to affect MSC functions, amplified MSC secretion only in microporous materials, despite a diffusivity similar to that of the nanoporous scaffold tested [84]. They concluded that microenvironments that allow for cell-cell interactions, and microporous scaffolds in particular, sensitize MSCs to external stimuli (e.g., growth factors) and enhance their paracrine response. In a similar study, Caldwell et al. investigated the role of scaffold porosity in cell secretion, using co-assembled PEG microgels with three distinct levels of microporosity (13 μ m vs 110 μ m vs 190 μ m) [85]. In the most porous hydrogels, MSCs resided as cell clusters, thereby increasing cell-cell interactions and displaying higher concentrations of secreted growth factors. This further confirmed that increasing the porosity of a scaffold in which MSCs are encapsulated can favor cell-cell interactions, thereby enhancing their secretion capacity. It is worth noting that, when increasing porosity, cell density must be carefully considered, at the risk of limiting cell-cell interactions and thereby cell secretion. Indeed, using 3D-printed collagen scaffolds with distinct pore sizes (400 μ m vs 500 μ m vs 600 μ m), it was shown that MSCs in scaffolds with medium pore size (500 μ m) properly aggregated between struts, while MSCs in the most porous scaffolds

preferentially adhered to the polymer rather than forming clusters [86]. The latter cell behavior resulted in a distinct secretion profile, with MSCs displaying lower secretion of several growth factors, such as VEGF and bFGF.

Overall, developing materials that promote specific cell adhesion patterns (e.g., oriented fibers) or cell-cell interactions (e.g., adjusted porosity) seems highly relevant to increase MSC secretion. Besides these two parameters, stiffness has been investigated as one of the key physical cues to influence the composition of the MSC secretome.

3.1.2. Soft hydrogels enhance immunomodulatory properties

The elastic modulus, or Young's modulus, is a measure of stiffness that reflects the ability of a material to resist to deformation upon an applied force. The influence of material stiffness on MSC proliferation or cell fate has been widely investigated. In particular, it was demonstrated that stiffer scaffolds promote MSC osteogenic differentiation while softer scaffolds promote proliferation and chondrogenic differentiation [106–108]. However, only few studies explored the effect of material stiffness on MSC secretome; and, among them, most of the studies were conducted using 2D culture models [87,88,109,110]. In addition, it is known that stiffness also influences other cell functions, such as cell viability and proliferation, affecting the results on secretion and making it difficult to draw proper conclusions. For example, MSCs encapsulated in poly (N-isopropylacrylamide) (PNIPAAm) hydrogels with different shear elastic moduli showed distinct secretion profiles but also distinct proliferation rates, hampering the comprehension of the relationship between stiffness and secretion activity [111].

Overall, soft scaffolds (≤ 5 kPa) showed a tendency to increase MSC secretion compared to stiff scaffolds in 2D culture conditions. Ji et al. cultured MSCs, in the absence of pro-inflammatory factors, on top of two distinct polyacrylamide (PAAm) hydrogels (0.5 kPa vs 200 kPa) functionalized with fibronectin, and observed a greater secretion of immunomodulatory and growth factors (e.g., PGE2, TSG-6, IDO, HGF or bFGF), as well as a greater expression of stemness markers (e.g., *Stro-1*), with the softer hydrogels [87]. The authors attributed this difference to distinct actin polymerization and cytoskeletal organization: on soft PAAm hydrogels, MSCs exhibited a rounded shape associated with a low cytoskeletal tension; whereas, on stiff hydrogels, cells were more elongated and displayed stress fibers. MSCs seeded on stiff hydrogels were further treated with an inhibitor of either actin polymerization (latrunculin A) or cytoskeletal tension (blebbistatin), both leading to the secretion of more soluble factors and an upregulation of stemness markers. The authors concluded that soft hydrogels may enhance MSC secretion through a greater maintenance of cell stemness, implying that differentiated cells lose secretion capacity. Using similar hydrogels, others correlated the reduction of cytoskeletal tension on soft surfaces with a higher concentration of intracellular ROS [88]. They showed that the mechano-regulated overexpression of ROS activates the HIF-1 α pathway, which in turn promotes cell secretion and survival (Fig. 2)

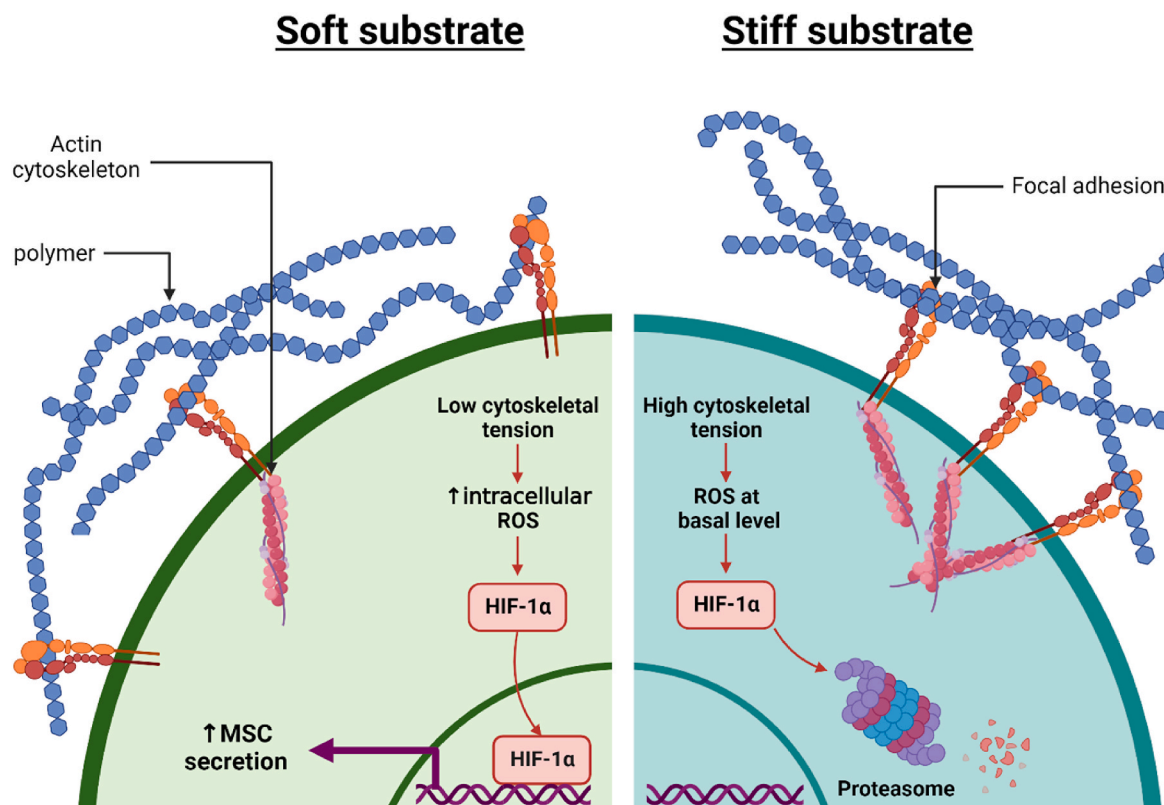


Fig. 2. Proposed mechanism of the influence of stiffness on MSC secretion through the activation of HIF-1 α (adapted from Ref. [88]).

[112]. This mechanism is reminiscent of the effect of hypoxic preconditioning of MSCs, which induces a greater secretion activity compared to normoxic conditions in a ROS-dependent manner [113, 114].

Although limited to few studies, the influence of stiffness on the secretome of MSCs cultured in 3D has also been reported [89,90,92, 111]. MSCs embedded in interpenetrating network hydrogels with shear elastic modulus of either 500 or 2500 Pa, showed no difference in their capacity to secrete PGE2 in the absence of pro-inflammatory factors [92]. This may be attributed to the limited range of shear elastic modulus studied, which may not have allowed to induce significant changes in cell behavior. Interestingly, Murphy et al. showed that MSC spheroids embedded in fibrin hydrogels that were either relatively soft (2 kPa) or stiff (45 kPa), exhibited distinct secretion profiles [89]. MSCs encapsulated in stiff hydrogels secreted preferentially VEGF, while MSCs in soft hydrogels secreted more PGE2. This outcome is extremely relevant for diseases such as OA, where the secretion of anti-inflammatory factors is crucial but that of pro-angiogenic factors might not be appropriate. In another study, the expression of a series of genes (e.g., *IL-6*, *IL-8*, *TSG-6*, *VEGF*) related to the paracrine functions of MSCs was evaluated after TNF- α stimulation, comparing alginate hydrogels with a stiffness of either 2 or 35 kPa [90]. While the expression of angiogenesis-related genes (i.e., *VEGF*, *ANGPT1*) did not differ with stiffness, an upregulation of the expression of some immunomodulation-related genes (e.g., *TSG-6*, *IL-6* and *IL-8*) could be observed with soft hydrogels. The authors further demonstrated that a soft matrix favors the clustering of TNF- α receptors, thereby increasing the binding of TNF- α and the activation of the transcriptional NF- κ B pathway, resulting in the upregulation of immunomodulation-related gene expression. More importantly, these two studies showed a similar increase in immunomodulatory factor secretion by MSCs encapsulated in soft scaffolds, with hydrogels differing in nature (fibrin vs alginate) but having similar levels of stiffness (both studies compared 2 kPa and \approx 40 kPa). This seems to indicate that these results were the sole effect of

changing stiffness in 3D culture, independently of the nature of the polymer. It is noteworthy that these results hardly fit with the aforementioned effects of fiber orientation on MSC secretion. Indeed, while fiber alignment was said to increase cell elongation and subsequently cell secretion, here the use of soft hydrogels led to rounder cells, which also enhanced cell secretion. This discrepancy may result from the different natures of the scaffolds (fibers vs hydrogels), as well as differences in composition, mechanical properties or specific cell-material interactions. It is also plausible that the two distinct mechanisms simply lead to similar results. Obviously, more work will be needed to fully unravel the role of stiffness and elongation in MSC secretion, as well as the underlying mechanisms.

