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Elastin-based biomaterials and mesenchymal stem cells

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Abstract

Elastin is the dominant mammalian elastic protein found in soft tissue. Elastin-based biomaterials have the potential to repair elastic tissues by improving local elasticity and providing appropriate cellular interactions and signaling. Studies that combine these biomaterials with mesenchymal stem cells have demonstrated their capacity to also regenerate non-elastic tissue. Mesenchymal stem cell differentiation can be controlled by their immediate environment, and their sensitivity to elasticity makes them an ideal candidate for combining with elastin-based biomaterials. With the growing accessibility of the elastin precursor, tropoelastin, and elastin-derived materials, the amount of research interest in combining these two fields has increased and, subsequently, is leading to the realization of a potentially new strategy for regenerative medicine.

1. Introduction

Regenerative medicine is a rapidly expanding area of modern medicine that aims to replace or repair organs through administration of cells that have regenerative and immunomodulative properties. These can include biologically active "matrices" that are capable of recruiting host cells, stem cells, or a combination of both. The use of biomaterials made from extracellular protein polymers is advantageous because they innately possess qualities desirable for tissue regeneration, such as supporting cellular activity, including cell signaling, and biodegradability where appropriate[1].

Mesenchymal stem cells (MSCs) have multilineage potential and have been intensely studied since their discovery. The combination of MSCs and scaffolds presents a new strategy for tissue regeneration. One avenue currently being explored is the combination of MSCs with elastin-based biomaterials, a class of protein biopolymers derived from elastin. Elastin is an important component of the extracellular matrix (ECM) predominantly found in soft elastic tissue (e.g. skin, blood vessels and lungs), and is produced from its monomer, tropoelastin. This review will focus on applications of elastin-based biomaterials and MSCs, discussing the profound impact of elasticity upon MSCs, giving background on the role of elastin in tissue repair, and detailing recent advances in research and applications combining the two.

2. Effects of elasticity on mesenchymal stem cells

Stem cells are unspecialized cells with the potential to differentiate into cells of multiple tissue lineages. They are essential in facilitating biological development and are heavily involved in repair and maintenance of tissue. MSCs were first isolated from bone marrow in the 1960s by Friedenstein *et al.* who described their ability to regenerate ossified bone, bone stroma and hematopoietic tissue[2]. MSCs are believed to reside in local cellular and molecular environmental niches which have not yet been isolated. The minimal requirements for

classifying MSCs are that the cells must adhere to tissue culture plastic under standard culture conditions, must be able to differentiate into osteoblasts, adipocytes and chondrocytes under standard differential conditions *in vitro*, and must exhibit markers CD105, CD73 and CD90 (≥95%) and lack expression of CD45, CD14, CD34, or CD11b, CD79 alpha or CD19 and HLA-DR (≤2% positive)[3].

Much research has focused on investigating MSC "plasticity" in terms of differentiation, which refers to the ability of MSCs to mature into cells other than those of their tissue origin[4]. Pittenger *et al.* showed the *in vitro* differentiation of human bone marrow MSCs (bmMSCs) into osteogenic, adipogenic and chondrogenic lineages by culturing cells in differential media and identifying the extent of differentiation through a combination of cell morphology, surface markers and histological methods[5]. Classically, media containing soluble growth factors that provide biochemical cues have been used to induce the differentiation of MSCs[5]. They were widely thought to be the determining factor of differentiation until Engler *et al.* showed the profound impact of matrix elasticity upon MSC morphology and lineage markers by using collagen-coated polyacrylamide gels with tunable stiffness to facilitate cell differentiation[6]. In the absence of differential media, bmMSCs grown on soft gel surfaces with Young's moduli of 0.1 – 1 kPa displayed branched neuron-like morphology and upregulated neuron-specific markers such as β3 tubulin and nestin[6]. BmMSCs grown on surfaces mimicking muscle stiffness with Young's moduli of 8 - 17 kPa or the osteoid stiffness with Young's moduli of 25 – 40 kPa displayed the appropriate respective myoblastic or osteogenic morphologies and transcriptional markers that indicated mechanically-directed differentiation[6]. The addition of differential media that did not promote the same lineage as the surface stiffness resulted in a mixed MSC phenotype and appeared to be influenced by both the physical and biochemical signals, highlighting the importance of matrix elasticity in

directing MSC activity[6]. This discovery significantly impacted the direction of MSC research, leading to the study of mechanical cues that affect MSC behavior.

