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Peptide-biofunctionalization of biomaterials for osteochondral tissue regeneration in early stage osteoarthritis: Challenges and opportunities

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Abstract

Osteoarthritis is a degenerative joint disease characterized by the progressive deterioration of articular cartilage, synovial inflammation and changes in periarticular and subchondral bone, being a leading cause of disability. Conventional treatments present several side effects and can involve the use of painkillers and non-steroidal anti-inflammatory drugs. In this sense, researchers have shifted the focus of new therapeutics to biomolecular agents and/or their combination with cells. However, the efficacy of these molecules is dependent on their metabolization which can differ according to the administration route and delivery approach. Consequently, tissue engineering strategy comprising the use of biomaterials can provide support for neo-tissue osteochondral repair/regeneration whilst conferring proper mechanical and functional features as well as protecting biomolecular agents from premature degradation. The current strategies and challenges used in biomaterials functionalization with peptides that can mimic ECM proteins or other natural soluble biomolecules, important to induce the complex interactions between cells and the ECM, are discussed herein. Many opportunities for treating OA are being explored by means of peptide-biofunctionalization of biomaterials which can be designed to be recognizable, induce differentiation, prevent infection, degrade at an intended rate or act as drug delivery systems for controlled release or even as simple triggers of cell behavior.

Keywords: biomaterials, osteoarthritis, osteochondral, mimetic peptides, immunomodulation.

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1. The osteoarthritic process.

Osteoarthritis (OA) is a degenerative and inflammatory disease that especially affects weight-bearing joints, typically knees, hips, hands, spine, and feet. OA is characterized by the progressive loss of articular cartilage, subchondral sclerosis, and proliferation of periosteal cells, leading to osteophyte formation and abnormal subchondral bone. [1,2] In its advanced stage, OA is manifested by the complete loss of cartilage causing eburnation, stiffness, and swelling.[3,4]

Several factors secreted by articular chondrocytes and synovial cells in OA can also contribute to increasing the disease severity. These include type-X collagen (Col-X), alkaline phosphatase (ALP), and RUNX2 (runt-related transcription factor 2), NOS-2 (nitric oxide synthase 2) and COX-2 (cyclooxygenase-2). Some catabolic enzymes are also expressed: matrix metalloproteinases (MMPs) 1, 2, 3, 8, 9 and 13; a disintegrin and metalloproteinase with thrombospondin motifs 4 and 5 (ADAMTS4 and ADAMTS5).[5,6] During OA the infiltration of mononuclear cells and the production of pro-inflammatory mediators, such as interleukins -1β and -6 (IL-1β, IL-6, respectively), tumor necrosis factor (TNF-α), and several chemokines also occurs. Additionally, degradation of type II collagen (Col-II) determines the irreversible progression of OA.[7] Similarly, osteoclasts also increase the levels of cathepsin K[8] and activate of the RANK/RANKL (Receptor-activator of Nuclear Factor kappa-β)[9,10] and other osteoclast-related molecules such as Colony-stimulating factor-1 receptor (c-fms), macrophage colony-stimulating factor (M-CSF), and osteocalcin (OCN).[11] Consequently, as OA progresses all the components of the osteochondral (OC) joint suffer chemical and cellular changes, which means that alterations/injuries in one single tissue will influence the entire joint, complicating the development of therapies that aim to treat a single phenomenon of OA (Figure 1). Therefore, the proper understanding of the biological and cellular processes associated with the pathophysiology of OA is of great importance to deep understand the progression of the disease and in order to develop more effective and targeted approaches.[12] Herein, we intend to provide an overview concerning the current cellular therapies used in OA. It is also summarized the existing methodologies that mimic and can improve OC tissue environment by means of using biomaterials functionalized with immunomodulatory and chemical cues.

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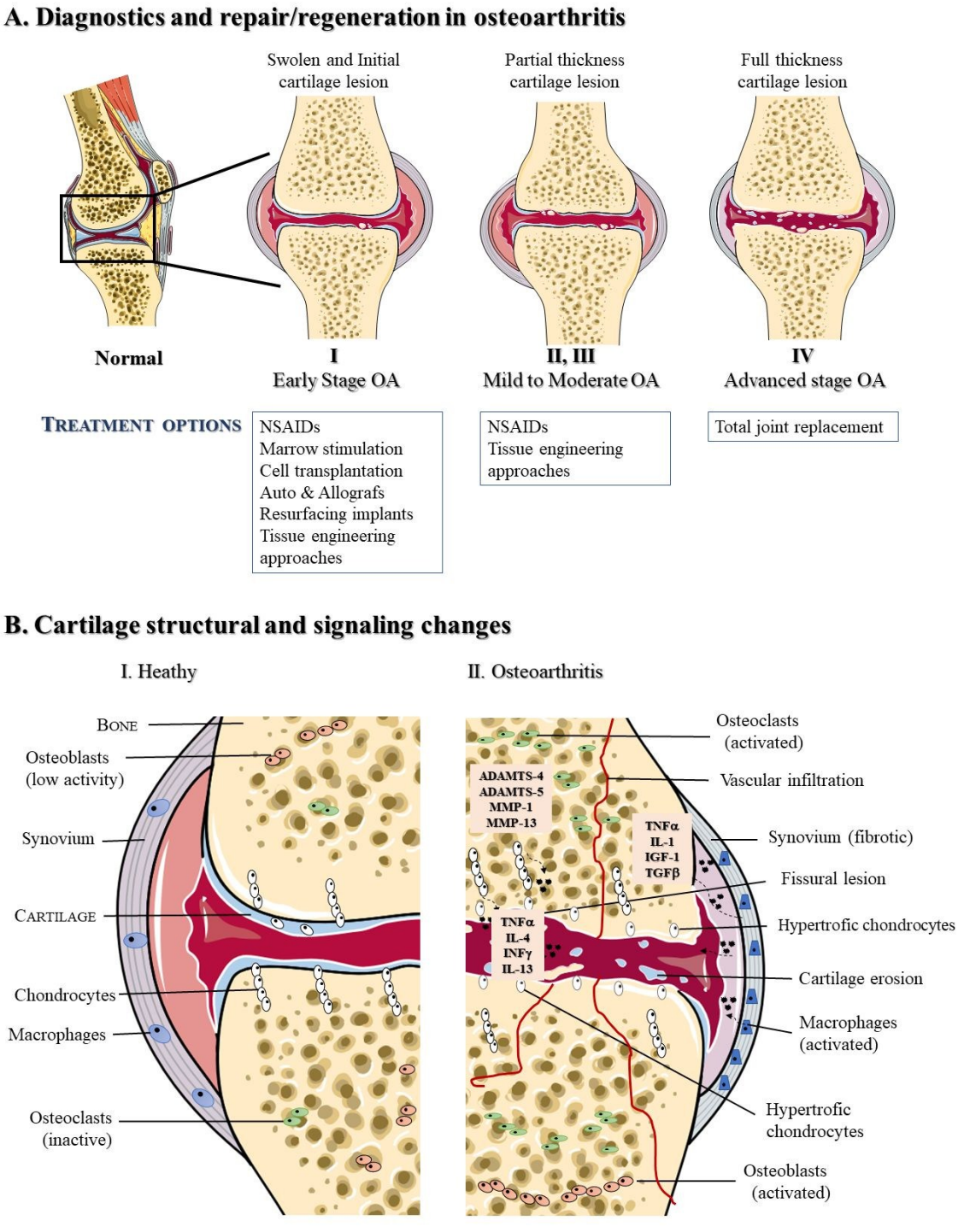

Figure 1: Sagittal section of the knee. A. Progression of OA: conditions and treatments in each stage. B. Schematic representation of cartilage structural and signaling changes between healthy (I) and osteoarthritic (II) scenarios. (Adapted with permission from[13]). IL=interleukin. ADAMTS=desintegrin and metalloproteinase with thrombospondin-like motifs. MMP=matrix metalloproteinase. TNF=tumour necrosis factor. IFN=interferon. IGF=insulin-like growth factor. TGF=transforming growth factor. VEGF=vascular endothelial growth factor.