3.1.2.1. Stress-relaxing materials enhance MSC secretion. While the influence of stiffness on cell behavior has become broadly recognized, the impact of the material viscoelasticity is still ambiguous. The viscoelasticity is the ability of a material to display both elastic and viscous properties, thereby dissipating the energy upon deformation in a time-dependent manner (i.e., stress relaxation) [115]. It is a major feature of the natural ECM [116], and its role in cell mechanotransduction has been recently investigated in vitro [117–119]. Viscoelasticity has been correlated with different cellular mechanisms such as cytoskeletal rearrangement, proliferation, differentiation and adhesion [119,120]. However, little is known about the interplay between viscoelasticity and MSC secretion activity. To produce viscoelastic materials, hydrogels in particular, polymers are usually crosslinked by non-covalent or dynamic covalent bonds that allow for network reorganization upon deformation [121,122]. Viscoelasticity is typically characterized by a stress relaxation time, which is the time it takes for a material to dissipate the stored energy after deformation. Hung et al. produced two distinct alginate hydrogels, one ionically-crosslinked (viscoelastic hydrogel) and the other one covalently-crosslinked (elastic hydrogel), displaying similar shear elastic moduli but distinct stress relaxation profiles [123]. The conditioned medium retrieved from MSCs cultured in the viscoelastic

hydrogel contained more VEGF than that from MSCs cultured in the purely elastic hydrogel, which in turn promoted higher endothelial cell proliferation in 2D culture conditions. This example highlights the role of viscoelasticity in the therapeutic potential of MSCs. Using similar alginate-based hydrogels, Vining et al. investigated the effect of stress relaxation on the expression of three immunomodulation-related genes (e.g., *COX-2*, *IL-1RN* and *TSG-6*) by TNF- α -stimulated MSCs [92]. The authors showed that a fast-relaxing hydrogel led to the upregulation of *COX-2* and *TSG-6* genes in combination with a higher secretion of PGE₂, by the encapsulated MSCs. These preliminary results revealed the major role of stress relaxation in MSC secretion capacity.

In general, modifying the physicochemical properties of a scaffold can greatly affect molecular diffusion, thereby impacting secretion measurements performed on supernatants. Yet, most studies have not assessed molecular diffusion, calling for caution regarding conclusions. Overall, porosity, stiffness and relaxation time seem to stand out as key parameters to tune MSC secretion. Porosity seems to mostly passively promote cell-cell interactions, which upregulates secretion, while soft and viscoelastic matrices can be more easily remodeled by encapsulated cells, triggering specific signaling pathways. Scaffold degradability is yet another factor. While it has widely been shown to influence cell behavior (e.g., migration, spreading, fate), only one study explored the role of degradability in MSC paracrine activity, showing a concomitant increase in degradability and secretion [124,125]. This paves the way toward new research on the underlying mechanisms between cell confinement, degradability, and cell secretion.

3.2. Biochemical cues to promote MSC secretion

3.2.1. Polymer composition plays a role yet to be explored

Like any other cells, MSCs have natural biochemical interactions with their surroundings, which affect their biological functions including secretion. However, the influence of scaffold composition on MSC secretion is still largely unknown. To date, we reported the only study that directly compared the effect of hydrogels made of two distinct polysaccharides (i.e., HPMC and alginate) on the secretion of MSCs [93]. We observed higher amounts of various factors of interest (i.e., IDO, PGE₂ and HGF) in the supernatants of cells encapsulated in HPMC hydrogels vs alginate hydrogels. However, the two hydrogels differed in their crosslinking mechanisms, as well as in their mechanical and diffusion properties, which did not allow us to conclude specifically on the influence of the nature of the polymer. To provide further insights on the influence of scaffold composition on MSC secretion, Drzeniek et al. developed collagen- and gelatin-based hydrogels displaying similar mechanical properties. Using a cytokine array, they showed that MSCs encapsulated in collagen-based hydrogels, in comparison to MSCs in gelatin-based hydrogels, secreted higher amounts of growth factors, including VEGF, HGF and TGF- β 1 [94]. Moreover, incorporating laminin in the collagen-based hydrogels further increased the secretion of VEGF, bFGF and leukemia inhibitory factor. Another study investigated the effect of collagen scaffolds, with and without chondroitin sulfate, and reported that the production of NO and PGE₂ increased in the presence of chondroitin sulfate [95]. This potentially highlights the beneficial use of glycosaminoglycans (GAGs) to enhance MSC secretion. Tuning cell-material interactions with bioactive polymers is a promising way to enhance and guide cell secretion. Yet, this avenue of research remains to be explored.

3.2.2. RGD and HAVDI peptides increase MSC survival and secretion

Designing biomaterials to mimic specific cell-ECM or cell-cell interactions is widely accepted as an effective approach to control cell behavior. MSCs both exert traction forces on ECM and interact with other cells through surface receptors, which regulate intracellular signaling pathways [56]. Thus, synthetic peptides inspired from natural ECM or cell surface receptor, are more and more often grafted onto scaffolds to recapitulate natural interactions [126–128]. While the role

of adhesive peptides in MSC proliferation [129] and fate [107,130] has been highlighted, their influence on MSC secretion is still largely unexplored. Various peptides have been investigated as means to modulate MSC secretion. Recently, it was shown that modifying a hydrogel (e.g., PEG, chitosan, alginate) with the RGD peptide, which mimics fibronectin and binds to various integrin receptors, can prolong the survival and retention of encapsulated MSCs in vivo [102,131], as well as enhance VEGF secretion [96]. Other integrin-binding peptides, such as GFOGER (found in type I collagen) or RRETAWA (found in fibronectin), have been shown to promote the osteo-reparative functions of MSCs [97, 98]. Specifically, the GFOGER peptide, which binds to various β 1-containing integrins, was found to increase the secretion of angiogenic and immunomodulatory factors (e.g., VEGF, IL-6, IL-8), promoting macrophage polarization toward an anti-inflammatory phenotype and improving bone repair in vivo. The RRETAWA peptide, which binds to the α 5 β 1 integrin, was found to play an important role in the upregulation of BMP-2 secretion. These results emphasize the role of ECM-activated integrins in the potential therapeutic activity of MSCs. Alternatively, specific transmembrane receptors could also be activated to boost MSC secretion. For instance, by mimicking the VEGF receptor-binding region, the QK peptide could enhance the secretion of proangiogenic factors by the MSCs and the proliferation of endothelial cells [123]. Overall, these studies provide appealing insights on the enhancement of MSC secretion by controlling cell-ECM interaction.

Several studies have also highlighted the upregulated secretion of MSCs when cultured as spheroids rather than in 2D, which was attributed to specific cell-cell interactions [132–135]. These interactions are mediated by transmembrane proteins involved in intercellular communication, and in particular the N-Cadherin protein. Thus, promoting N-Cadherin interactions was explored as a means to increasing MSC secretion. HAVDI is a peptide sequence found in the first extracellular domain of the N-cadherin transmembrane that can be used to recapitulate the N-cadherin protein binding site. To confirm that the HAVDI peptide can mimic N-Cadherin and influence MSC secretion, Caldwell et al. encapsulated MSCs in PEG hydrogels, with or without immobilized HAVDI peptide, and measured the secretion of eighty growth factors [85]. MSCs embedded in the HAVDI-modified hydrogels showed an increased production of almost all quantified growth factors (e.g., IL-10, TGF- β 1, GDNF, VEGF), notably reaching a 10-fold increase in the secretion of more than thirty of them. Using HAVDI-modified alginate hydrogels, others corroborated this upregulation on several secreted growth factors of interest (e.g., VEGF, IL-10) [84]. These results demonstrate the capacity of immobilized peptides to trigger the activation of intracellular signaling pathways on isolated cells that are equivalent to the mechanisms resulting from cell-cell interactions in spheroids. To date, incorporating the HAVDI peptide is one of the most promising approaches to maximize the secretion of anti-inflammatory and growth factors by MSCs.

In general, using biochemical cues, especially binding peptides, to activate specific immunomodulatory signaling pathways constitutes a promising approach to enhance the therapeutic efficacy of MSCs. But many questions remain. Most of the reported studies focused on the secretion of a small number of growth factors, showing a global reduction or increase. However, the secretion of only a specific set of molecules will most likely be necessary for an appropriate therapeutic effect. The feasibility of guiding MSCs toward such specific secretion with biomaterials in a therapeutic context has yet to be shown. This will require wider and more systematic secretome analyses, possibly benefiting from the recent development of omics technologies.

4. Guidelines for a rational material-assisted OA cell therapy

In this section, we discuss the properties of the ideal biomaterial for a material-assisted MSC therapy for OA. Beyond cytocompatibility, designing a delivery vehicle for intra-articular MSC therapy requires to carefully consider the practical aspects of the surgical procedure, the

specificity of an intra-articular cell therapy, and all of the aforementioned cell-material interactions coming into play.

Primarily, the considered materials must enable safe and easy cell encapsulation, necessitating non-toxic chemical tools. Regarding hydrogels, this requires the use of cytocompatible crosslinking mechanisms that are efficient under physiological pH and temperature, without the need for purification. “Click” reactions often meet these criteria and are therefore powerful tools for hydrogel design [136–138]. Ideally, these reactions should also be bioorthogonal, which means that they should not alter or not be altered by biological components and biochemical processes [139]. Bioorthogonal “click” reactions constitute a short list of chemical reactions (e.g., inverse electron-demand Diels-Alder, Strain-promoted azide-alkyne cycloaddition), extensively studied in the field of 3D cell culture and regenerative medicine [140, 141]. These tools offer the advantage to be widely applicable to any natural or synthetic polymers, provide highly tunable materials, and allow for the additional grafting of peptides, drugs and fluorophores. However, using innovative chemical tools comes at the cost of slow clinical translation, requiring dissuasive yet necessary years of validation prior to application. This explains why the current clinical studies focusing on material-assisted cell therapy for OA rarely looked for optimal material design, in favor of scaffolds already on the market. For instance, high molecular weight HA solutions have long been investigated and used as viscosupplements [142], and have therefore been envisioned as potential delivery vehicles for the intra-articular injection of MSCs [143–146]. The association of MSCs and HA led to the temporary reduction of inflammation and OA severity [147,148]; yet, viscous HA solutions lack stability and can hardly be tuned in terms of composition, mechanical properties and specific cell-material interactions, limiting researchers in their rational design. In this context, the ideal material for optimal intra-articular MSC therapy will likely take advantage of the most advanced synthetic tools (Fig. 3).

Regarding the practical aspects of the surgical procedure, the newly designed material should ideally be delivered in a minimally-invasive manner, to avoid open surgery and limit the risks of complications. This requires the use of either injectable materials, i.e., materials that can be formed ahead of surgery and yet be injectable via a fine-gauge needle, or in-situ forming materials, i.e., materials whose viscous precursors can be injected. Pre-formed injectable materials may be more

appropriate and practical as they would allow clinicians to load syringes in advance and use them when desired, without the risk of cell sedimentation or untimely gelation. Suitable injectable hydrogels can be designed using non-covalent [149] or dynamic covalent crosslinking [150]. Alternatively, micro-encapsulation techniques can be used to obtain sufficiently small objects to circumvent any injection issue [151, 152]. Micro-encapsulation also minimizes the distance between the encapsulated MSCs and the host environment. In addition to enabling minimally-invasive surgery, injectable materials have attracted attention to improve cell survival upon injection by reducing the shear forces cells are subjected to [153]. Despite limited and sometimes contradictory literature on this subject, this opens interesting perspectives for cell therapy improvement. Once injected and formed in vivo, the material is expected to physically maintain cells at the injection site, limiting cell leakage and therefore maximizing their local therapeutic effect.