Of noteworthy consideration is that most of the above experiments were performed on 2D substrates, whilst the usual environment of cells in tissue is 3D. The difference in cell activity between 2D and 3D environments has become an area of intense study because results of 2D experiments do not necessarily translate well to 3D experiments[7]. For example, although matrix elasticity influences MSC differentiation similarly in both 2D and 3D scaffolds, the morphology of cells in 3D experiments is markedly different to those grown in a 2D environment[8-10]. A likely explanation for this is that MSCs adopt apical-basal polarity on 2D surfaces, unlike in their native 3D environment[7], meaning that the cell's adhesions to the substrate are limited to the base of the cell rather than spread across the majority of its surface[11]. As many of the cell's adhesion receptors also serve as mechanosensors, the dimensionality of scaffolds becomes important in eliciting cellular responses, including stem cell fate, signal transduction, ion flux and gene transcription[12]. As the intricacies of the relationship between cell fate and dimensionality are unraveled, scaffolds that better simulate the cells' natural 3D environment may be fabricated, furthering the depth of knowledge about MSC activity.

The impact of matrix elasticity extends beyond MSC differentiation. For example, the cell cycle of three distinct progenitor cells (osteoblasts, fibroblasts and mammary epithelial cells) was found to be affected by substrate elasticity in all cases[13]. Cell cycle progression was found to be partly controlled by phosphorylation of retinoblastoma protein (a regulator of the G1/S stage of the cell cycle) in a manner dependent on substrate elasticity[13]. Another study found that MSCs appear to be quiescent on soft substrates with Young's moduli of approximately 250 Pa, similar to that of fat and bone marrow[14]. MSCs cultured on materials with other Young's moduli values below 1 kPa have yielded similar results[15, 16]. A further

study using muscle stem cells demonstrated stem cell self-renewal on hydrogels that mechanically resembled soft muscle tissue with Young's moduli of approximately 12 kPa[17]. These data are suggestive of microenvironmental stem cell niches being defined by unique matrix elasticity, and that the elasticity may play a role in regulating stem cell quiescence. Under this assumption, the presence of MSC niches in stiffer tissues could then be explained by the existence of niches as specialized "pockets" of regulatory ECM that have an elasticity different to the rest of the tissue in that anatomical location.

The composition of the ECM has also been found to impact MSC activity. For example, laminin surface coatings have been found to enhance neuronal stem cell migration, expansion and differentiation, unlike fibronectin coatings[18]. This suggests unique roles for ECM proteins in regulating differentiation, possibly by providing extra signals to drive the cell cycle. Additionally, the presence of elastin around stem cell niches, such as hair follicles in skin[19] or through vasculature of the bone[20], presents circumstantial evidence for its potential involvement in regulating stem cells. Since elastin is involved in cell signaling to the ECM[21], it may also have a role in regulating the fate of MSCs.

Cells sense substrates including the ECM through their external receptors. An example of these receptors are integrins, a major class of adhesive cell receptors, which are linked to the cell cytoskeleton through a network of proteins[22]. The binding of integrins to cell receptors is linked to cytoskeletal activities such as spreading and morphology, which are used as a measure of biocompatibility of substrates. The large assortment of integrins that arise from combinations of α and β subunits means that these receptors will only bind substrates when appropriate, because they have different binding preferences towards different substrates. For example, collagen I is bound by integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$[23], tropoelastin is bound by $\alpha_v\beta_3$[24] and $\alpha_v\beta_5$[21], and laminin is bound by $\alpha_6\beta_1$[25]. The specific sequences bound by integrins have been used to functionalize biomaterials, especially "blank-slate" materials such as poly(ethylene