2. Up-to-date cellular therapies

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Cellular therapies have emerged due to the non-satisfactory results to repair articular cartilage and restore full joint function obtained using conventional FDA-approved therapies. Autologous chondrocyte implantation (ACI) was the method of selection for many years.[14] It consists of harvesting a piece of cartilage from a non-bearing weight area, isolating the cells, their *in vitro* expansion and re-implantation of the cell suspension on the damaged area. Nevertheless, this frequently resulted in uneven distribution of the cells, and as a result, matrix-induced autologous chondrocyte implantation (MACI), intending to implant these cells on biodegradable membranes, was proposed.[15,16] However, other issues like availability, donor site morbidity and dedifferentiation represent ongoing challenges.[17,18] In this sense, mesenchymal stem cells (MSCs) started being used for local delivery to treat OA joints, due to their ability of self-renewal and differentiation into chondrocytes.[19] However, besides the loss of phenotype, there is a risk of pathogen contamination in cell expansion and MSC can induce teratoma formation.[20] Microfracture (MF) represents an option to treat small cartilage defects ($< 2 – 3$ cm^2). MF removes the calcified cartilage layer and drills into the surface of the underlying bone until the extravasation of blood, which releases bone marrow and MSCs that can contribute to the repair of the OC defect. However, the regenerated tissue is mainly fibrocartilaginous with a disorganized matrix .[21] Mosaicplasty (MP) is based on the extraction of OC autographs from non-weight bearing joints and re-implantation on the injured side. This process is limited bydonor cartilage reserves.[22]

FDA guidelines impose that when working with cells minimum manipulation must be applied. In this sense, platelet-rich plasma (PRP) prepared from autologous blood has been applied in several repairs and regenerative processes including angiogenesis, anti-inflammation, chondrogenesis and bone remodeling.[23,24] PRP excludes the concerns of immunologic reactions and disease transmission due to its autologous origin. It also represents an economic procedure with high recovery potential, easy handling and offers natural growth factors (GF), cytokines, chemokines, ECM proteins, ascorbic acid, MMP inhibitors, and nucleotides.[24] The clinical use of PRP-based therapies can decrease joint pain and stiffness and improve the quality of life of patients with OA. However, the benefits will vary with the volume of PRP, the presence of a 3D scaffold to support cells, number and interval of injections.[25]

Exosomes (Exos) and microvesicles/microparticles (MPs) derived from MSCs have paracrine effects associated to a chondroprotective effect in collagenase-induced OA models.[26] Exos (30-100 nm) are generated from the cell membrane during endocytic internalization, before being released, while MPs (0.05–1.0 μm) are released from cell membranes upon activation and/or apoptosis, expressing markers from the parental cell.[27] Cosenza and co-workers demonstrated that Exos and MPs from murine BM-MSCs were able to re-establish chondrocyte homeostatic state, protect chondrocytes from apoptosis and stimulate macrophage polarization towards an anti-inflammatory phenotype. RT-qPCR quantification showed that Exos and MPs could recover

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the expression of chondrocyte markers (Col-II, aggrecan) and inhibit the catabolic (MMP-13, ADAMTS5) and inflammatory (iNOS) markers.[28] In a more recent study, Consenza and coworkers showed that Exos and MPs have similar immunosuppressive effect, decrease T and B cells and induce Regulatory T cell (Treg).[29] The authors observed that Exos, but not MPs, significantly decrease arthritis in an *in vivo* model for collagenase-induced OA (CIOA).[30] In brief, the potent immunosuppressive effect of Exos can open up novel therapeutic possibilities for managing the treatment of OA patients.

3. Biofunctionalization strategies to improve the efficacy of biomaterials

Administration of simple cell transplantation originates low survival rates, phenotypic instability, regulatory issues, and high costs. Therefore, tissue engineering (TE) uses biomaterials to provide support and guide neo-tissue development whilst conferring proper mechanical and functional features. The rationale is that biomaterials can stimulate tissue repair without the hurdles of using cell-based therapies. However, clinical translation can be hampered by some degree of immune response or foreign-body response, associated with infection or allergic reaction, which end in the rejection of the biomaterial. Other limitations include mechanical failure and teratoma formation. Even though the choice of the biomaterial is essential, we must bear in mind that the cell-biomaterial interface must mimic the environment in which it will be used in order to allow to precisely control cell fate. Biomaterials must behave as bioactive instructive scaffolds with the capacity to dictate cell behavior. In this sense, biomaterials can be modified through physicochemical modification and functionalized using mimetic peptides to promote desirable features. Biomaterials can become recognizable by the host and promote cell proliferation and migration, expand or degrade at an intended rate, acting as controlled release drug delivery systems or simple triggers of cell behaviour.[31,32]

3.1 Cellular recognition and adhesion peptides

In the last years, it has been demonstrated that the ECM is not only the natural scaffold that holds the cells into the living organisms but also it holds several proteins, proteoglycans and other signaling molecules important to modulate a wide range of cellular behaviors such as adhesion, proliferation, migration, and differentiation.[33] One of the most important motifs for cell recognition and binding to many integrins is RGD (Arginyl-glycyl-aspartic acid) which was identified by Pierschbacher and Rouslahti as an essential cell adhesion peptide sequence in fibronectin.[34,35] The RGD immobilization into scaffolds has shown to enhance cell recognition.[36,37] Therefore, the use of cell-adhesive molecules (CAM) in conjunction with RGD has been employed to mimic cell interactions and to enhance cell adhesion. CAMs are grouped into several families, namely, immunoglobulins, cadherins, integrins, selectins and ECM proteins

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(Table 1).[38,39,48,40–47] These proteins can mediate cell adhesion by heterophilic (between cells of a different type) and homophilic (between cells of a single type) interactions. CAMs have specific affinities for cell surface glycoproteins, helping the specific location of cells within the tissues and transport of biochemical information to coordinate homeostasis including signal transduction, cellular communication, and recognition.[49] CAMs also play a role in embryogenesis, inflammatory and immune responses, and apoptosis, which means they are involved in several pathologies such as diabetes, cancer or osteoarthritis, specifically due to alterations in the specific binding to cell receptors.

Inflammatory diseases as OA possess deregulations in their endothelium remodeling. Specifically, they present an improved permeability to lipoproteins and other plasma components involved in the increase of the adhesive capacity of leukocyte adhesion molecules (integrins, L-selectin, and platelet endothelial cell adhesion molecule 1 (PECAM-1)) and endothelial adhesion molecules (including vascular cell adhesion molecule (VCAM-1), endothelial intercellular adhesion molecule-1 (ICAM-1), E-selectin and P-selectin).[50] OA is characterized by the presence of several inflammatory cytokines that are known to stimulate ICAM-1 in the synovium.[51,52] On the other hand, VCAM-1, the surface sialoglycoprotein expressed on chondrocytes and synovial fibroblasts, was shown to function as soluble predictor of the long-term risk (~15-year) of knee and hip OA.[53] The immobilization of this adhesion molecule in poly(lactic acid) scaffolds (PLLA) increased the attachment of MSCs, due to the interaction of VCAM-1 with the integrin α4β7, expressed by these cells.[54] Similarly, PECAM-1 is highly expressed on endothelial cells, however, but even though the vascular permeability is increased in OA, this adhesion molecule does not suffer any up-regulation when compared with normal synovium.[55] Very late antigen-4 (VLA-4) is expressed on the surface of most leukocytes and mediates cell-cell and cell-ECM interactions as well as the release of several MMPs. In RA, VLA-4 arbitrates the adhesion and migration of circulatory monocytes and lymphocytes across the activated vascular endothelium and inflamed joints. Studies have shown that the blockage of this integrin represents an useful therapeutic approach to treat arthritis.[56]

N-cadherins are very important in mediating cell-cell interactions in mesenchymal condensation. Consequently, hyaluronic acid (HA) hydrogels have been conjugated with mimetic peptides containing the HAV tripeptide sequence (His-Ala-Val), which is the conserved motif of type I cadherins. This type of biomaterial has proved to promote the chondrogenesis of encapsulated human mesenchymal stem cells (hMSCs) in long-term cultures (28 days).[57] Additionally, other studies have demonstrated the effectiveness of this mimetic peptide on the regulation of the osteogenesis of hMSCs.[58,59] Zhu and coworker functionalized HA hydrogels with HAV and RGD peptides and showed an enhancement of osteogenesis, which was attributed to improved cell-cell communication and gene expression through connexin-mediated gap junctions.[39]