In the context of OA, long-lasting therapeutic effects are desired, with cells responding specifically and periodically to inflammatory flares over months to years. This necessitates materials that are stable for long periods of time, resistant to an inflammatory environment containing enzymes (e.g., matrix metalloproteinases) and ROS, and able to endure the repeated and high mechanical loads constantly exerted on joints. Designing such materials could benefit from the most recent developments in the design of self-healing hydrogels, which have the ability to dissipate energy via temporary bond breaking and network rearrangement [154], and tough hydrogels, which most often make use of interpenetrating networks to reach mechanical strength equivalent to that of cartilage [155]. Designing a long-lasting material that promotes the desired cell-material interactions may be challenging because the presence of degrading enzymes may exclude the use of bioactive polymers, such as hyaluronic acid and collagen, for long-term stability. If successful, such a stable material would provide a suitable environment for cell therapy, allowing for prolonged therapeutic effect and avoiding repeated intra-articular injections. The permeability of the biomaterial must also be taken into consideration in view of a factor-secreting MSC therapy. For efficient OA flare mitigation, the hydrogel must allow the diffusion of molecules over a large range of molecular weights (from ≈ 300 Da to 70 kDa). This includes the diffusion of nutrients and pro-inflammatory molecules from the outside to the inside of the hydrogel, as well as the diffusion of the therapeutic factors and EVs

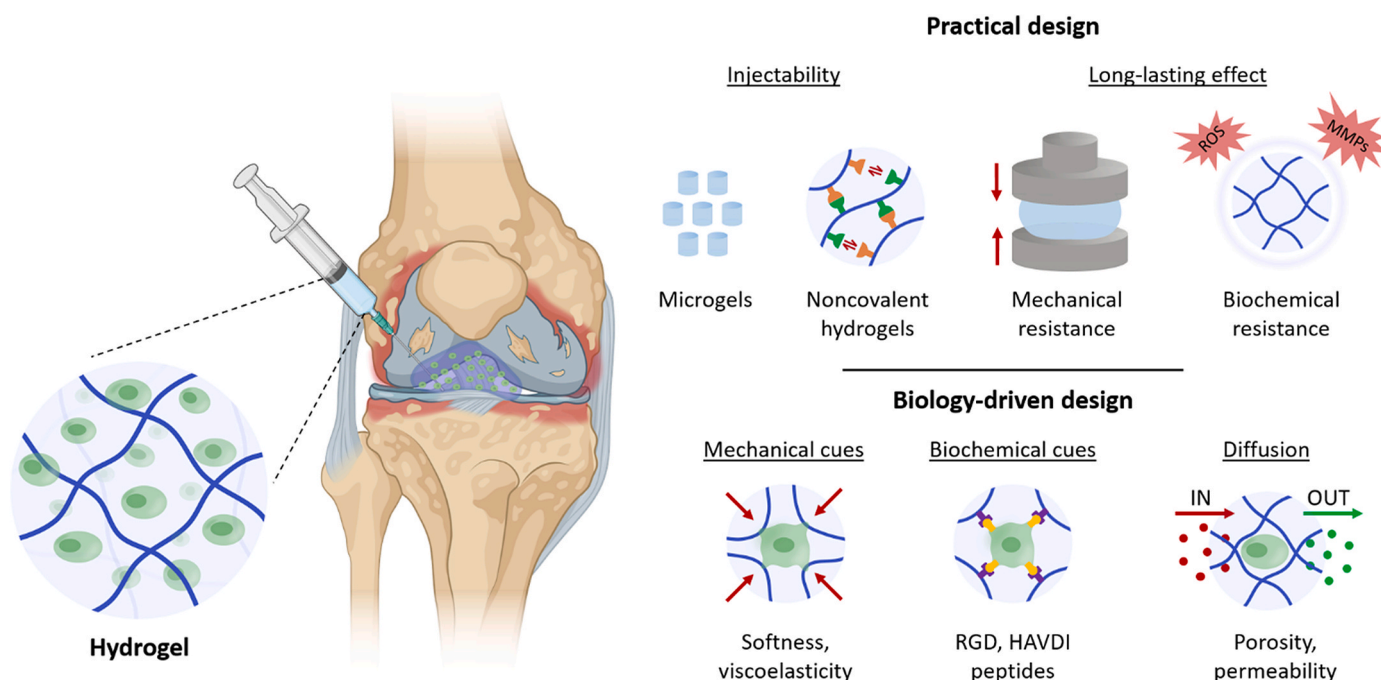


Fig. 3. Guidelines for an intra-articular, material-assisted cell therapy of OA.

secreted by the encapsulated cells toward the surrounding diseased tissues. However, scaffold permeability has not been clearly put into question for MSC delivery to date. It could be anticipated that injecting a large volume of hydrogel may partially hamper the paracrine activity of MSCs; and encapsulating cells into smaller particles might then be more appropriate. While the two approaches have yet to be compared, it was already shown that MSCs encapsulated in microgels can be effectively stimulated with inflammatory factors (TNF- α and INF- γ) to adopt an immunomodulatory phenotype *in vitro* [156]. Finally, controlling cell fate is also crucial for long-lasting effects. Regarding factor secretion, undifferentiated MSCs have been shown to secrete more immunomodulatory factors than chondrocytes and chondrogenically primed MSCs [157], leading to better outcomes on models of osteochondral defect [158,159]. Furthermore, differentiated cells could become immunodetectable, which is undesirable. Thus, for long-lasting factor secretion and safety reasons, the undifferentiated phenotype of MSCs must be maintained. While it is known that microenvironment dictates cell fate, only a handful of articles have investigated the influence of scaffold properties on cell stemness maintenance, calling for further investigations [116, 160,161].

From the basic knowledge on cell-material interactions presented in this review, we can enounce some interesting material features to enhance the factor-secreting cell therapy of OA. In terms of composition, chondroitin sulfate has been correlated with the modulation of MSC immunomodulatory properties. Thus, despite their possible accelerated degradation in the inflammatory environment, GAGs might be more suitable than other polymers as primary building blocks. Considering integrin binding and activation, two peptide sequences, RGD and HAVDI, have been shown to promote cell survival and mimic cell-cell interactions, respectively, and improve MSC therapeutic ability. Although more work on adhesive peptides in the context of OA cell therapy is expected, these two sequences can already be considered. As an alternative to the HAVDI peptide, it is worth noting that cell-cell interactions can be promoted by the encapsulation of spheroids, possibly obviating the need for peptide immobilization and the complex synthetic strategies that come along with it. Regarding the mechanical properties of the envisioned scaffold, we can hypothesize that, for a given biomaterial, it exists an optimal stiffness and/or viscoelasticity to promote appropriate factor secretion by MSCs. Current knowledge suggests that MSC secretion ability is governed by how easily cells can remodel their microenvironment and/or interact with other cells, highlighting the preferable use of soft (≤ 5 kPa) and possibly fast-relaxing hydrogels, which may be compatible with the required capacity to endure repeated loads. Interestingly, the effect of soft and fast-relaxing materials on MSC secretion seems to correlate with elevated concentrations of intracellular ROS, echoing the mechanism by which hypoxia enhances MSC survival and secretion [112–114,162]. Caution should be taken here, as a high dose of ROS is also known to lead to more cell death [163,164]. This highlights the overall need for the right amount of stress on MSCs to maximize their therapeutic effect without affecting basic biological functions. Finally, in addition to soluble factors, MSCs exert their therapeutic functions through the secretion of EVs, which may add new design criteria to the list. The influence of the material composition and matrix mechanics on the secretion, diffusion and therapeutic ability of EVs is largely unknown, opening new avenues of research. As preliminary insights for future material design, it is worth mentioning that HA as a polymer has been correlated with the regulation of EV internalization by fibroblast-like synoviocytes [165]. It was also suggested that increasing the stress relaxation of a hydrogel could facilitate the diffusion of EVs throughout the hydrogel network [166]. These preliminary studies suggest that material design could enhance the therapeutic efficacy of encapsulated EVs, and therefore enhance that of EVs secreted by encapsulated MSCs.

5. Limits and perspectives

A careful analysis of the literature allowed us to unveil what could be the future of material design for material-assisted OA cell therapy. Yet, while the knowledge on MSC secretion and cell-material interactions is burgeoning, our understanding remains limited. The mechanisms behind OA development are complex, and far from being fully unraveled. Various factors (e.g., PGE2, IDO, NO, TSG-6) have been identified as anti-inflammatory, but there are few factors identified as specifically *anti*-OA to date [71,72]. In this context, modulating factor secretion via cell-material interactions may be illusive. Also, if some immunomodulatory and growth factors appear to be relevant for OA, angiogenic and osteogenic factors must be avoided; yet, there is little evidence that systems can be tailored for the secretion of specific factors, with most studies reporting a global increase or decrease of all of the investigated cytokines. This makes specific cytokine secretion a major challenge for OA treatment; and large cytokine arrays and proteomic analyses will have to be carefully and systematically carried out to confirm and adjust the effect of any newly designed material. More importantly, maximizing at any costs the secretion of factors by MSCs under inflammatory conditions could eventually lead to detrimental overshooting treatments, emphasizing the need for controlling the dose secreted in time and space, which is beyond current technological capabilities. This also highlights the need for materials that can help cells to stop secreting factors between inflammation flares, which has not been investigated in the context of OA. Finally, regarding the investigation of the desired biological outcomes, results must be carefully put into perspective with other biological mechanisms that could alter our understanding, such as cell viability, proliferation and differentiation, all known to be highly dependent on scaffold properties. Thus, only systematically mapping all of the critical biological processes will allow to draw the careful conclusions required to move toward the clinic. Overall, unprecedented collaborations between material scientists, biologists and clinicians will be needed to tackle a biomedical challenge that is yet to be truly explored.