glycol) which do not normally encourage cell adhesion[26, 27]. These materials are useful for investigating the effects of either functional ligands or entire ECM proteins, which are inherently difficult to isolate from the mechanical properties of a pure protein scaffold since the two are closely intertwined. Hwang *et al.* found MSCs encapsulated in PEG gels containing different ECM constituents differentiated depending on the component available for cellular binding[28], thus allowing some discrete observation of the effects of particular ECM ligands. Rowlands *et al.* investigated the combined impact of substrate stiffness and ECM signaling upon MSCs, by using polyacrylamide hydrogels covalently coated with ECM proteins[29]. Discrete combinations of hydrogel stiffness and protein coatings were used to examine the factors required to direct MSC osteogenesis or myogenesis[29]. Rowlands *et al.* found that MyoD1, a myogenic marker, was expressed at a high level on surfaces of 80 kPa regardless of the ECM protein, yet the area of spreading was significantly different between ECM coatings[29], highlighting the capacity ECM of components to direct MSC lineage and gene expression. Altrock *et al.* observed that hematopoetic stem cells altered their signaling and rearranged their distribution of integrins depending on the nanopatterning of fibronectin coatings[30]. Further studies investigated the nanopatterning of RGDfK ligands on PEG gels and found that osteoblasts spread poorly when ligands were over 73 nm apart due to limited integrin clustering[31]. The importance of the arrangement of ligands adds a further layer of complexity to directing MSC function. Although the ligand and mechanical signaling aspects of the ECM are closely interrelated, these advances come closer to understanding the subtle cues that are innate to the ECM and are likely to give rise to a new class of biomaterial that is highly instructive and can precisely direct cellular activity.

Two long-standing issues of regenerative biomaterials are the rejection of an implant by the patient's immune system, and that angiogenesis needs to occur in order for the implant to host viable cells long-term. The secretion of trophic and immunomodulatory molecules can

be affected by substrate stiffness[32-34]. Therefore, focus has been on creating biomaterials that direct the MSC secretome, which can lead to modulating immune cells or promoting trophic activities such as angiogenesis to improve patient outcomes[35-37].

MSCs are of interest in both these areas because they secrete a wide variety of biochemical molecules including soluble growth factors, including TGF-β1, VEGF, insulin-like growth factor, platelet-derived growth factor, and fibroblast growth factor. An example of the influence of substrate elasticity upon the trophic behavior of MSCs is the increased secretion of VEGF on stiff surfaces that resemble muscle and harder tissues (20 kPa – 40 kPa) compared to softer surfaces resembling neural tissue (0.5 – 2 kPa)[32, 33]. A study involving retinal cells noted that an increase in matrix elasticity correlated to an increase in VEGF mRNA and *in vivo* retinal angiogenesis, and additionally elucidated the involvement of filamin, a mechanotransucing protein, through inhibition with short interfering RNA[34]. Although the exact link between elasticity and VEGF secretion has not been established in MSCs, these initial results suggest the feasibility of a scaffold that can eventually be designed with appropriate mechanical properties to promote angiogenesis in regenerated tissue.

MSCs are also of special focus in immunomodulation. They are capable of suppressing the proliferation of B cells, dendritic cells and natural killer cells[38], modulating CD^{8+} T cells[39], and halting monocyte differentiation into specialized immune cells[40]. Therefore, MSCs have the potential to be applied to patients with diseases where the immune response poses a significant barrier for the wellbeing of patients, such as graft-vs-host disease, Crohn's disease, myocardial infarctions and tissue transplantation[41]. However, MSCs can also secrete pro-inflammatory cytokines in certain situations; MSCs grown on substrates of medium stiffness resembling muscle tissue (10 – 20 kPa), secreted pro-inflammatory cytokines, interleukin 6 and interleukin 8[32, 33]. It is apparent that substrate elasticity requires a fine degree of tuning if MSCs are to be therapeutically relevant, especially for patients who may

be immunocompromised or taking immunosuppressive treatment. To further illustrate the complexity of design considerations of a scaffold for implantation, it is of note that these cytokines are also involved in promoting angiogenesis[42, 43], which is of significance for tissue repair. Thus, further investigation is required to assess whether the pro-angiogenic nature of these cytokines would outweigh the elevation of inflammation *in vivo* to promote sufficient healing in patients.