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Integrin receptors, a family of cell adhesion molecules, are essential for adhesion-dependent cell regulation. These heterodimers composed by α and β transmembrane subunits, form several receptors with high affinity for many ECM proteins. It is important to note that each ECM protein can also bind to one or more integrin heterodimers. For example, fibronectin binds to α3β1, α4β1, α5β1, and αVβ1 integrins while collagen binds to α1β1, α2β1, α10β1, and α11β1 integrins. The presence of a certain group of integrins will define the composition of the ECM, as well as biochemical signals and factors.[60] For instance, integrins α1β1, α2β1, α3β1, α5β1 and α10β1 influence chondrocyte attachment to Col-II whereas α3β1, α4β1, α5β1 and αvβ3 have been immunologically detected in human osteoblasts.[40,61] In OA there are some changes in the expression of integrins. α1β1 and α3β1 seem to experience an increase [62] alongside with the appearance of α2β1, α4β1 and some α6β1[63]. This phenomenon is not well understood but it is believed that it relates with the change in biochemical signaling molecules involved in OA environments. In particular, GF and cytokines that stimulate integrin expression as well as the changes in the ECM and hypertrophy of the chondrocytes.[60]

Selectins are a class of cell adhesion molecules constituted by three structurally and functionally different types: Platelet (P)-selectin, Endothelial (E)-selectin and leukocyte (L)-selectin. They have a common structure, which consists of a N-terminal Ca^{2+}-dependent lectin-type domain, an EGF domain, variable numbers of short repeats homologous to complement-binding sequences, a single transmembrane region and a short cytoplasmic domain.[64] P-selectins are involved in the rolling and adhesion between endothelial cells and neutrophils and when in deficient number accelerate the development of OA. This fact suggests that the plasma level of P-selectins serve as a biomarker to evaluate the disease progression.[65] Similarly, E-selectins, only expressed on endothelial cells after activation IL-1, TNFα or bacterial lipopolysaccharides, facilitate inflammatory cell infiltration such as neutrophils, monocytes, and T cells.[66] In OA, increased angiogenesis indirectly promotes the infiltration of inflammatory cells and may contribute to the progression of the disease.[67,68] L-selectins are crucial for the migration of leukocytes in inflammation and the recirculation of lymphocytes between lymphoid tissues and the bloodstream. These adhesion molecules have been reported to be increased in patients with RA.[69]

ECM proteins are typically fibrillary and provide a complex structural and functional network capable to interact with several cell surface receptors.[70] To illustrate, laminin, the major basement component of the cell membranes, has been applied in several TE approaches. Farrukh *et al*. produced biofunctionalized polyacrylamide gels coupled with polylysine (via its amine group) and the laminin-derived CAM, IKVAV (via its thiol group), to study its effect on neuronal survival and neurite outgrowth.[47] The produced structures were optimized regarding their mechanical properties, specifically, the elasticity, which enhanced the biological action of IKVAV in neurogenesis. Collagen, another ECM protein, is widely used in medical applications,

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including tissue regeneration. However, its animal origin represents a big limitation especially in terms of antigenicity and pathogenicity. Moreover, this protein represents a nonspecific cell adhesion protein with low thermal stability. To overcome these problems, collagen-like polypeptides such as poly(prolyl-hydroxyprolyl-glycyl) and (poly(Pro-Hyp-Gly)) have been synthesized.[45] This peptide forms a collagen-like triple-helical structure with good biodegradability and biocompatibility enhancing full-thickness dermal wound epithelialization in rabbit ear.[71] The integrin-binding ligand GFOGER derived from collagen type I (Col-I) has been described to improve osteointegration and osteogenic differentiation *in vivo*.[72] Wojtowicz and coworkers used collagen-mimetic peptide, GFOGER, to coat synthetic PCL scaffolds to promote bone formation in critical-size segmental defects in rats.[73] The produced scaffolds significantly increased bone formation in non-healing femoral defects compared to controls, in the absence of exogenous cells or GF. Another Col-I derived peptide, DGEA, was covalently bound to alginate via carbodiimide chemistry to assess the ability to improve cell adhesion and induce osteogenesis in MSCs as an alternative to co-delivery of soluble GF. The data showed that MSCs encapsulated in hydrogels with DGEA ligands alone resulted in significantly increased osteocalcin production and mineral deposition, but this ligand did not facilitate cell adhesion by itself. These data suggest that the presentation of DGEA ligand is a feasible approach for selectively inducing an osteogenic phenotype.[74]

Table 1: List of the most common cell adhesion peptides used in TE approaches.

Cell-adhesion molecules	Function	Subfamilies	Available peptides	Ref
Immunoglobulins	Proteins linked to recognition, binding and adhesion of the cells. They are located on the surface of the immune cells.	E-selectin, ICAM-1, VCAM-1, PECAM-1, VLA-4, neural-cell adhesion molecule (NCAM), Mac-1, etc.	-	38
Cadherins	Transmembrane glycoprotein calcium-dependent to bind immunoglobulin repeats through a homophilic manner.	Epithelial (E)-, placental (P)-, and neural (N)-cadherins. There are also desmosomal cadherins and proto-cadhers.	H2N-LRAHAVDVNG-amide, HAVDI	57
Integrin receptor	Glycoproteins that combine heterodimeric transmembrane receptors with α and β subunits on the cell surface. Important for immune cell activation, adhesion and migration, as well as the signaling process from inside-out and outside-in.	Fibronectin receptor (FNR), vitronectin receptor (VNR), VLA-4, and lymphocyte function-associated antigen-1 (LFA-1).	-	40
Selectins	The selectins belong to a family of divalent cation-dependent glycoproteins. They play an important role in the leukocyte trafficking and platelet binding.	E-selectin, L-selectin, and P-selectin.	Sialylated and fucosylated carbohydrates, H-CQKLDKSFSMIK-OH	41,42
ECM proteins	The ECM possesses a great number of proteins responsible for a substantial range of cells and tissues functions. It represents a dynamic repository for GF, functions as adhesive substrate, senses and transduces mechanical signals.	**Fibronectin** is a glycoprotein that plays an important role in several stages of wound healing, with its main function in cellular adhesion and migration.	GRGDS, PHSRN, PRARI, PKRGDL, NGRAHA, DAPS, FAP-P	43,75
		Vitronectin is one of the most abundant cell adhesion glycoproteins in plasma.	CKKQRFRHRNRKG	44
		Collagen is the most abundant proteins in the animal kingdom. It limits the distensibility of tissues owing to its vast tensile strengths of collagen fibrils.	DGEA, GFOGER, poly (Pro-Hyp-Gly)	45
		Laminin is a heterotrimeric glycoprotein with binding regions to collagen, integrins, proteoglycans and other cellular domains.	YIGSR, IKVAV, AGTFALRGDNPQG (A99), RKRLQVQLSIRT (AG73)	46,47
		Elastin is responsible for elasticity in several tissues like skin, cartilage, arteries and ligaments.	VAPG	48