6. Conclusion

In this review, we have highlighted how biomaterial design could be a key to successfully translate MSC-based OA therapy to the clinic. We particularly emphasized the need to better understand and control cell-material interactions, along with more practical aspects such as the injectability and stability of the delivery system. Interestingly, these concepts can be easily transposed to MSC therapy for any other immune or inflammatory disorder. However, the question of disease-specific MSC secretion remains to be addressed, and the community still has to answer whether paracrine functions should and/or can be precisely tuned. Until then, MSCs will be used as another “therapeutic black box”, limiting their translation and applications. While tremendous efforts need to be made, the successful design of material-assisted cell therapies will open the way to more futuristic programmable cell therapy, where cell bioengineering combined with innovative delivery vehicles will allow cell secretion on demand, controlled in time and space.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

- [1] S.R. Jafarzadeh, D.T. Felson, Updated estimates suggest a much higher prevalence of arthritis in US adults than previous ones, *Arthritis Rheumatol.* (2018), 70176, <https://doi.org/10.1016/j.physbeh.2017.03.040>, 185–192.
- [2] J.H. Salmon, A.C. Rat, J. Sellam, M. Michel, J.P. Eschard, F. Guillemin, D. Jolly, B. Fautrel, Economic impact of lower-limb osteoarthritis worldwide: a systematic review of cost-of-illness studies, *Osteoarthritis Cartilage* 24 (2016) 1500–1508, <https://doi.org/10.1016/j.joca.2016.03.012>.
- [3] J.W.J. Bijlsma, F. Berenbaum, F.P.J.G. Lafeber, Osteoarthritis: an update with relevance for clinical practice, *Lancet* 377 (2011) 2115–2126, [https://doi.org/10.1016/S0140-6736\(11\)60243-2](https://doi.org/10.1016/S0140-6736(11)60243-2).
- [4] N. de l'Escalopier, P. Anract, D. Biau, Surgical treatments for osteoarthritis, *Ann. Phys. Rehabil. Med.* 59 (2016) 227–233, <https://doi.org/10.1016/j.rehab.2016.04.003>.
- [5] A.I. Caplan, Mesenchymal stem cells, *J. Orthop. Res.* 9 (1991) 641–650, <https://doi.org/10.1201/b13978>.
- [6] M.F. Pittenger, D.E. Discher, B.M. Péault, D.G. Phinney, J.M. Hare, A.I. Caplan, Mesenchymal stem cell perspective: cell biology to clinical progress, *Npj Regen. Med.* 4 (2019) 22, <https://doi.org/10.1038/s41536-019-0083-6>.
- [7] Y. Shi, Y. Wang, Q. Li, K. Liu, J. Hou, C. Shao, Y. Wang, Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases, *Nat. Rev. Nephrol.* 14 (2018) 493–507, <https://doi.org/10.1038/s41581-018-0023-5>.
- [8] F. Hached, C. Vinatier, C. Le Visage, H. Gondé, J. Guicheux, G. Grimandi, A. Billon-Chabaud, Biomaterial-assisted cell therapy in osteoarthritis: from mesenchymal stem cells to cell encapsulation, *Best Pract. Res. Clin. Rheumatol.* 31 (2017) 730–745, <https://doi.org/10.1016/j.berh.2018.05.002>.
- [9] A.A. Foster, L.M. Marquardt, S.C. Heilshorn, The diverse roles of hydrogel mechanics in injectable stem cell transplantation, *Curr. Opin. Chem. Eng.* 15 (2017) 15–23, <https://doi.org/10.1016/j.coche.2016.11.003>.
- [10] K. Toupet, M. Maumus, J.A. Peyrafitte, P. Bourin, P.L.E.M. Van Lent, R. Ferreira, B. Orsetti, N. Pirot, L. Castella, C. Jorgensen, D. Noël, Long-term detection of human adipose-derived mesenchymal stem cells after intraarticular injection in SCID mice, *Arthritis Rheum.* 65 (2013) 1786–1794, <https://doi.org/10.1002/art.37960>.
- [11] S.R. Caliani, J.A. Burdick, A practical guide to hydrogels for cell culture, *Nat. Methods* 13 (2016) 405–414, <https://doi.org/10.1038/nmeth.3839>.
- [12] C.M. Madl, S.C. Heilshorn, Engineering hydrogel microenvironments to recapitulate the stem cell niche, *Annu. Rev. Biomed. Eng.* 20 (2018) 21–47, <https://doi.org/10.1146/annurev-bioeng-062117-120954>.
- [13] S. Mazzitelli, L. Capretto, F. Quinci, R. Piva, C. Nastruzzi, Preparation of cell-encapsulation devices in confined microenvironment, *Adv. Drug Deliv. Rev.* 65 (2013) 1533–1555, <https://doi.org/10.1016/j.addr.2013.07.021>.
- [14] S.R. Goldring, M.B. Goldring, Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage bone crosstalk, *Nat. Rev. Rheumatol.* 12 (2016) 632–644, <https://doi.org/10.1038/nrrheum.2016.148>.
- [15] T.D. Spector, C. Cooper, Radiographic assessment of osteoarthritis in population studies: whether kelly and lawrence? *Osteoarthritis Cartilage* 1 (1993) 203–206, [https://doi.org/10.1016/S1063-4584\(05\)80325-5](https://doi.org/10.1016/S1063-4584(05)80325-5).
- [16] K.B. Gupta, J. Duryea, B.N. Weissman, Radiographic evaluation of osteoarthritis, *Radiol. Clin.* 42 (2004) 11–41, [https://doi.org/10.1016/S0033-8389\(03\)00169-6](https://doi.org/10.1016/S0033-8389(03)00169-6).
- [17] S.A. Mazza, K.D. Brandt, P.A. Dieppe, M. Doherty, B.P. Katz, K.A. Lane, Effect of alignment of the medial tibial plateau and x-ray beam on apparent progression of osteoarthritis in the standing anteroposterior knee radiograph, *Arthritis Rheum.* 44 (2001) 1786–1794, [https://doi.org/10.1002/1529-0131\(200108\)44:8<1786::AID-ART315>3.0.CO;2-L](https://doi.org/10.1002/1529-0131(200108)44:8<1786::AID-ART315>3.0.CO;2-L).
- [18] D. Hayashi, A. Guermazi, C.K. Kwok, Clinical and translational potential of MRI evaluation in knee osteoarthritis, *Curr. Rheumatol. Rep.* 16 (2014) 391–406, <https://doi.org/10.1007/s11926-013-0391-6>.
- [19] D. Hayashi, F.W. Roemer, A. Guermazi, Imaging for osteoarthritis, *Ann. Phys. Rehabil. Med.* 59 (2016) 161–169, <https://doi.org/10.1016/j.rehab.2015.12.003>.
- [20] A. Guermazi, H. Alizai, M.D. Crema, S. Trattnig, R.R. Regatte, F.W. Roemer, Compositional MRI techniques for evaluation of cartilage degeneration in osteoarthritis, *Osteoarthritis Cartilage* 23 (2015) 1639–1653, <https://doi.org/10.1016/j.joca.2015.05.026>.
- [21] A. Guermazi, F.W. Roemer, I.K. Haugen, M.D. Crema, D. Hayashi, MRI-based semiquantitative scoring of joint pathology in osteoarthritis, *Nat. Rev. Rheumatol.* 9 (2013) 236–251, <https://doi.org/10.1038/nrrheum.2012.223>.
- [22] S. Glyn-Jones, A.J.R. Palmer, R. Agricola, A.J. Price, T.L. Vincent, H. Weinans, A. J. Carr, Osteoarthritis, *Lancet* 386 (2015) 376–387, [https://doi.org/10.1016/S0140-6736\(14\)60802-3](https://doi.org/10.1016/S0140-6736(14)60802-3).
- [23] B. Xia, Di Chen, J. Zhang, S. Hu, H. Jin, P. Tong, Osteoarthritis pathogenesis: a review of molecular mechanisms, *Calcif. Tissue Int.* 95 (2014) 495–505, <https://doi.org/10.1007/s00223-014-9917-9>.
- [24] L. Roseti, G. Desando, C. Cavallo, M. Petretta, B. Grigolo, Articular cartilage regeneration in osteoarthritis, *Cells* 8 (2019) 1305, <https://doi.org/10.3390/cells8111305>.
- [25] E.G.J. Ripmeester, U.T. Timur, M.M.J. Caron, T.J.M. Welting, Recent insights into the contribution of the changing hypertrophic chondrocyte phenotype in the development and progression of osteoarthritis, *Front. Bioeng. Biotechnol.* 6 (2018), <https://doi.org/10.3389/fbioe.2018.00018>.
- [26] S. Chen, P. Fu, H. Wu, M. Pei, Meniscus, articular cartilage and nucleus pulposus: a comparative review of cartilage-like tissues in anatomy, development and function, *Cell Tissue Res.* 370 (2017) 53–70, <https://doi.org/10.1007/s00441-017-2613-0>.
- [27] T.J. McKee, G. Perlman, M. Morris, S.V. Komarova, Extracellular matrix composition of connective tissues: a systematic review and meta-analysis, *Sci. Rep.* 9 (2019) 1–15, <https://doi.org/10.1038/s41598-019-46896-0>.
- [28] T.M. Quinn, H.J. Häuselmann, N. Shintani, E.B. Hunziker, Cell and matrix morphology in articular cartilage from adult human knee and ankle joints suggests depth-associated adaptations to biomechanical and anatomical roles, *Osteoarthritis Cartilage* 21 (2013) 1904–1912, <https://doi.org/10.1016/j.joca.2013.09.011>.
- [29] A.J. Sophia Fox, A. Bedi, S.A. Rodeo, The basic science of articular cartilage: structure, composition, and function, *Sport Health* 1 (2009) 461–468, <https://doi.org/10.1177/1941738109350438>.
- [30] D.B. Burr, Anatomy and physiology of the mineralized tissues: role in the pathogenesis of osteoarthritis, *Osteoarthritis Cartilage* 12 (2004) 20–30, <https://doi.org/10.1016/j.joca.2003.09.016>.
- [31] D.B. Burr, M.B. Schaffler, The involvement of subchondral mineralized tissues in osteoarthritis: quantitative microscopic evidence, *Microsc. Res. Tech.* 37 (1997) 343–357, [https://doi.org/10.1002/\(SICI\)1097-0029\(19970515\)37:4<343::AID-JEMT9>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<343::AID-JEMT9>3.0.CO;2-L).
- [32] R.F. Loeser, Integrins and chondrocyte-matrix interactions in articular cartilage, *Matrix Biol.* 39 (2014) 11–16, <https://doi.org/10.1016/j.matbio.2014.08.007>.
- [33] T. Pap, J. Bertrand, Syndecans in cartilage breakdown and synovial inflammation, *Nat. Rev. Rheumatol.* 9 (2013) 43–55, <https://doi.org/10.1038/nrrheum.2012.178>.
- [34] S. Honsawek, S. Malila, P. Yuktanandana, A. Tanavalee, B. Deepaisarsakul, J. Parvizi, Association of MMP-3 (-1612 5A/6A) polymorphism with knee osteoarthritis in Thai population, *Rheumatol. Int.* 33 (2013) 435–439, <https://doi.org/10.1007/s00296-012-2371-y>.
- [35] N.H. Lim, E. Meinjohann, M. Meldal, G. Bou-Gharios, H. Nagase, In vivo imaging of MMP-13 activity in the murine destabilised medial meniscus surgical model of osteoarthritis, *Osteoarthritis Cartilage* 22 (2014) 862–868, <https://doi.org/10.1016/j.joca.2014.04.006>.
- [36] L. Troeberg, H. Nagase, Proteases involved in cartilage matrix degradation in osteoarthritis, *Biochim. Biophys. Acta, Proteins Proteomics* 1824 (2012) 133–145, <https://doi.org/10.1016/j.bbapap.2011.06.020>.
- [37] P. Verma, K. Dalal, ADAMTS-4 and ADAMTS-5: key enzymes in osteoarthritis, *J. Cell. Biochem.* 112 (2011) 3507–3514, <https://doi.org/10.1002/jcb.23298>.
- [38] C. Maes, E. Araldi, K. Haigh, R. Khatri, R. Van Looveren, A.J. Giaccia, J.J. Haigh, G. Carmeliet, E. Schipani, VEGF-independent cell-autonomous functions of HIF-1 α regulating oxygen consumption in fetal cartilage are critical for chondrocyte survival, *J. Bone Miner. Res.* 27 (2012) 596–609, <https://doi.org/10.1002/jbmr.1487>.
- [39] S. Hu, C. Zhang, L. Ni, C. Huang, D. Chen, K. Shi, H. Jin, K. Zhang, Y. Li, L. Xie, M. Fang, G. Xiang, X. Wang, J. Xiao, Stabilization of HIF-1 α alleviates osteoarthritis via enhancing mitophagy, *Cell Death Dis.* 11 (2020) 481–497, <https://doi.org/10.1038/s41419-020-2680-0>.
- [40] R. Liu-Bryan, R. Terkeltaub, Emerging regulators of the inflammatory process in osteoarthritis, *Nat. Rev. Rheumatol.* 11 (2015) 35–44, <https://doi.org/10.1038/nrrheum.2014.162>.
- [41] C.R. Scanzello, S.R. Goldring, The role of synovitis in osteoarthritis pathogenesis, *Bone* 51 (2012) 249–257, <https://doi.org/10.1016/j.bone.2012.02.012>.
- [42] S. Yang, J. Kim, J.-H. Ryu, H. Oh, C.-H. Chun, B.J. Kim, B.H. Min, J.-S. Chun, Hypoxia-inducible factor-2 α is a catabolic regulator of osteoarthritic cartilage destruction, *Nat. Med.* 16 (2010) 687–693, <https://doi.org/10.1038/nm.2153>.
- [43] T. Saito, A. Fukai, A. Mabuchi, T. Ikeda, F. Yano, S. Ohba, N. Nishida, T. Akune, N. Yoshimura, T. Nakagawa, K. Nakamura, K. Tokunaga, U. Chung, H. Kawaguchi, Transcriptional regulation of endochondral ossification by HIF-2 α during skeletal growth and osteoarthritis development, *Nat. Med.* 16 (2010) 678–686, <https://doi.org/10.1038/nm.2146>.
- [44] L.J. Sandell, Etiology of osteoarthritis: genetics and synovial joint development, *Nat. Rev. Rheumatol.* 8 (2012) 77–89, <https://doi.org/10.1038/nrrheum.2011.199>.
- [45] M.B. Goldring, S.R. Goldring, Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis, *Ann. N. Y. Acad. Sci.* 1192 (2010) 230–237, <https://doi.org/10.1111/j.1749-6632.2009.05240.x>.
- [46] K. Takebe, M. Rai, E. Schmidt, L. Sandell, The chemokine receptor CCR5 plays a role in post-traumatic cartilage loss in mice, but does not affect synovium and bone, *Osteoarthritis Cartilage* 23 (2015) 454–461.
- [47] C.R. Scanzello, E. Umoh, F. Pessler, C. Diaz-Torne, T. Miles, E. DiCarlo, H. G. Potter, L. Mandl, R. Marx, S. Rodeo, S.R. Goldring, M.K. Crow, Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stage disease, *Osteoarthritis Cartilage* 17 (2009) 1040–1048, <https://doi.org/10.1016/j.joca.2009.02.011>.