The relationship between MSCs and matrix elasticity offers an explanation as to why administering MSCs as an infusion, into either tissue or the bloodstream, have yielded mixed results. Although some studies reported improvements in wound healing[44-47], others have shown that undirected administration of MSCs can cause serious and adverse effects such as ossification of cardiac tissue[48, 49]. Although MSCs are capable of homing to injured tissue to participate in wound repair[50, 51], damage through injury or disease can compromise the elasticity of tissue by aberrant ECM formation, leading to changed biochemical conditions that may not necessarily direct MSCs in a way that is beneficial for patients[49].

3. Elastin and tropoelastin: role in tissue repair and maintenance

3.1. Properties and interactions

The structure of tropoelastin is composed of several regions that directly contribute to its inherent mechanical properties. Tropoelastin is a highly elastic protein; it is capable of extending to approximately eight times its resting length with no evident hysteresis[52]. A spring-like coil adjacent to the N-terminus primarily contributes to this elasticity. The molecule is entropically driven to recoil after stretching because the configuration of water changes when hydrophobic regions are exposed upon stretching[53], decreasing the number of possible structural conformations[54]. Further flexibility arises from a hinge region, which has a less ordered structure compared to other regions in tropoelastin[55-57]. The hinge region

contains key lysine residues that participate in cross-linking to form elastin[58, 59], adding stiffness to elastin fibers as demonstrated by the difference in Young's moduli between tropoelastin and natural mature elastin (~3kPa and 300-600 kPa respectively)[52, 60]. The stiffness of elastin-containing biomaterials can be modified with different manufacturing conditions and cross-linking treatments, resulting in materials with uniquely tailored Young's moduli from 8 kPa to 20 MPa[61]. The role of the bridge region in tropoelastin is less understood, but as the disruption of domains in this region leads to limited elastin formation, it may have involvement in elastin assembly[62, 63].

The full extent of interactions between cells and elastin derivatives is only partially understood. However, it is well accepted that elastin derivates, such as tropoelastin, provide an important platform for supporting the activities of many cells including fibroblasts, endothelial cells and smooth muscle cells[19]. The most well characterized cell-tropoelastin interactions are mediated through cell receptors such as elastin-binding protein (EBP)[64], glycosaminoglycans (GAGs)[65] and integrins[21, 24].

EBP is a transmembrane protein[66] that is an inactive, alternatively spliced variant of β-galactosidase capable of binding XGXXPG sequences in elastin such as VGVAPG[67, 68]. Binding of EBP to extracellular elastin fragments, formed through injury, triggers cell activity such as myofibrillogenesis mediated by vascular smooth muscle cells[69, 70] and chemotaxis of fibroblasts and monocytes[67]. EBP also has a chaperone role during elastin formation by limiting tropoelastin degradation and intracellular aggregation[68].

Other major protein receptors of elastin are integrins, which are involved in cell adhesion, migration and proliferation, as previously discussed[71]. Integrins often bind RGD motifs of other ECM components[72]. However, tropoelastin lacks RGD sequences and binds to integrin

$\alpha_v\beta_3$ through its C-terminus[24], and has also recently been shown to interact with fibroblast integrin $\alpha_v\beta_5$ through a central region[21].

Lastly, elastogenic cell-surface GAGs[73] are thought to be involved in elastin assembly[74, 75]. Negative charges on GAGs may interact with positive residues on tropoelastin, such as lysine and arginine, to neutralize charges that may otherwise repel each other during elastin assembly[76] and enhance the critical concentration of tropoelastin in solution required to form sphere-like structures important for elastin formation[77]. However, GAG-tropoelastin interactions have been mostly observed with bovine tropoelastin, which contains additional exons 34 and 35[24]; the binding between GAGs and human elastin has yet to be fully explored.