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The RGD sequence is recognized by many integrin receptors involved in cell adhesion but mainly binds to integrin αvβ3. RGD sequence is present in several ECM proteins, including vitronectin (VN), fibronectin (FN), fibrinogen collagen, laminin, and von Willebrand Factor and has been extensively applied as CAM in several biomaterials.[76,77] The multifunctional protein FN promotes ECM formation, cell attachment, adhesion, migration, and differentiation.[78] Recently, polyacrylate substrates modeled with RGD were applied to induce MSC differentiation into chondrocytes without the typical cares of using GF and seeding aggregates of cells to induce chondrogenesis.[79] After a few days, the human and mouse MSC formed multi-layer aggregates with the expression of Col-II, Sox9 and aggrecan suggesting that with the proper artificial environment chondrogenesis can still proceed.[80] VN is another ECM protein that binds to GAGs. This protein interacts with thrombin and antithrombin III during coagulation and acts as an inhibitor of the complement system. The N-terminal end of VN also presents an RGD sequence, which interacts with a specific cell-surface receptor. Van and co-workers took advantage of this fact and grafted the synthetic RGD oligopeptide derived from VN protein (Ac-KGGPQVTRGDVFTMP) onto carboxymethyl chitosan coated in PCL nanofibrous meshes through standard NHS/EDC chemistry. The resultant mesh was evaluated regarding its potential in proliferation and osteogenic differentiation of human pluripotent stem cells (hPSC) showing osteogenic phenotype after culture.[81] Varum and coworkers used vitronectin-derived peptide (VDP) coated in polystyrene plates to direct the growth and differentiation of hPSC into neural progenitor cells (hNPCs). The coated plates showed a long-term propagation and direct neural differentiation of hNPCs. It also allows the theoretical expansion of hNPCs to the necessary quantities (>10^{10}) for regenerative medicine purposes or drug screening.[82] VDP arbitrates cell binding through interactions with the vitronectin binding integrin α_v-containing integrins and GAG side chain of cell surface proteoglycans.[83] Another potential protein used as CAM is elastin, one of the constituent proteins of the medial layer of the vascular wall. In this sense, Gobin and West reported the use of the elastin-derived peptide, Val-ala-pro-gly (VAPG), for biospecific cell adhesion of smooth muscle cells.[48] Therefore, the peptide was grafted to non-adhesive photo-polymerizable polyethylene glycol (PEG) hydrogels. That work showed an increased spread of smooth muscle cells with the increase of VAPG concentration. It was also observed that either endothelial cells, fibroblast or platelets were not able to adhere to these hydrogels showing the specificity of this non-integrin ligand. Finally, selectins are proteins involved in lymphocyte homing and inflammation processes. Selectins are responsible for the binding of leukocytes (tethering) and their consequent rolling into the inner surface of vessel walls. In this sense, these CAM represent an opportunity to effectively alter the progression of inflammatory responses.[84] Juenet *et al*. produced polymer nanoparticles using the polysaccharide fucoidan (Fuco-NPs) to target P-selectin.[42] These nanostructures showed enhanced thrombolytic efficiency with overexpression of P-selectin.

Combinations of several CAM can also be used to bind to different cell receptors. This strategy could be helpful in controlling cell function and to identify synergic effects between CAMs, as demonstrated in the study of Weber and coworkers, in which the influence of CAMs sequences on encapsulated β-cell survival and insulin release was studied [85]. To this end, PEG hydrogels were conjugated with different peptides such as IKLLI, IKVAV, LRE, PDSGR, RGD, YIGSR, and DGEA prior to the encapsulation of MIN6 β-cell. Cells encapsulated in hydrogels without any adhesion peptide presented low survival, nevertheless, when the sequences IKLLI and IKVAV or other laminin sequences (LRE, PDSGR, RGD, and YIGSR) were added, cells preserved their viability and increase insulin production. CAMs combinations IKLLI–IKVAV, IKVAV–YIGSR, and PDSGR–YIGSR were tested to explore synergistic effects, but only PDSGR and YIGSR suggests synergistic role showing a statistically significant difference in glucose-stimulated insulin secretion of β-cells.[85,86] Therefore, the use of CAMs on implanted biomaterials have proved to be useful to regulate *in vitro* and *in vivo* cell adhesion mechanisms and can be harnessed to direct tissue reparative responses including cell differentiation.

3.2 Cellular differentiation peptides

The process of cell differentiation is of great importance in tissue regeneration and cell turnover. It allows stem cells to specialize into a specific cell type and to segregate the component of specific tissues. Several proteins such as GFs and specific peptides sequences derived from active domains of bone or cartilage ECM, usually involved in the regulation of the cell differentiation process, can promote tissue renewal and regeneration. This process happens through their direct interaction with cell receptors, activating particular signaling pathways (Table 2).[73,87–95] Besides their success, GFs present some limitations regarding their low protein stability, short circulating half-life, the rapid rate of cellular internalization and side effects. Therefore, alternatives to the use of the whole proteins have been investigated due to the evidence that using smaller GF fragments or mimetic peptides motivates receptor-mediated signal transduction.[96] The use of peptidomimetic molecules entails great advantages. They can be designed to have the proper conformation that elicits the required biological response with higher affinity, more stability, and better pharmacokinetic parameters than the original proteins or active peptidic sequences.[97]

On the other hand, osteogenic differentiation has been accomplished by using bone morphogenetic proteins (BMPs), transforming growth factor (TGF-β), ascorbic acid, insulin-like growth factor 2 (IGF-2), osteogenic growth peptide (OGP), Col-I, parathyroid hormone (PTH), and platelet-derived growth factor (PDGF).[87,89–91] The phosphoprotein osteopontin (OPN) is found mostly in non-collageneous ECM proteins and in mineralized tissues such as bone and it has been proved to be involved in cell attachment to the ECM as well as a chemoattractant for

bone cells.[98,99] Shin and coworkers developed a biodegradable cross-linkable hydrogel with OPN-derived peptide (ODP) DVDVPDGR-GDSLAYG to repair tissue defects.[98] Results from this work indicate the presence of biomimetic interactions between the hydrogel OPF-ODP and osteoblasts, as well as the critical role of the RGD sequence in the binding to ODP. On the other hand, TGF-β mimetic peptides are important for chondrogenic differentiation, matrix deposition, and collagen synthesis. These ligands have proved to be capable to direct MSCs to produce cartilage matrix.[19] The work of Renner and coworkers used two peptides renamed as TGF1 and TGF2 (ANVAENA and LIAEAK, respectively) to mimic TGF- β1.[94] The culture of pellets of MSCs with the referred peptides leads to the expression of higher levels of the gene of Col-I when compared to negative controls. Chen and co-workers investigated the effect of IGF-2 on BMP-9-induced bone formation on MSCs.[100] Data presented in this study showed an increase in ALP activity and the expression of OCN and OPN, as well as BMPR-Smad (receptors for BMP and TGF-β, respectively) reporter activity and the nuclear translocation of Smad1/5/8. PDGF is the first factor released in wounds. It is involved in the connective tissue healing, including bone regeneration. It has been reported that PDGF, a potent mitogen, can increase the proliferation and differentiation of cultured animal osteoblasts as well as the calcium release.[101] Pountos and co-workers have shown that PDGF-BB (in high doses) enhanced osteogenesis in a more advantageous way than other GF, due to its intervention both in the proliferation and osteogenic differentiation. However, that work also shows that the highest osteogenic response was obtained with BMP-2 and BMP-7.[102] On the other hand, Saska and co-workers developed a 3D printed scaffold based on poly(3-hydroxybutyrate) (PHB) were produced by selective laser sintering (SLS) and functionalized with osteogenic growth peptide (OGP).[103] Data from *in vitro* experiments using rat bone marrow stem cells showed good cell viability/proliferation in all the PHB scaffolds. Additionally, SEM images demonstrated morphological differentiation in the groups containing OGP. In another work, Pigossi and co-workers have shown that the biofunctionalization of bacterial cellulose-hydroxyapatite (BC-HA) with OGP in critical-size calvarial defects in mice increases osteoblast differentiation/activity due to a better bioactivity of these biomaterials, having a positive effect on bone regeneration.[104] MicroCT analysis after 60 and 90 days showed a high percentage of bone formation. Enhanced expression of the bone biomarkers AlpL, Spp1 and Tnfrs11b were also observed during this period of time. Zouani and coworkers used hydrogels of poly(acrylamide-co-acrylic acid) chemically grafted with a BMP-2 mimetic peptide (RKIPKASSVPTELSAISMLYL).[87] The work intended to evaluate the effect of mechanical factors on cell differentiation, even though the hydrogels having the immobilized peptide *per se* induced the differentiation of hMSCs only into osteoblast-like cells. However, data demonstrated clearly that in softer gels (0.5–3.5 kPa), the effect of the BMP-2 mimetic peptide is inhibited, and no differentiation occurs. This suggests that the correct organization of F-actin cytoskeleton via focal adhesion contacts for bone differentiation is dictated by the stiffness of the

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environment (mechanical properties), which is necessary to trigger BMP-induced smad1/5/8 phosphorylation and nuclear translocation processes, important for osteogenic lineage commitment. In another study, Yewle and coworkers used oxidized parathyroid hormone (PTH_{1-34}) due to its high bone-binding affinity and conjugated it to hydrazine bisphosphonates (HBPs) via the N-terminal aldehyde to a variable length and lipophilicity.[90] The PTH–HBP conjugates were immobilized on bone wafers to simulate the bone surface, and the bioactivity of PTH_{1-34} was proved by the ability of apatite formation and an increase of pre-osteoblastic cell interaction when compared with groups having adsorbed PTH and no PTH (control). Finally, the bioactivity was altered neither by the length nor by lipophilicity of HBPs.