- [48] J.M. Cuellar, G.J. Scuderi, V.G. Cuellar, S.R. Golish, D.C. Yeomans, Diagnostic utility of cytokine biomarkers in the evaluation of acute knee pain, *J. Bone Jt. Surg. Am.* 91 (2009) 2313–2320, <https://doi.org/10.1206/JBJS.H.00835>.
- [49] M.B. Goldring, M. Otero, Inflammation in osteoarthritis, *Curr. Opin. Rheumatol.* 23 (2011). https://journals.lww.com/co-rheumatology/Fulltext/2011/09000/Inflammation_in_osteoarthritis.12.aspx.
- [50] J. Bondeson, S.D. Wainwright, S. Lauder, N. Amos, C.E. Hughes, The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis, *Arthritis Res. Ther.* 8 (2006) 1–12, <https://doi.org/10.1186/ar2099>.
- [51] N. Joshi, J. Yan, S. Levy, S. Bhagchandani, K.V. Slaughter, N.E. Sherman, J. Amiraault, Y. Wang, L. Riegel, A. He, T.S. Rui, M. Valic, P.K. Vemula, O. R. Miranda, O. Levy, E.M. Gravalles, A.O. Aliprantis, J. Ermann, J.M. Karp, Towards an arthritis flare-responsive drug delivery system, *Nat. Commun.* 9 (2018) 1–11, <https://doi.org/10.1038/s41467-018-03691-1>.
- [52] C. Yuan, Z. Pan, K. Zhao, J. Li, Z. Sheng, X. Yao, H. Liu, X. Zhang, Y. Yang, D. Yu, Y. Zhang, Y. Xu, Z.-Y. Zhang, T. Huang, W. Liu, H. Ouyang, Classification of four distinct osteoarthritis subtypes with a knee joint tissue transcriptome atlas, *Bone Res* 8 (2020) 38–48, <https://doi.org/10.1038/s41413-020-00109-x>.
- [53] C.G. Boer, K. Hatzikotoulas, L. Southam, L. Stefánsdóttir, Y. Zhang, R. Coutinho de Almeida, T.T. Wu, J. Zheng, A. Hartley, M. Teder-Laving, A.H. Skogholt, C. Terao, E. Zengini, G. Alexiadis, A. Barysenka, G. Björnsdóttir, M.E. Gabrielsen, A. Gilly, T. Ingvarsson, M.B. Johnsen, H. Jonsson, M. Kloppenburg, A. Luetge, S. H. Lund, R. Mägi, M. Mangino, R.R.G.H.H. Nelissen, M. Shivakumar, J. Steinberg, H. Takuwa, L.F. Thomas, M. Tuerlings, G.C. Babis, J.P.Y. Cheung, J.H. Kang, P. Kraft, S.A. Lietman, D. Samartzis, P.E. Slagboom, K. Stefansson, U. Thorsteinsdóttir, J.H. Tobias, A.G. Uitterlinden, B. Winsvold, J.-A. Zwart, G. Davey Smith, P.C. Sham, G. Thorleifsson, T.R. Gaunt, A.P. Morris, A.M. Valdes, A. Tsezou, K.S.E. Cheah, S. Ikegawa, K. Hveem, T. Esko, J.M. Wilkinson, I. Meulenbelt, M.T.M. Lee, J.B.J. van Meurs, U. Styrkársdóttir, E. Zeggini, Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations, *Cell* 184 (2021) 4784–4818.e17, <https://doi.org/10.1016/j.cell.2021.07.038>.
- [54] M. Gnechi, P. Danieli, G. Malpasso, M.C. Ciuffreda, Paracrine mechanisms of mesenchymal stem cells in tissue repair, *Methods Mol. Biol.* (2016) 123–146, https://doi.org/10.1007/978-1-4939-3584-0_7.
- [55] O. Levy, R. Kuai, E.M.J. Siren, D. Bhere, Y. Milton, N. Nissar, M. de Biasio, M. Heinelt, B. Reeve, R. Abdi, M. Alturki, M. Fallatah, A. Almalik, A.H. Alhasan, K. Shah, J.M. Karp, Shattering barriers toward clinically meaningful MSC therapies, *Sci. Adv.* 6 (2020) 1–19, <https://doi.org/10.1126/sciadv.aba6884>.
- [56] K.H. Vining, D.J. Mooney, Mechanical forces direct stem cell behaviour in development and regeneration, *Nat. Rev. Mol. Cell Biol.* 18 (2017) 728–742, <https://doi.org/10.1038/nrm.2017.108.Mechanical>.
- [57] A.I. Caplan, D. Correa, The MSC: an injury drugstore, *Cell Stem Cell* 9 (2011) 11–15, <https://doi.org/10.1016/j.stem.2011.06.008>.
- [58] A.I. Caplan, J.E. Dennis, Mesenchymal stem cells as trophic mediators, *J. Cell. Biochem.* 98 (2006) 1076–1084, <https://doi.org/10.1002/jcb.20886>.
- [59] D. Polchert, J. Sobinsky, G. Douglas, M. Kidd, A. Moadsiri, E. Reina, K. Genrich, S. Mehrotra, S. Setty, B. Smith, A. Bartholomew, IFN- γ activation of mesenchymal stem cells for treatment and prevention of graft versus host disease, *Eur. J. Immunol.* 38 (2008) 1745–1755, <https://doi.org/10.1002/eji.200738129>.
- [60] N. Song, M. Scholtemeijer, K. Shah, Mesenchymal stem cell immunomodulation: mechanisms and therapeutic potential, *Trends Pharmacol. Sci.* 41 (2020) 653–664, <https://doi.org/10.1016/j.tips.2020.06.009>.
- [61] C. Manferdini, F. Paoletta, E. Gabusi, L. Gambari, A. Piacentini, G. Filardo, S. Fleury-Cappellero, A. Barbero, M. Murphy, G. Lisignoli, Adipose stromal cells mediated switching of the pro-inflammatory profile of M1-like macrophages is facilitated by PGE₂: in vitro evaluation, *Osteoarthritis Cartilage* 25 (2017) 1161–1171, <https://doi.org/10.1016/j.joca.2017.01.011>.
- [62] R. Kalluri, V.S. LeBleu, The biology, function, and biomedical applications of exosomes, *Science* 300 (2020) 367, <https://doi.org/10.1126/science.aau6977>.
- [63] Z.G. Zhang, B. Buller, M. Chopp, Exosomes — beyond stem cells for restorative therapy in stroke and neurological injury, *Nat. Rev. Neurol.* 15 (2019) 193–203, <https://doi.org/10.1038/s41582-018-0126-4>.
- [64] T. Li, Y. Yan, B. Wang, H. Qian, X. Zhang, L. Shen, M. Wang, Y. Zhou, W. Zhu, W. Li, W. Xu, Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis, *Stem Cell. Dev.* 22 (2013) 845–854, <https://doi.org/10.1089/scd.2012.0395>.
- [65] S. Bruno, C. Grange, F. Collino, M.C. Deregibus, V. Cantaluppi, L. Biancone, C. Tetta, G. Camussi, Microvesicles derived from mesenchymal stem cells enhance survival in a lethal model of acute kidney injury, *PLoS One* 7 (2012), <https://doi.org/10.1371/journal.pone.0033115>.
- [66] B. Zhang, Y. Yin, R.C. Lai, S.S. Tan, A.B.H. Choo, S.K. Lim, Mesenchymal stem cells secrete immunologically active exosomes, *Stem Cell. Dev.* 23 (2014) 1233–1244, <https://doi.org/10.1089/scd.2013.0479>.
- [67] J. Dittmer, B. Leyh, Paracrine effects of stem cells in wound healing and cancer progression (review), *Int. J. Oncol.* 45 (2014) 1789–1798, <https://doi.org/10.3892/ijo.2014.2385>.
- [68] S.H. Ranganath, O. Levy, M.S. Inamdar, J.M. Karp, Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease, *Cell Stem Cell* 10 (2012) 244–258, <https://doi.org/10.1016/j.stem.2012.02.005>.
- [69] M. Maumus, C. Manferdini, K. Toupet, J.A. Peyrafitte, R. Ferreira, A. Facchini, E. Gabusi, P. Bourin, C. Jorgensen, G. Lisignoli, D. Noël, Adipose mesenchymal stem cells protect chondrocytes from degeneration associated with osteoarthritis, *Stem Cell Res.* 11 (2013) 834–844, <https://doi.org/10.1016/j.scr.2013.05.008>.