3.2. Elastin and elasticity in repair and disease

Elastin does not comprise the majority of most tissue other than large elastic blood vessels and ligaments[78], yet its contribution to tissues is of such importance that when its structure is compromised, there are serious repercussions for the function of tissue containing aberrant elastin[19]. Elastin can be damaged by external mechanical and thermal forces[79], or internally by diseases such as atherosclerosis of arteries and calcification of native heart valves[80, 81]. The breakdown of elastin, the most durable ECM protein, is thought to elicit a powerful signal that involves all stages of the wound repair pathway: inflammation, repair and remodeling[19, 82, 83].

Remarkably, fetal tissue wounds repair scarlessly wounds to fetal tissue unlike wounds in adult tissue. This is likely due to dissimilarities between the tissues, for example, repression of immune cells in fetal tissue[19]. A significant concern that arises during adult tissue repair is a deficiency in elasticity, which results in *de novo* tissue that is both mechanically and functionally compromised[19]. A well-studied example is the reduction of elasticity in the lungs of asthma patients through airway remodeling[84]. Disarrayed elastin formed in poorly

remodeled airways elevates inflammation and increases the number of smooth muscle cells and blood vessels, leading to overall airway wall thickening and reduced elasticity[84]. A similar situation occurs during atherosclerosis, where elastin damage through low density lipoproteins leads to anomalous remodeling in the arteries, causing an impairment of function that contributes to strokes and thrombosis[80]. Histological analysis of dermal scarring such as hypertrophic scars, burn-related keloids, and other trauma show fragmented elastin that has been poorly remodeled in inappropriate dermal layers[85]. For example, a significantly larger amount of aberrant elastin is deposited in the deep dermis as keloids, contributing to the lack of local elasticity in this type of scarring[85].

Elastin-based biomaterials have primarily been investigated due to their potential to introduce elasticity into poorly regenerated tissue, with the aim of imbuing them with improved function comparable to tissue reformed during fetal wound healing[86, 87]. Further knowledge of the cellular interactions and mechanical properties of elastin will allow the creation of biomaterials that are able to mimic a range of functions required for tissue regeneration.

3.3. Sources of elastin and its derivatives

Elastin-based biomaterials can be fabricated from natural, recombinant and synthetic sources. They have been recently reviewed in-depth[88], therefore, this section will briefly describe the sources of elastin available and the current technologies available for creating biomaterials.

Natural elastin and tropoelastin are difficult to source for use in biomaterial fabrication; the expression of tropoelastin is largely repressed in adults[89], nor is it readily isolated because it is insoluble in its native form[90]. Due to this, human elastin has often been purified from donated cadavers. Consequently, finding alternative sources of elastin and tropoelastin was warranted. Due to interspecies conservation of many important sequences in the tropoelastin gene[91], animal-derived tropoelastin has become a widespread alternative to natural human

tropoelastin. Generally, however, isolating elastin from animals requires inhibiting the natural cross-linking process involving lysyl oxidase and is often achieved through administering a copper deficient diet to animals[92]. Being inefficient and ethically questionable, there have been other strategies to source animal-based elastin, for example chemically treating synthetic-soluble elastins such as α-elastin[93].

A second alternative to human elastin employs recombinant human tropoelastin DNA in *E. coli* systems[94]. Optimization of the genetic codons fo protein synthesis in *E. coli* has allowed synthetic human tropoelastin to be more readily obtained through bacterial culture, yielding high purity recombinant human tropoelastin[95]. This recombinant synthetic human tropoelastin has been demonstrated to be functionally similar to native tropoelastin in its ability to form mature elastic fibres[96]. Natural and recombinant full-length tropoelastin have been most commonly used in dermal and cardiovascular applications[61].