Chondrogenic differentiation commonly uses GF such as TGF-β1, TGF-β3, BMP-2 and IGF-1.[105,106] Combination of TGF-β3 and BMP-2 or TGF-β with IGF-1 has proved to be superior in chondrogenic differentiation of MSC when compared with TGF-β3 alone, due to the synergetic effect of both peptides.[107,108] Renner and Liu used mimetic peptides of chondrogenic GF in culture media to show its potential on chondrogenesis.[94] BMP-derived peptide (KIPKASSVPTELSAISTLYL) alone improved GAG production. TGF2 peptide (LIANAK) did not have any significant effect in chondrogenesis either alone or in combination with the other peptides. However, the interaction between TGF1 (ANVAENA) and IGF (GRVDWLQRNANFYDWFVAELG) peptides gave a higher average GAG content than controls. Zheng and coworkers used derived PRG (two-unit RGD cell adhesion motif) self-assembled peptide scaffolds with recombinant LAP-MMP-mTGF-β3 protein (latency-associated peptide, LAP) to produce cartilage *in vivo* through the differentiation of adipose-derived stem cells (ASCs) in chondrocytes.[109] The idea is to take advantage of the MMP-cleavage signal (PLGLWA) to release TGF-β3 from the recombinant fusion protein. After 21 days in culture, the hybrids with differentiated chondrocytes were injected subcutaneously into nude mice and retrieved after 4 days. The specific immunostaining demonstrated an enhanced accumulation of both ACAN and COL2A1 for recombinant fusion protein group, which means it was more effective in the formation of cartilage matrix than the groups with TGF-β3 alone or the control.

Different from cartilage regeneration, TGF-β has been reported to be an important driving force for synovial fibrosis in OA and contributes to the articular cartilage pathology.[110] TGF-β is released from damaged joints during OA development. This GF is responsible for the formation of nestin-positive MSC clusters, similar to early progenitor cells that form marrow osteoid islets accompanied by high levels of angiogenesis. In murine experimental OA models the overexpression of TGF-β1 in osteoblasts, induces OA with subchondral bone sclerosis and cartilage destruction. On the other hand, inhibition of TGF-β activity in subchondral bone stabilized the subchondral bone microarchitecture and attenuated the degeneration of articular cartilage.[111]

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The works presented here showed that mimetic peptides can also be useful in the clinics both when chondrogenic and osteogenic differentiation is envisaged, without the hurdles of using entire, complex proteins. In fact, inducing bone or cartilage formation with the biofunctionalization of OC biomaterials with such molecules may increase the rate of success of these tissue-engineered strategies due to the possibility of modulating the cells microenvironment and tuning their fate into an intended phenotype.

Table 2: List of natural-inspired osteogenic and chondrogenic differentiation peptides used in the biofunctionalization of biomaterials.

	Parental molecule	Peptide sequence	Function	Ref
Osteogenic Peptides	PTH	SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF	Hormone that plays important physiological roles, including calcium regulation and bone remodeling.	90
	OCN	γEPRRγEVCγEL	A small noncollagenous protein secreted by osteoblasts. Responsible for the synthesis and deposition of the mineralized, collagen-rich matrix that composes bone tissue.	92
	OGP	ALKRQGRTLYGFGG	A key factor in the mechanism of the systemic osteogenic response to local bone marrow injury. It promotes bone cell proliferation and differentiation.	91
	BMP-2	RKIPKASSVPTELSAISMLYL	Belongs to the TGF-β superfamily and induces differentiation of osteoblasts precursor cells into more mature osteoblasts-like cells.	87
	BMP-7	GQGFSYPYKAVFSTQ	Peptide also known as osteogenic protein-1, is a member of the TGF-β superfamily, and promotes osteoblast differentiation or osteogenesis.	89
	BMP-9	RKVGKASSVPTKLSPISILYK	Important for the development of the skeleton and the maintenance of haemostasis during bone remodeling.	88
	Col-I	GFOGER	The most abundant protein synthesized by osteoblasts. Promotes osteoblastic differentiation via specific binding of the α2β1 integrin receptor.	73
Chondrogenic peptides	TGF-β1	ANVAENA	An important function in the development, growth, maintenance and repair of articular cartilage. Deregulation of its signaling and responses has been shown to be involved in OA.	94
	IGF-1	GRVDWLQRNANFYDWFVAELG	Enhances the synthesis of proteoglycans and up-regulates the genetic expression of Col-II. Reduces the production of MMP-13.	94
	FGFR1	GPPDWHWKAMTH	Regulates the maintenance of cartilage and plays a critical role in joint homeostasis and OA development.	93
	Col-II	WYRGRL	A major component of hyaline cartilage. Promotes cell proliferation, ECM deposition and wound healing of chondrocytes.	95

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3.3 Antimicrobial peptides

Medical devices and biomaterials to be implanted inside the body should be able to promote adsorption of a layer of adhesion proteins (e.g. FN, VN, fibrinogen, albumin, and immunoglobulins) to its surface, favoring unwanted effects namely, microbial colonization.[112] In order to avoid bacterial infection, researchers have been studying natural antibacterial peptides and proteins (AMPs), also called host defense peptides in higher eukaryotic organisms, as surface coatings of implants.[113] The idea is to take advantage of these natural highly conserved molecules, which are present in all organisms (from prokaryotes to humans) and function as the first line of defense against bacteria, fungi, yeasts, and viruses.[114] In this way, AMPs are promising due to their low cytotoxicity, good biocompatibility, immunomodulatory activities, high efficacy and the development of resistance is less frequent than with antibiotics (Table 3).[115–124] One approach to surpass some of the limitations associated with their natural forms, namely, lack of stability, manufacturing costs, large size (>20 aa), possible toxic effects when used in high concentrations, short half-life, and susceptibility to proteases, is the production of synthetic mimetic AMPs.[125]

Table 3: Summary of natural human antimicrobial peptides and proteins (AMPs)

AMP	Location	Size (residues)	Function	Ref
Dermcidin	Eccrine sweat/skin.	47	Prevents local and systemic invasion of pathogens by the activation of pro-inflammatory cytokines TNF-α, IL-8, interferon-inducible protein 10 and macrophage inflammatory protein-3 α in keratinocytes.	115
LL-37 (cleaved cathelicidin hCAP-18)	Granules of neutrophils, lymphocytes and monocytes, squamous epithelia, epididymis, seminal plasma and lungs.	37	Presents synergistic antibacterial effects with the defensins, and is a chemotactic agent for neutrophils, monocytes, and T cells.	116
HBD3 (β – Defensin 3)	Keratinocytes, tonsilar tissue, epithelia of the respiratory, gastrointestinal and genitourinary tracts.	45	Exhibit antibacterial activities towards Gram-negative and Gram-positive bacteria as well as an ability to act as a chemo-attractant. It Inhibits lipid II in peptidoglycan biosynthesis.	117
HD5 (α-defensin 5)	Small intestine.	32	Disruption of cell division events in Gram-negative bacteria.	118
Histatin-5	Saliva.	24	Inhibits proteases such MMP-2 and MMP-9 and prevents the formation of reactive oxygen species (ROS) in fungus and bacteria.	119
HNP-1 (α-defensin 1)	Neutrophil azurophilic granules.	30	Induces DNA, RNA and lipid II in proteoglycan biosynthesis failure in bacteria.	120
Lactoferricin B	Gastrointestinal tract.	25	Inhibits phosphorylation of the two-component systems to suppress bacterial growth.	121
Lysozyme	Saliva, tears, intestine.	130	Hydrolyses specific residues in peptidoglycan cell wall of the bacteria.	122
PR-39	Neutrophils and wound fluids.	39	Inhibits DNA and proteins biosynthesis, translation and septation. Affects nucleotides and coenzymes transport and metabolism by the inhibition of NADPH oxidase activity.	123
tPMP-1 (thrombin-activated platelet microbicidal protein-1)	Platelet granules.	72	Promotes activation of autolytic enzymes in bacterial cells and reduces the capacity of bacteria and fungi to adhere to platelets.	124