- [70] H. Suga, H. Eto, T. Shigeura, K. Inoue, N. Aoi, H. Kato, S. Nishimura, I. Manabe, K. Gonda, K. Yoshimura, IFATS collection: fibroblast growth factor-2-induced hepatocyte growth factor secretion by adipose-derived stromal cells inhibits postinjury fibrogenesis through a c-jun N-terminal kinase-dependent mechanism, *Stem Cell.* 27 (2009) 238–249, <https://doi.org/10.1634/stemcells.2008-0261>.
- [71] M. Maumus, C. Manferdini, K. Toupet, P. Chuchana, L. Casteilla, M. Gachet, C. Jorgensen, G. Lisignoli, D. Noël, Thrombospondin-1 partly mediates the cartilage protective effect of adipose-derived mesenchymal stem cells in osteoarthritis, *Front. Immunol.* 8 (2017), <https://doi.org/10.3389/fimmu.2017.01638>.
- [72] M. Ruiz, K. Toupet, M. Maumus, P. Rozier, C. Jorgensen, D. Noël, TGFB1 secreted by mesenchymal stromal cells ameliorates osteoarthritis and is detected in extracellular vesicles, *Biomaterials* 226 (2020), 119544, <https://doi.org/10.1016/j.biomaterials.2019.119544>.
- [73] S. Cosenza, M. Ruiz, K. Toupet, C. Jorgensen, D. Noël, Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis, *Sci. Rep.* 7 (2017) 1–12, <https://doi.org/10.1038/s41598-017-15376-8>.
- [74] P. Mancuso, S. Raman, A. Glynn, F. Barry, J.M. Murphy, Mesenchymal stem cell therapy for osteoarthritis: the critical role of the cell secretome, *Front. Bioeng. Biotechnol.* 7 (2019) 1–9, <https://doi.org/10.3389/fbioe.2019.00009>.
- [75] U. Nöth, A.F. Steinert, R.S. Tuan, Technology Insight: adult mesenchymal stem cells for osteoarthritis therapy, *Nat. Clin. Pract. Rheumatol.* 4 (2008) 371–380, <https://doi.org/10.1038/ncprheum0816>.
- [76] A.R. Armiento, M.J. Stoddart, M. Alini, D. Eglin, Biomaterials for articular cartilage tissue engineering: learning from biology, *Acta Biomater.* 65 (2018) 1–20, <https://doi.org/10.1016/j.actbio.2017.11.021>.
- [77] L. Zhang, J. Hu, K.A. Athanasios, The role of tissue engineering in articular cartilage repair and regeneration, *Crit. Rev. Biomed. Eng.* 37 (2009) 1–57, <https://doi.org/10.1615/CritRevBiomedEng.v37.i1-2.10>.
- [78] J. Yang, Y.S. Zhang, K. Yue, A. Khademhosseini, Cell-laden hydrogels for osteochondral and cartilage tissue engineering, *Acta Biomater.* 57 (2017) 1–25, <https://doi.org/10.1016/j.actbio.2017.01.036>.
- [79] D.M. Jensen, Diagnosis and treatment of definitive diverticular hemorrhage (DDH), *Am. J. Gastroenterol.* 113 (2018) 1570–1573, <https://doi.org/10.1038/s41395-018-0061-4>.
- [80] C.-Y. Lin, Y.-L. Wang, Y.-J. Chen, C.-T. Ho, Y.-H. Chi, L.Y. Chan, G.-W. Chen, H.-C. Hsu, D.W. Hwang, H.-C. Wu, S.-C. Hung, Collagen-binding peptides for the enhanced imaging, lubrication and regeneration of osteoarthritic articular cartilage, *Nat. Biomed. Eng.* 6 (2022) 1105–1117, <https://doi.org/10.1038/s41551-022-00948-5>.
- [81] N. Su, P.L. Gao, K. Wang, J.Y. Wang, Y. Zhong, Y. Luo, Fibrous scaffolds potentiate the paracrine function of mesenchymal stem cells: a new dimension in cell-material interaction, *Biomaterials* 141 (2017) 74–85, <https://doi.org/10.1016/j.biomaterials.2017.06.028>.
- [82] S. Wan, X. Fu, Y. Ji, M. Li, X. Shi, Y. Wang, FAK- and YAP/TAZ dependent mechanotransduction pathways are required for enhanced immunomodulatory properties of adipose-derived mesenchymal stem cells induced by aligned fibrous scaffolds, *Biomaterials* 171 (2018) 107–117, <https://doi.org/10.1016/j.biomaterials.2018.04.035>.
- [83] T.H. Qazi, D.J. Mooney, G.N. Duda, S. Geissler, Biomaterials that promote cell-cell interactions enhance the paracrine function of MSCs, *Biomaterials* 140 (2017) 103–114, <https://doi.org/10.1016/j.biomaterials.2017.06.019>.
- [84] T.H. Qazi, D.J. Mooney, G.N. Duda, S. Geissler, Niche-mimicking interactions in peptide-functionalized 3D hydrogels amplify mesenchymal stromal cell paracrine effects, *Biomaterials* 230 (2020), 119639, <https://doi.org/10.1016/j.biomaterials.2019.119639>.
- [85] A.S. Caldwell, V.V. Rao, A.C. Golden, K.S. Anseth, Porous bio-click microgel scaffolds control hMSC interactions and promote their secretory properties, *Biomaterials* 232 (2020), 119725, <https://doi.org/10.1016/j.biomaterials.2019.119725>.
- [86] T.H. Qazi, L. Tytgat, P. Dubruel, G.N. Duda, S. Van Vlierberghe, S. Geissler, Extrusion printed scaffolds with varying pore size as modulators of MSC angiogenic paracrine effects, *ACS Biomater. Sci. Eng.* 5 (2019) 5348–5358, <https://doi.org/10.1021/acsbiomater.9b00843>.
- [87] Y. Ji, J. Li, Y. Wei, W. Gao, X. Fu, Y. Wang, Substrate stiffness affects the immunosuppressive and trophic function of hMSCs: via modulating cytoskeletal polymerization and tension, *Biomater. Sci.* 7 (2019) 5292–5300, <https://doi.org/10.1039/c9bm01202h>.
- [88] H. Yang, N.M.J. Cheam, H. Cao, M.K.H. Lee, S.K. Sze, N.S. Tan, C.Y. Tay, Materials stiffness-dependent redox metabolic reprogramming of mesenchymal stem cells for secretome-based therapeutic angiogenesis, *Adv. Healthc. Mater.* 8 (2019) 1–12, <https://doi.org/10.1002/adhm.201900929>.
- [89] K.C. Murphy, J. Whitehead, D. Zhou, S.S. Ho, J.K. Leach, Engineering fibrin hydrogels to promote the wound healing potential of mesenchymal stem cell spheroids, *Acta Biomater.* 64 (2017) 176–186, <https://doi.org/10.1016/j.actbio.2017.10.007>.
- [90] S.W. Wong, S. Lenzini, M.H. Cooper, D.J. Mooney, J.W. Shin, Soft extracellular matrix enhances inflammatory activation of mesenchymal stromal cells to induce monocyte production and trafficking, *Sci. Adv.* 6 (2020) 1–13, <https://doi.org/10.1126/sciadv.aaw0158>.
- [91] S.H. Lim, H.-Q. Mao, Electrospun scaffolds for stem cell engineering, *Adv. Drug Deliv. Rev.* 61 (2009) 1084–1096, <https://doi.org/10.1016/j.addr.2009.07.011>.
- [92] K.H. Vining, A. Stafford, D.J. Mooney, Sequential modes of crosslinking tune viscoelasticity of cell-instructive hydrogels, *Biomaterials* 188 (2019) 187–197, <https://doi.org/10.1016/j.biomaterials.2018.10.013>.

- [93] F. Hached, C. Vinatier, P.G. Pinta, P. Hulin, C. Le Visage, P. Weiss, J. Guicheux, A. Billon-Chabaud, G. Grimandi, Polysaccharide hydrogels support the long-Term viability of encapsulated human mesenchymal stem cells and their ability to secrete immunomodulatory factors, *Stem Cell. Int.* 2017 (2017) 6–8, <https://doi.org/10.1155/2017/9303598>.
- [94] N.M. Drzeniek, A. Mazzocchi, S. Schlickeiser, S.D. Forsythe, G. Moll, S. Geißler, P. Reinke, M. Gossen, V.S. Gorantla, H.-D. Volk, S. Soker, Bio-instructive hydrogel expands the paracrine potency of mesenchymal stem cells, *Biofabrication* 13 (2021), 045002, <https://doi.org/10.1088/1758-5090/ac0a32>.
- [95] B. Corradetti, F. Taraballi, S. Minardi, J. Van Eps, F. Cabrera, L.W. Francis, S. A. Gazze, M. Ferrari, B.K. Weiner, E. Tasciotti, Chondroitin sulfate immobilized on a biomimetic scaffold modulates inflammation while driving chondrogenesis, *Stem Cells Transl. Med.* 5 (2016) 670–682.
- [96] S.S. Ho, K.C. Murphy, B. Binder, C. Vissers, J.K. Leach, Increased survival and function of mesenchymal stem cell spheroids entrapped in instructive alginate hydrogels, *Stem Cells Transl. Med.* 5 (2016) 773–781.
- [97] A.Y. Clark, K.E. Martin, J.R. Garcia, C.T. Johnson, H.S. Theriault, W.M. Han, D. W. Zhou, E.A. Botchwey, A.J. Garcia, Integrin-specific hydrogels modulate transplanted human bone marrow-derived mesenchymal stem cell survival, engraftment, and reparative activities, *Nat. Commun.* 11 (2020) 1–14, <https://doi.org/10.1038/s41467-019-14000-9>.
- [98] S. Xin, C.A. Gregory, D.L. Alge, Interplay between degradability and integrin signaling on mesenchymal stem cell function within poly(ethylene glycol) based microporous annealed particle hydrogels, *Acta Biomater.* 101 (2020) 227–236, <https://doi.org/10.1016/j.actbio.2019.11.009>.
- [99] K.R. Hixon, T. Lu, S.A. Sell, A comprehensive review of cryogels and their roles in tissue engineering applications, *Acta Biomater.* 62 (2017) 29–41, <https://doi.org/10.1016/j.actbio.2017.08.033>.
- [100] J. Xue, D. Pisignano, Y. Xia, Maneuvering the migration and differentiation of stem cells with electrospon nanofibers, *Adv. Sci.* 7 (2020) 1–17, <https://doi.org/10.1002/adv.202000735>.
- [101] C. Lin, K. Xu, Y. He, B. Tao, Z. Yuan, K. Li, X. Li, Z. Xia, K. Cai, A dynamic matrix potentiates mesenchymal stromal cell paracrine function: via an effective mechanical dose, *Biomater. Sci.* 8 (2020) 4779–4791, <https://doi.org/10.1039/d0bm01012j>.
- [102] A. Skardal, S.V. Murphy, K. Crowell, D. Mack, A. Atala, S. Soker, A tunable hydrogel system for long-term release of cell-secreted cytokines and bioprinted in situ wound cell delivery, *J. Biomed. Mater. Res. Part B Appl. Biomater.* 105 (2017) 1986–2000, <https://doi.org/10.1002/jbm.b.33736>.
- [103] L.J. Eggermont, Z.J. Rogers, T. Colombani, A. Memic, S.A. Bencherif, Injectable cryogels for biomedical applications, *Trends Biotechnol.* 38 (2020) 418–431, <https://doi.org/10.1016/j.tibtech.2019.09.008>.
- [104] W. Sun, B. Starly, A.C. Daly, J.A. Burdick, J. Groll, G. Skeldon, W. Shu, Y. Sakai, M. Shinohara, M. Nishikawa, J. Jang, D.W. Cho, M. Nie, S. Takeuchi, S. Ostrovidov, A. Khademhosseini, R.D. Kamm, V. Mironov, L. Moroni, I. T. Ozbolat, The bioprinting roadmap, *Biofabrication* 12 (2020), <https://doi.org/10.1088/1758-5090/ab5158>.
- [105] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, *Nat. Biotechnol.* 32 (2014) 773–785, <https://doi.org/10.1038/nbt.2958>.
- [106] W.S. Toh, T.C. Lim, M. Kurisawa, M. Spector, Modulation of mesenchymal stem cell chondrogenesis in a tunable hyaluronic acid hydrogel microenvironment, *Biomaterials* 33 (2012) 3835–3845, <https://doi.org/10.1016/j.biomaterials.2012.01.065>.
- [107] J. Lee, O. Jeon, M. Kong, A.A. Abdeen, J.-Y. Shin, H.N. Lee, Y. Bin Lee, W. Sun, P. Bandaru, D.S. Alt, K. Lee, H. Kim, S.J. Lee, S. Chatterji, S.R. Shin, E. Alsborg, A. Khademhosseini, Combinatorial screening of biochemical and physical signals for phenotypic regulation of stem cell-based cartilage tissue engineering, *Sci. Adv.* 6 (2020), <https://doi.org/10.1126/sciadv.aaz5913> eaz5913.
- [108] N. Huebsch, P.R. Arany, A.S. Mao, D. Shvartsman, O.A. Ali, S.A. Bencherif, J. Rivera-Feliciano, D.J. Mooney, Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate, *Nat. Mater.* 9 (2010) 518–526, <https://doi.org/10.1038/nmat2732>.
- [109] M. Nasser, Y. Wu, Y. Danaoui, G. Ghosh, Engineering microenvironments towards harnessing pro-angiogenic potential of mesenchymal stem cells, *Mater. Sci. Eng., C* 102 (2019) 75–84, <https://doi.org/10.1016/j.msec.2019.04.030>.
- [110] F.P. Seib, M. Prewitz, C. Werner, M. Bornhäuser, Matrix elasticity regulates the secretory profile of human bone marrow-derived multipotent mesenchymal stromal cells (MSCs), *Biochem. Biophys. Res. Commun.* 389 (2009) 663–667, <https://doi.org/10.1016/j.bbrc.2009.09.051>.
- [111] L. Cai, R.E. Dewi, A.B. Goldstone, J.E. Cohen, A.N. Steele, Y.J. Woo, S. C. Heilshorn, Regulating stem cell secretome using injectable hydrogels with in situ network formation, *Adv. Healthc. Mater.* 5 (2016) 2758–2764, <https://doi.org/10.1002/adhm.201600497>.
- [112] Z. Fábíán, The effects of hypoxia on the immune-modulatory properties of bone marrow-derived mesenchymal stromal cells, *Stem Cell. Int.* 2019 (2019), <https://doi.org/10.1155/2019/2509606>.
- [113] D.E. Lee, N. Ayoub, D.K. Agrawal, Mesenchymal stem cells and cutaneous wound healing: novel methods to increase cell delivery and therapeutic efficacy, *Stem Cell Res. Ther.* 7 (2016) 1–8, <https://doi.org/10.1186/s13287-016-0303-6>.
- [114] H.H. Qin, C. Filippi, S. Sun, S. Lehec, A. Dhawan, R.D. Hughes, Hypoxic preconditioning potentiates the trophic effects of mesenchymal stem cells on co-cultured human primary hepatocytes, *Stem Cell Res. Ther.* 6 (2015) 1–12, <https://doi.org/10.1186/s13287-015-0218-7>.
- [115] O. Chaudhuri, Viscoelastic hydrogels for 3D cell culture, *Biomater. Sci.* 5 (2017) 1480–1490, <https://doi.org/10.1039/c7bm00261k>.
- [116] O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S.A. Bencherif, J.C. Weaver, N. Huebsch, H.P. Lee, E. Lippens, G.N. Duda, D.J. Mooney, Hydrogels with tunable stress relaxation regulate stem cell fate and activity, *Nat. Mater.* 15 (2016) 326–334, <https://doi.org/10.1038/nmat4489>.
- [117] M. Cantini, H. Donnelly, M.J. Dalby, M. Salmeron-Sanchez, The plot thickens: the emerging role of matrix viscosity in cell mechanotransduction, *Adv. Healthc. Mater.* 9 (2020), <https://doi.org/10.1002/adhm.201901259>.
- [118] S. Tang, H. Ma, H. Tu, H. Wang, P. Lin, K.S. Anseth, Adaptable fast relaxing boronate-based hydrogels for probing cell–matrix interactions, *Adv. Sci.* 5 (2018), 1800638, <https://doi.org/10.1002/adv.201800638>.
- [119] O. Chaudhuri, J. Cooper-White, P.A. Janmey, D.J. Mooney, V.B. Shenoy, Effects of extracellular matrix viscoelasticity on cellular behaviour, *Nature* 584 (2020) 535–546, <https://doi.org/10.1038/s41586-020-2612-2>.
- [120] A.R. Cameron, J.E. Frith, J.J. Cooper-White, The influence of substrate creep on mesenchymal stem cell behaviour and phenotype, *Biomaterials* 32 (2011) 5979–5993, <https://doi.org/10.1016/j.biomaterials.2011.04.003>.
- [121] L. Teng, Y. Chen, Y.G. Jia, L. Ren, Supramolecular and dynamic covalent hydrogel scaffolds: from gelation chemistry to enhanced cell retention and cartilage regeneration, *J. Mater. Chem. B* 7 (2019) 6705–6736, <https://doi.org/10.1039/c9tb01698h>.
- [122] X. Zhao, N. Huebsch, D.J. Mooney, Z. Suo, Stress-relaxation behavior in gels with ionic and covalent crosslinks, *J. Appl. Phys.* 107 (2010), 063509, <https://doi.org/10.1063/1.3343265>.
- [123] B.P. Hung, T. Gonzalez-Fernandez, J.B. Lin, T. Campbell, Y. Bin Lee, A. Panitch, E. Alsborg, J.K. Leach, Multi-peptide presentation and hydrogel mechanics jointly enhance therapeutic duo-potential of entrapped stromal cells, *Biomaterials* 245 (2020), 119973, <https://doi.org/10.1016/j.biomaterials.2020.119973>.
- [124] R.J. Wade, J.A. Burdick, Engineering ECM signals into biomaterials, *Mater. Today* 15 (2012) 454–459, [https://doi.org/10.1016/S1369-7021\(12\)70197-9](https://doi.org/10.1016/S1369-7021(12)70197-9).
- [125] S. Khetan, M. Guvendiren, W.R. Legant, D.M. Cohen, C.S. Chen, J.A. Burdick, Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels, *Nat. Mater.* 12 (2013) 458–465, <https://doi.org/10.1038/nmat3586>.
- [126] M.P. Lutolf, J.A. Hubbell, Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering, *Nat. Biotechnol.* 23 (2005) 47–55, <https://doi.org/10.1038/nbt1055>.
- [127] N. Huebner, T.R. Dargaville, A. Forget, Discovering cell-adhesion peptides in tissue engineering: beyond RGD, *Trends Biotechnol.* 36 (2018) 372–383, <https://doi.org/10.1016/j.tibtech.2018.01.008>.
- [128] J. Lam, T. Segura, The modulation of MSC integrin expression by RGD presentation, *Biomaterials* 34 (2013) 3938–3947, <https://doi.org/10.1016/j.biomaterials.2013.01.091>.
- [129] F. Karimi, A.J. O'Connor, G.G. Qiao, D.E. Heath, Integrin clustering matters: a review of biomaterials functionalized with multivalent integrin-binding ligands to improve cell adhesion, migration, differentiation, angiogenesis, and biomedical device integration, *Adv. Healthc. Mater.* 7 (2018) 1–28, <https://doi.org/10.1002/adhm.201701324>.
- [130] N. Huebsch, P.R. Arany, A.S. Mao, D. Shvartsman, O.A. Ali, S.A. Bencherif, D. J. Mooney, Harnessing traction-mediated manipulation of the cell-matrix interface to control stem cell fate, *Nat. Mater.* 9 (2010) 518–526, <https://doi.org/10.1038/nmat2732>.
- [131] J. Dhillon, S.A. Young, S.E. Sherman, G.I. Bell, B.G. Amsden, D.A. Hess, L. E. Flynn, Peptide-modified methacrylated glycol chitosan hydrogels as a cell- viability supporting pro-angiogenic cell delivery platform for human adipose-derived stem/stromal cells, *J. Biomed. Mater. Res., Part A* 107 (2019) 571–585, <https://doi.org/10.1002/jbm.a.36573>.
- [132] Z. Cesarz, K. Tamama, Spheroid culture of mesenchymal stem cells, *Stem Cell. Int.* 2016 (2016) 1–11, <https://doi.org/10.1155/2016/9176357>.
- [133] V. Miceli, M. Pampalona, S. Vella, A.P. Carreca, G. Amico, P.G. Conaldi, Comparison of immunosuppressive and angiogenic properties of human amnion-derived mesenchymal stem cells between 2D and 3D culture systems, *Stem Cell. Int.* 2019 (2019) 1–16, <https://doi.org/10.1155/2019/7486279>.
- [134] S. Sart, A.C. Tsai, Y. Li, T. Ma, Three-dimensional aggregates of mesenchymal stem cells: cellular mechanisms, biological properties, and applications, *Tissue Eng. B Rev.* 20 (2014) 365–380, <https://doi.org/10.1089/ten.teb.2013.0537>.
- [135] E.J. Lee, S.J. Park, S.K. Kang, G.H. Kim, H.J. Kang, S.W. Lee, H.B. Jeon, H.S. Kim, Spherical bullet formation via E-cadherin promotes therapeutic potency of mesenchymal stem cells derived from human umbilical cord blood for myocardial infarction, *Mol. Ther.* 20 (2012) 1424–1433, <https://doi.org/10.1038/mt.2012.58>.
- [136] E. Kim, H. Koo, Biomedical applications of copper-free click chemistry: in vitro, in vivo, and ex vivo, *Chem. Sci.* 10 (2019) 7835–7851, <https://doi.org/10.1039/c9sc03368h>.
- [137] H.C. Kolb, M.G. Finn, K.B. Sharpless, Click chemistry: diverse chemical function from a few good reactions, *Angew. Chem. Int. Ed.* 40 (2001) 2004–2021, [https://doi.org/10.1002/1521-3773\(20010601\)40:11<2004::AID-ANIE2004>3.0.CO;2-5](https://doi.org/10.1002/1521-3773(20010601)40:11<2004::AID-ANIE2004>3.0.CO;2-5).
- [138] J.E. Hein, J.C. Tripp, L.B. Krasnova, K.B. Sharpless, V.V. Fokin, Copper(I)-catalyzed cycloaddition of organic azides and 1-iodoalkynes, *Angew. Chem. Int. Ed.* 48 (2009) 8018–8021, <https://doi.org/10.1038/jid.2014.371>.
- [139] E.M. Sletten, C.R. Bertozzi, From mechanism to mouse: a tale of two bioorthogonal reactions, *Acc. Chem. Res.* 44 (2011) 666–676, <https://doi.org/10.1021/ar200148z>.
- [140] C.M. Madl, S.C. Heilshorn, Bioorthogonal strategies for engineering extracellular matrices, *Adv. Funct. Mater.* 28 (2018) 1706046–1706067, <https://doi.org/10.1002/adfm.201706046>.