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The unique feature of antimicrobial peptides is that the molecular mechanism of antimicrobial action does not rely on specific interactions with receptors or enzymes in bacteria. Moreover, microorganisms are less likely to develop resistance against these rapidly-acting synthetic peptides.[126] In general, these synthetic mimetic peptides are resistant to proteolysis and have prolonged lifetime and activity in physiological conditions. Mohamed and co-workers investigated the antibacterial activity of two synthetic peptides against multidrug-resistant staphylococci (WR12, 12 aa, exclusively composed of arginine and tryptophan, and D-IK8, 8 aa, β-sheet peptide).[127] WR12 and D-IK8 were able to reduce the TNF-α level by 50% and 44%, respectively, while also reducing IL-6 by 48% and 42%, respectively. These two peptides showed better antimicrobial activity than some conventional antibiotics (Vancomycin, Kanamycin, Erythromycin, Ciprofloxacin, Trimethoprim, and Linezolid), having potential to be used for different clinical applications, including resilient Methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Lim and coworkers studied another synthetic peptide, CW11, rich in arginine and tryptophan that proved to display potent antimicrobial activity against *E. coli, P. aeruginosa and S. aureus* via membrane disruption.[128] The peptide was chemically immobilized onto a polydimethylsiloxane (PDMS) surface and showed to be non-cytotoxic, with a hemolysis rate of ~2.0%. Interleukin 37 (IL-37) is a cationic peptide that unlike other antimicrobial peptides, is protected from proteolytic degradation, due to its ability to form aggregates and lipid bilayers.[129] In the study of Ding and coworkers, the correlation between IL-37 levels and OA was investigated in order to evaluate the anti-inflammatory effects of IL-37 in peripheral blood mononuclear cells (PBMCs) and synovial cells (SCs) from patients with erosive inflammatory OA (EIOA), patients with primary generalized OA (PGOA), and healthy controls (HCs).[130] The results showed an increase in the mRNA levels of IL-37 in the blood of EIOA patients compared with PGOA and HCs. Positive correlations were observed between IL-37 and the pro-inflammatory cytokines TNF-α, IL-1β and IL-6. These findings suggested that the IL-37 levels can be induced by pro-inflammatory cytokines *in vitro*, which act as positive feedback loops for increasing the production of IL-37.

Therefore, the natural AMPs are important components of the innate immune system, and as such, are involved in some human diseases.[131] In OA, AMPs such as HBD-3 present a non-anti-inflammatory function in the articular diseased joints. Varoga and coworker refer that after HBD-3 stimulation of osteoarthritic chondrocytes, MMP suffers an up-regulation, which suggests that this β-defensins may be involved in catabolic pathways of articular cartilage. The results indicate that HBD-3 can contribute to ECM degradation by the activation of MMP-1, -3, -13, and reduction of TIMP-1 and -2 expression.[132] The human peptide hCAP18 is an AMP produced in synovial membrane under the inflammatory conditions of OA.[133] In this type of environment, the hCAP18 is released and cleaved by proteinase 3 in neutrophils and kallikrein in keratinocytes, producing the alpha-helical LL-37 peptide, which recruits inflammatory cells and induces

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inflammatory responses, and activates M1 macrophages. Besides its pro-inflammatory functions, LL-37 also has antibacterial and immunomodulatory functions. This peptide allows the neutralization of TLR4 activation by LPS, the down-modulation of inflammatory cytokine responses and prevents invasion and inflammatory responses to pathogenic bacteria.[129]

4.4 Immune system improvement: anti-inflammatory peptides

After an injury, the tissue-resident macrophages produce DAMP (damage-associated molecular patterns) that are passively released from necrotic cells initiating inflammation.[134] Neutrophils are the first inflammatory cells recruited at a site of injury, enhancing host defense and wound detection while removing contaminants and promoting inflammation and monocyte/macrophage recruitment.[135] Macrophages have both pathogenic and protective functions in many biological processes. It has been proved that organized and well-regulated macrophage response is determinant for tissue remodelling and functional recovery. Macrophages present two types of phenotypes with different functions. Pro-inflammatory macrophages – M1, produced by exposure to interferon gamma (IFN-γ) and TNF-α) – which can become polarized by IL-4, IL-10, IL-13, TGF-β and glucorticoids changing their phenotype to anti-inflammatory macrophages – M2.[136] In this sense, it is understandable that deregulation of M1 leads to uncontrolled inflammation process (e.g., inflammatory bowel disease, RA, and OA) while the modulation of macrophages to M2 can contribute to regeneration through a crosstalk with Tregs. This, in turn, helps to sustain the anti-inflammatory/anti-fibrotic phenotype via the secretion of anti-inflammatory cytokines such as IL-10. [137,138] A recent study discovered that colony stimulating factor-1 (CSF1R) is the key to prevent fibrosis without interfering with other macrophage functions and when grafted into scaffolds improves the biomaterial biocompatibility without the need for broad immunosuppression.[139]

The most targeted method for immunomodulation of macrophages destiny has been to release factors such as interleukins IL-4, IL-10 or steroids into controlled released systems. Spiller and co-workers designed scaffolds for bone regeneration based on the decellularized bone with physically adsorbed IFN-γ with fast release to promote the M1 phenotype, followed by a more sustained release of IL-4 attached via biotin-streptavidin binding to promote the M2 phenotype. The scaffolds were able to promote the polarization of M1 to M2 and although there was an overlapping in the release of IFN- γ and IL4 to some extent, the subcutaneous implantation in murine models was able to increase vascularization, as intended. This is due to M1 macrophages being responsible for the initiation of angiogenesis while M2 macrophages promote vessel maturation (Figure 2).[140]

Along these lines, the specific control of the immune system through the use of biomaterials has raised some attention, especially due to the lack of effectiveness and limitations associated with the use of cellular therapies and GF as previously referred.[141] Consequently, chemical

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modifications of implanted biomaterials have been used to modulate cell response and behavior.[142] In this sense, Rostam and coworkers assessed the impact of different surface chemistries on macrophages polarization using untreated hydrophobic polystyrene (PS) and hydrophilic O_2 plasma-etched polystyrene (O_2-PS40) surfaces cultured with human monocytes for 6 days.[143] The report demonstrated that the surface O_2-PS40 polarized monocytes towards M1 phenotype as evidenced by the higher expression of the pro-inflammatory transcription factors, STAT1 and IRF5, as well as produced the highest levels of IL-6 and IL-1β. The hydrophobic PS surface exhibited M2 phenotype with high expression of mannose receptor and production of the anti-inflammatory cytokines IL-10 and CCL18. As referred, increased quantities of M2 macrophages within the remodelling site has been associated with better outcomes after the implantation of biomaterials improving the regeneration process. However, it is widely accepted that the prolonged presence of M2 can origin foreign body giant cells (FBGCs), which are detrimental to biomaterials due to their ability to increase oxidative damage and recruit inflammatory cells. In this sense, the understanding of the control of the M2: M1 ratio is the key point to design the next generation of immuno-informed biomaterials that enhance tissue integration, remodeling, and regeneration.[144]

In OA, both macrophages and fibroblasts play a prominent role in the progression of the disease. *In vitro* studies have shown that macrophages derived from bone-marrow and peritoneum secrete enzymes that may be responsible for cartilage degeneration.[145,146] Utomo and coworkers demonstrated that M1 aggravates the progression of cartilage degeneration and M2 does not directly affect OA cartilage or inhibit the effects of M1 on cartilage.[147] That study suggests that the products of M2 macrophages itself may not be potent enough to suppress inflammation in OA cartilage, however, the interaction between different phenotypes of macrophages may be necessary to trigger a proper regenerative response.

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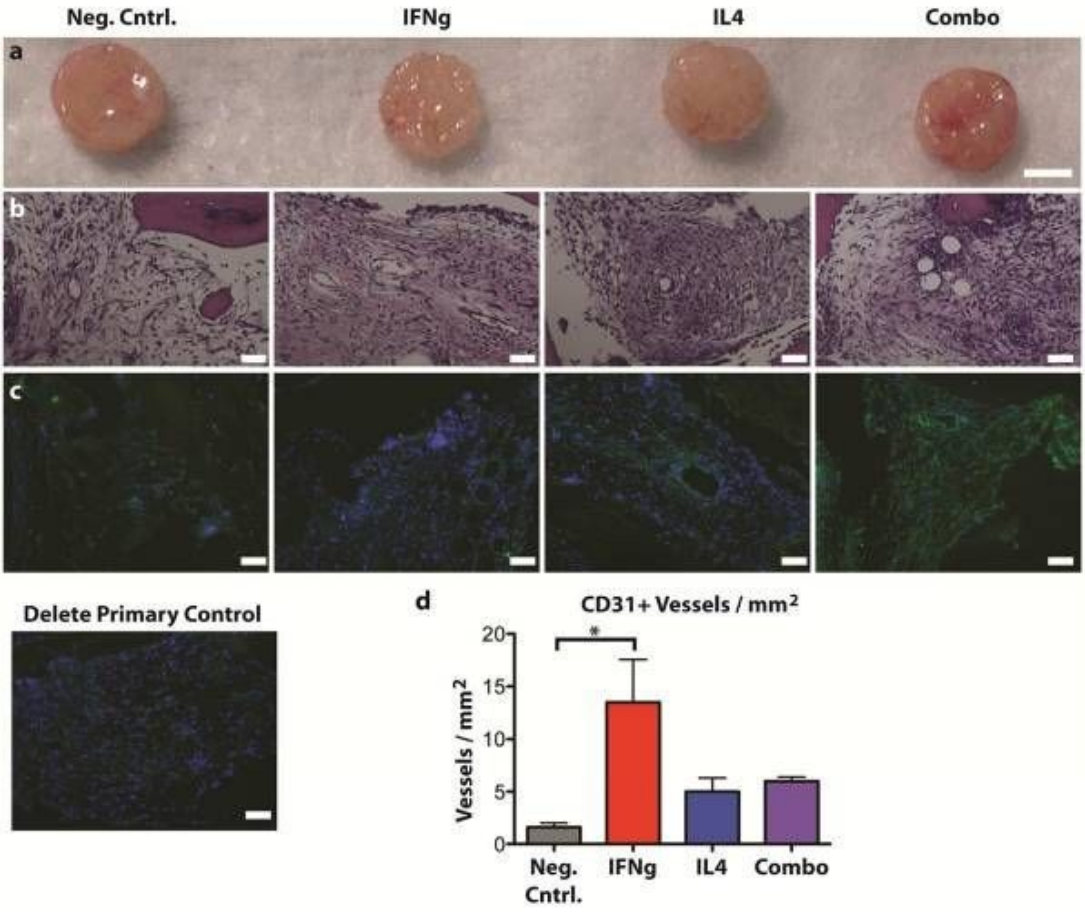

Figure 2: Vascularization *in vivo* after 2-weeks of implantation in a murine subcutaneous model. a) Gross view, (b) H&E staining, (c) immunohistochemical analysis for the endothelial cell marker CD31 (green) and counterstained with DAPI (blue), and (d) quantification of the number of CD31+ vessels per mm2 (mean +/− SEM, n=3, * significance in one way ANOVA and Tukey's post-hoc analysis, $p<0.05$). Scale bars 100µm. Adapted from [140].

The data concerning the use of chemically modified peptides suggests that specific changes in the chemistry of biomaterial surfaces can be a powerful tool to regenerate osteoarthritic joints through the modulation of macrophage phenotype, since there is a homeostasis between macrophage and other cell types that researchers can optimize to enhance the regenerative or protective capacity of these inflammatory cells.

4.5 Degradation enzymes

Natural ECM provides the structural framework to allow the homeostasis between the formation and maintenance of the tissues. ECM is able to dynamically be degraded and synthesized by the cells, in both healthy and diseased conditions, which is essential for cell adhesion, migration, proliferation, and differentiation.[148] Therefore, the use of ECM-based materials such as fibrinogen, collagen, chondroitin, heparin or HA grants this important feature providing an advantage to these materials. However, it is also possible to incorporate

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biochemically relevant degradation cues that guide the cellular behavior to accomplish a proper remodeling process. Moreover, the use of specific chemical or physical modifications, as well as the use of mimetic peptides able to activate or take advantage of the degradation enzymes present in certain environments can be advantageous to treat some physiologic processes.[149] With this in mind, Pereira and coworkers synthesized a photo-polymerizable pectin hydrogel with the adhesive peptide RGD and the MMP-cleavable peptide (CGPQG↓IWGQC).[150] They observed that the rate of cell spreading and elongation in these cell-degradable gels depends on two factors, the MMP-mediated degradation, and the hydrogel stiffness. Hydrogels with a low degree of crosslinking are less stiff, more contractile and improved the elongation of the cells. Conversely, in stiffer hydrogels, the spreading is mediated by the MMP-assisted degradation, which means that as the hydrogel degrades the cells are enabled to penetrate inside the network of its structure and properly spread (Figure 3). On the other hand, Parmar and coworker prepared collagen hydrogels (based on collagen produced in *Streptococcus* with HA motifs to mimic the cartilage environment) and grafted them with different ratios of MMP7-degradable peptide (PLELRA) and the aggrecanase ADAMTS4-cleavable peptide (RDTEGEARGSVIDR) in order to match the degradation rate of hMSCs and improve chondrogenesis.[151] The rationale of this design is based on chondrogenesis, because hMSCs express MMP7 in the early stage of the process, while ADAMTS4 has increased activity in mature cartilage, and the authors intended to the scaffolds be degraded in a chondrogenic-driven process providing the release of the newly produced chondrocytes. Regarding the sGAG content, it was significantly higher for the MMP7: ACAN(25:75)-HIHA-Scl2 hydrogels. The data from immunohistochemical staining showed that Col-II was noticeably increased when compared with the controls (Col I and Col X) indicating the promotion of chondrogenesis in all hydrogels.

These works evidence the importance of protease-mediated cleavage in biological processes as well as the need to include biochemically relevant cues to assure the proper degradation of biofabricated scaffolds produced with non-absorbable materials. In OA, it is thought that proteolytic enzymes are the key mediators of the degradation of cartilage components. Two major classes of proteases are the main players in collagen and proteoglycan cleavage: MMPs secreted in the synovial space contribute to collagen degradation; members of both MMP and ADAMTS families oversee the degradation of proteoglycans which are also susceptible to the action of many other proteases due to their extended core protein conformation.[152] The knowledge of the involved proteases in OA might allow researchers the precise introduction of specific protease-cleavable peptides, depending on the type of material or stage of chondrogenesis. This can enable a controlled degradation of the produced scaffolds and allow them to fill OA defects by taking advantage of the diseased environment in which they will be inserted.