- [141] M.R. Arkenberg, H.D. Nguyen, C.-C. Lin, Recent advances in bio-orthogonal and dynamic crosslinking of biomimetic hydrogels, *J. Mater. Chem. B* 8 (2020) 7835–7855, <https://doi.org/10.1039/D0TB01429J>.
- [142] V. Lègré-Boyer, Viscosupplementation: techniques, indications, results, *Orthop. Traumatol. Surg. Res.* 101 (2015) S101–S108, <https://doi.org/10.1016/j.otsr.2014.07.027>.
- [143] C. Feng, X. Luo, N. He, H. Xia, X. Lv, X. Zhang, D. Li, F. Wang, J. He, L. Zhang, X. Lin, L. Lin, H. Yin, J. He, J. Wang, W. Cao, R. Wang, G. Zhou, W. Wang, Efficacy and persistence of allogeneic adipose-derived mesenchymal stem cells combined with hyaluronic acid in osteoarthritis after intra-articular injection in a sheep model, *Tissue Eng.* 24 (2018) 219–233, <https://doi.org/10.1089/ten.tea.2017.0039>.
- [144] J.M. Murphy, D.J. Fink, E.B. Hunziker, F.P. Barry, Stem cell therapy in a caprine model of osteoarthritis, *Arthritis Rheum.* 48 (2003) 3464–3474, <https://doi.org/10.1002/art.11365>.
- [145] X. Lv, J. He, X. Zhang, X. Luo, N. He, Z. Sun, H. Xia, V. Liu, L. Zhang, X. Lin, L. Lin, H. Yin, D. Jiang, W. Cao, R. Wang, G. Zhou, W. Wang, Comparative efficacy of autologous stromal vascular fraction and autologous adipose-derived mesenchymal stem cells combined with hyaluronic acid for the treatment of sheep osteoarthritis, *Cell Transplant.* 27 (2018) 1111–1125, <https://doi.org/10.1177/0963689718773333>.
- [146] M. Sato, K. Uchida, H. Nakajima, T. Miyazaki, A.R. Guerrero, S. Watanabe, S. Roberts, H. Baba, Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain Guinea pigs with spontaneous osteoarthritis, *Arthritis Res. Ther.* 14 (2012) 1–9, <https://doi.org/10.1186/ar3735>.
- [147] E.-R. Chiang, H.-L. Ma, J.-P. Wang, C.-L. Liu, T.-H. Chen, S.-C. Hung, Allogeneic mesenchymal stem cells in combination with hyaluronic acid for the treatment of osteoarthritis in rabbits, *PLoS One* 11 (2016), e0149835, <https://doi.org/10.1371/journal.pone.0149835>.
- [148] D. Xing, J. Wu, B. Wang, W. Liu, W. Liu, Y. Zhao, L. Wang, J.J. Li, A. Liu, Q. Zhou, J. Hao, J. Lin, Intra-articular delivery of umbilical cord-derived mesenchymal stem cells temporarily retard the progression of osteoarthritis in a rat model, *Int. J. Rheum. Dis.* 2020 (2020) 1–10.
- [149] C.B. Rodell, A.L. Kaminski, J.A. Burdick, Rational design of network properties in guest-host assembled and shear-thinning hyaluronic acid hydrogels, *Biomacromolecules* 14 (2013) 4125–4134, <https://doi.org/10.1021/bm401280z>.
- [150] T.C. Tseng, L. Tao, F.Y. Hsieh, Y. Wei, I.M. Chiu, S.H. Hsu, An injectable, self-healing hydrogel to repair the central nervous system, *Adv. Mater.* 27 (2015) 3518–3524, <https://doi.org/10.1002/adma.201500762>.
- [151] F. Nativel, D. Renard, F. Hached, P.-G. Pinta, C. D'Arros, P. Weiss, C. Le Visage, J. Guicheux, A. Billon-Chabaud, G. Grimandi, Application of millifluidics to encapsulate and support viable human mesenchymal stem cells in a polysaccharide hydrogel, *Int. J. Mol. Sci.* 19 (2018) 1952–1968, <https://doi.org/10.3390/ijms19071952>.
- [152] D. Dhamecha, R. Movsas, U. Sano, J. Menon, Applications of Alginate microspheres in therapeutics delivery and cell culture: past, present and future, *Int. J. Pharm.* 569 (2019) 118627–118640, <https://doi.org/10.1016/j.ijpharm.2019.118627>.
- [153] B.A. Aguado, W. Mulyasmita, J. Su, K.J. Lampe, S.C. Heilshorn, Improving viability of stem cells during syringe needle flow through the design of hydrogel cell carriers, *Tissue Eng.* 18 (2012) 806–815, <https://doi.org/10.1089/ten.tea.2011.0391>.
- [154] S. Talebian, M. Mehrali, N. Taebnia, C.P. Pennisi, F.B. Kadumudi, J. Foroughi, M. Hasany, M. Nikkhal, M. Akbari, G. Orive, A. Dolatshahi-Pirouz, Self-healing hydrogels: the next paradigm shift in tissue engineering? *Adv. Sci.* 6 (2019) 1801664–1801735, <https://doi.org/10.1002/adv.201801664>.
- [155] S. Fuchs, K. Shariati, M. Ma, Specialty tough hydrogels and their biomedical applications, *Adv. Healthc. Mater.* 9 (2020) 1–33, <https://doi.org/10.1002/adhm.201901396>.
- [156] A.S. Mao, B. Özkale, N.J. Shah, K.H. Vining, T. Descombes, L. Zhang, C. M. Tringides, S.W. Wong, J.W. Shin, D.T. Scadden, D.A. Weitz, D.J. Mooney, Programmable microencapsulation for enhanced mesenchymal stem cell persistence and immunomodulation, *Proc. Natl. Acad. Sci. U. S. A* 116 (2019) 15392–15397, <https://doi.org/10.1073/pnas.1819415116>.
- [157] A.E. Ryan, P. Lohan, L.O. Flynn, O. Treacy, X. Chen, C. Coleman, G. Shaw, M. Murphy, F. Barry, M.D. Griffin, T. Ritter, Chondrogenic differentiation increases antidonor immune response to allogeneic mesenchymal, *Stem Cell Transplantation* 22 (2014) 655–667, <https://doi.org/10.1038/mt.2013.261>.
- [158] I. Akgun, M.C. Unlu, O.A. Erdal, T. Ogut, M. Erturk, E. Ovali, F. Kantarci, G. Caliskan, Y. Akgun, Matrix-induced autologous mesenchymal stem cell implantation versus matrix-induced autologous chondrocyte implantation in the treatment of chondral defects of the knee : a 2-year randomized study, 251–263, <https://doi.org/10.1007/s00402-014-2136-z>, 2015.
- [159] Y. Park, C. Ha, J. Kim, Comparison of undifferentiated versus chondrogenic pre-differentiated mesenchymal stem cells derived from human umbilical cord blood for cartilage repair in a rat model, 451–461, <https://doi.org/10.1177/0363546518815151>, 2019.
- [160] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, *Cell* 126 (2006) 677–689, <https://doi.org/10.1016/j.cell.2006.06.044>.
- [161] C. Yang, M.W. Tibbitt, L. Basta, K.S. Anseth, Mechanical memory and dosing influence stem cell fate, *Nat. Mater.* 13 (2014) 645–652, <https://doi.org/10.1038/nmat3889>.
- [162] J. Paquet, M. Deschepper, A. Moya, D. Logeart-Avramoglou, C. Boisson-Vidal, H. Petite, Oxygen tension regulates human mesenchymal stem cell paracrine functions, *Stem Cells Transl. Med.* 4 (2015) 809–821, <https://doi.org/10.5966/sctm.2014-0180>.
- [163] S. Ryter, H. Kim, A. Hoetzel, J. Park, K. Nakahira, X. Wang, A. Choi, Mechanisms of cell death in oxidative stress, *Antioxidants Redox Signal.* 9 (2007) 49–89, <https://doi.org/10.1089/ars.2007.9.49>.
- [164] M. Redza-Dutordoir, D.A. Averill-Bates, Activation of apoptosis signalling pathways by reactive oxygen species, *Biochim. Biophys. Acta Mol. Cell Res.* 1863 (2016) 2977–2992, <https://doi.org/10.1016/j.bbamcr.2016.09.012>.
- [165] E. Ragni, C. Perucca Orfei, P. De Luca, G. Lugano, M. Viganò, A. Colombini, F. Valli, D. Zaccetti, V. Bollati, L. De Girolamo, Interaction with hyaluronan matrix and miRNA cargo as contributors for in vitro potential of mesenchymal stem cell-derived extracellular vesicles in a model of human osteoarthritic synoviocytes, *Stem Cell Res. Ther.* 10 (2019) 1–17, <https://doi.org/10.1186/s13287-019-1215-z>.
- [166] S. Lenzini, R. Bargi, G. Chung, J.W. Shin, Matrix mechanics and water permeation regulate extracellular vesicle transport, *Nat. Nanotechnol.* 15 (2020) 217–223, <https://doi.org/10.1038/s41565-020-0636-2>.