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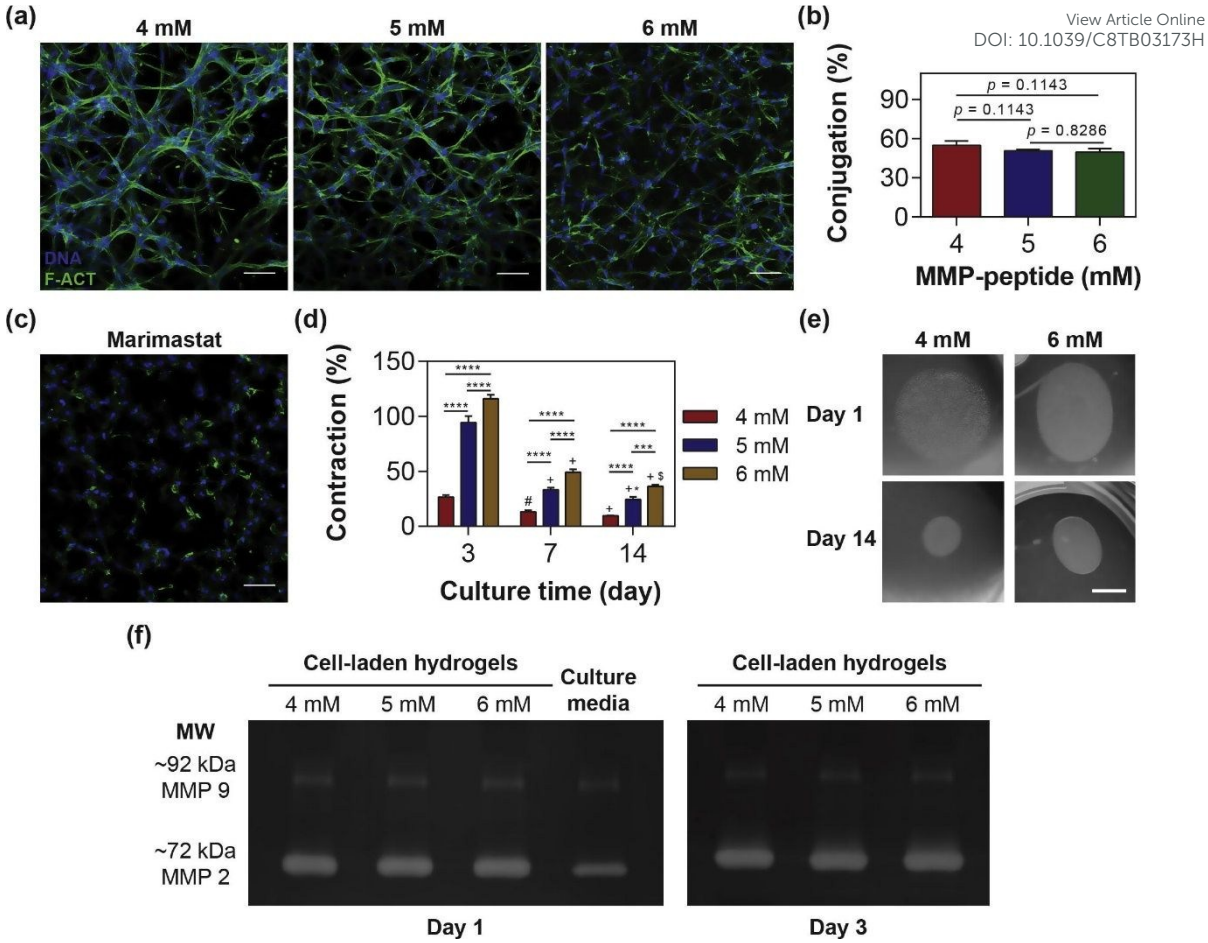

Figure 3: Encapsulated dermal fibroblasts within MMP-degradable NorPEC hydrogels. (a) Confocal microscopy images of fibroblasts stained for DAPI (blue) and F-actin (green) at day 14 detailing the influence of MMP-peptide concentration (4, 5 and 6 mM MMP-cleavable peptide, 2.5% NorPEC, 2 mM RGD peptide, 20 s UV) Scale bar: 50 µm. (b) Influence of bulk MMP-degradable peptide content on the conjugation efficiency of RGD-peptide during photo-polymerization. (c) Confocal microscopy images at day 7. Scale bar: 50 µm. (d) Effect of MMP-peptide concentration on the contraction of hydrogels. (e) Photographs of low and highly crosslinked hydrogels at day 1 and 14. Scale bar: 2.5 mm. (f) Gelatin-zymograms of conditioned media collected at day 1 and 3. Statistical analysis using two-way ANOVA with Tukey's multiple comparisons test (***$p < .001$, ****$p < .0001$); compared to day 3: $^{+}p < .0001$, $^{\#}p < .001$; compared to day 7: *$p < .01$, $^{\$}p < .001$. Adapted from [150]. Copyright 2017 Acta Materialia Inc. Published by Elsevier Ltd.

4. Perspective remarks and future trends

While OA conventional treatments are capable of reducing the symptoms but do not stop or reverse the disease, cellular therapies using MSCs arose as potential alternatives due to their great potential to regenerate OA joints. However, there are some risks associated with cell therapies, namely the loss of cell phenotype, the risk of pathogen contamination in cell expansion, due to fetal bovine serum (FBS) and GF used, as well as teratoma formation. On the other hand, Exos represent natural delivery systems with small size, a feature that can avoid phagocytosis or degradation by macrophages, thus overcoming problems associated with nanoparticle systems.

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However, still little is known about the role of Exos in health and disease, which makes it complicated to predict their long-term safety and therapeutic effect. The heterogenicity in composition shows immunogenicity (immunostimulatory or immunosupressive) especially due to their possible involvement in tumor progression and growth.

Table 4: Sum up of the immobilization strategies and concentrations used for biofunctionalization of peptides.

Peptide	Immobilization	Concentration	Ref.
VCAM-1	Physical adsorption.	4 µg/mL	54
HAV	Michael-type addition.	-	57
GRGDS + PHSRN	Grafting using DMF and DIPEA.	0.25 mg/mL	45
A99 + AG73	Carbodiimide chemistry.	A99/AG73: ratio of 9:1, total 0.2 mM	46
IKVAV	Carbodiimide chemistry.	IKVAV/PL (100/10 µg/mL)	47
VAPG	Covalent immobilization.	1.4 mol/mL	48
GFOGER	Coating of titanium surfaces.	20 µg/ml	72
DGEA	Carbodiimide chemistry.	1% (w/v)	74
Ac-KGGPQVTRGDVFTMP	Carbodiimide chemistry.	1 mM	81
DVDVPDGR-GDSLAYG	Chemical cross-linking.	0.1, 0.5, 1 mmol/g	98
H_2N-ALKRQGRTLYGFGG-OH	Adsorption.	10^{-5} mol/L	103
RKIPKASSVPTELSAISMLYL	Carbodiimide chemistry.	10^{-3} M	87
CGPQGIWGQC	Thiol-ene photo-click reaction.	4–6 mM	150
PLELRA and RDTEGEARGSVIDR.	Ionic cross-linking.	75:25, 50:50, and 25:75 M ratio.	151

In this sense, strategies making use of biofunctionalized biomaterials with mimetic peptides, aiming to develop *in situ* tissue regeneration strategies that guide and support neo-tissue whilst conferring proper mechanical and functional features, may allow overcoming these hurdles. However, the creation of ECM-mimetic scaffolds still remains one of the major challenges regenerative medicine faces and the chosen biomaterials need to be conveniently modified to precisely control cell fate and tissue remodeling. Thus, the use of mimetic peptides, derived from the active domains of soluble or ECM proteins, represents a massive advantage in OC repair and regeneration, opening several possibilities to allow biomaterials to be properly integrated into the desired tissue. In particular, mimetic peptides can avoid the use of GF, FBS and other molecules responsible for cell differentiation and organization. It also circumvents the drawbacks of the immune system activation due to the origin of the cells (autologous or allogenic), pathogen contamination, loss of clinical grade or potential tumor formation. These

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peptides are commonly non-toxic and can be designed to have the proper conformation and temporal-spatial regulation to elicit the required biological response with higher affinity, more stability, and better pharmacokinetic parameters than the original biomolecules. Specifically, the mimetic peptide can improve cell adhesion, induce cell differentiation, prevent infection or inflammation processes and enable dynamic remodelling of the ECM by using peptides that target sequence for a specific enzyme such as MMPs. Biofunctionalized biomaterials with mimetic peptides will provide cues to stem cells present in the injured tissues to restore their structure and function. Therefore, these peptides can either direct the attachment of cells or can be released as soluble ligands at a certain rate to exert their effect. Either way, the liberation of these mimetic peptides will depend on the strategy used to entrap the peptide into the biomaterial, which is of utmost importance (Table 4). For quick bulk release, the simplest strategy is to adsorb the peptides to the surface of the biomaterial, but the entrapment within the structure of the biomaterial will reduce the diffusion rate. The covalent binding is also a possibility. It allows a more finely controlled release rate of the mimetic peptide that will depend on the degradation rate of the biomaterial as well as the specific affinity of the binding domain of the peptide. However, some issues like the density of ligands used, the distance between the ligand and the biomaterial, as well as the biological half-life and clearance of the peptides are important issues to consider in covalent bonding of bioactive molecules to biomaterials. These parameters can determine both their biological efficiency and/or *in vivo* stability. Another possible limitation of using peptides and proteins is related to their natural degradation by endogenous proteases, which is usually overcome through the design of mimetic peptides. Beyond these facts, it is necessary to question whether these peptides are enough to regulate cell fate under OA conditions. This query emerged from the literature overviewed herein, in which all the studies use healthy cell models from different sources instead of using disease models. The use of OA models would be of great benefit to properly predict the efficiency of the chosen biomaterial-peptide system to treat OC defects. For a thorough understanding of this approach, scientists need to combine specific expertise from cell biology, chemistry, material and biomechanical sciences with the clinical challenges to develop strategies that precisely control the host regenerative response.

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