A third class of elastin derived-biomaterials are elastin-like polypeptides (ELPs). These are usually based on repeating penta- or hexapeptide sequences containing a VPGXG pattern (where X is any amino acid other than proline) that are present in hydrophobic regions of tropoelastin[97-99]. They can be chemically synthesized or produced by recombinant technology, and have been researched due to their self-association properties to engineer a variety of materials because they are biocompatible[100], biodegradable[100] and low in toxicity[101]. One of the distinguishing features of ELPs is their inverse temperature sol-gel transition, allowing the formation of materials under mild conditions[97, 98, 102]. Conjugating specific peptide sequences to ELPs facilitates cell binding[103-105], and can be tailored to bind specific cells[45,106]. These motifs include the RGD, which is a ligand of many major integrins such as $\alpha_v\beta_3$[107], and other sequences such as RGDS or REDV to encourage specific cell binding[44,45]. The minimalist approach to building ELP scaffolds has resulted in highly

tailored surfaces that promote cell activity including adhesion and differentiation[107-110], and similar discoveries in the future could provide further customization of therapeutic scaffolds.

3.4. Elastin-based biomaterials

The current applications of elastin-based biomaterials are wide and varied. The forms in which elastin derivatives have been utilized can be classified as hydrogels, electrospun scaffolds, and material coatings.

Although many polymers have been researched for clinical applications, the surface properties of some polymers may be inadequate as a proper biological interface, especially in the context of cell signaling. Hence, it is not only advantageous but sometimes necessary to provide an active biological interface between an implant and the *in vivo* environment. In cardiovascular applications, tropoelastin, elastin and elastin-derivatives have been shown to readily adsorb onto different polymer substrates and form non-thrombogenic coatings[111]. Plasma activated coatings have also been employed to cardiovascular stents in conjunction with tropoelastin, demonstrating improved biocompatibility, facilitating endothelial cell adhesion and proliferation, and lowering thrombogenicity of metal alloy stents[112, 113].

Elastin and its derivatives have also been employed in fabricating hydrogel scaffolds. A number of cross-linking options are available for the formation of hydrogel scaffolds including traditional chemical cross-linkers, such as glutaraldehyde[104, 114] and bis(sulfosuccinimidyl) suberate (BS3)[115], to more novel chemical-free cross-linking strategies, such as pH instigated cross-linking[116] and photocrosslinking[117]. Subsequent to the variations in fabrication, tropoelastin, ELP and elastin-based hydrogel scaffolds have demonstrated great tunability in terms of microstructure[118, 119], mechanical properties[117, 120], and biological function[121, 122].

Electrospinning is another common technique for producing biomaterials. It results in nano- and micro-diameter fibers from a protein solution that can be cross-linked to provide structural stability[87]. The mechanical strength, pore size and surface structure of the materials can be controlled by flow rate and concentration of the solution, applied voltage and distance to target[123,124]. Elastin derivatives can be co-spun with other polymers, natural or synthetic, to alter the properties of the resulting scaffold, resulting in a greater range of mechanical properties[87, 125, 126] and biological activity[87, 127].

4. Applications of MSCs in combination with elastin-based biomaterials

As discussed, elastin in the ECM has crucial roles in cell adhesion, migration and proliferation[61, 63]. Elastin and its derivates have been employed in numerous biomaterials to provide cellular attachment sites and enhance tissue flexibility and biocompatibility[128]. The uses of elastin-based biomaterials have expanded, from "classical" elastic tissue regeneration to regenerating non-elastic tissue such as bone[107]. This has been, in part, achieved through harnessing the multilineage potential of MSCs[120, 121, 129, 130]. Considering elastin and MSCs are both integral to tissue and wound repair, it is expected that some elastin-based biomaterials are capable of supporting the cellular activities of MSCs, though the precise nature of these interactions awaits characterization. MSCs and elastin-based biomaterials have been separately discussed in-depth in recent reviews[88, 123, 131]; therefore, this review section will discuss advances pertaining to the combination of MSCs and elastin-based biomaterials.

4.1. Bone

MSC-mediated regeneration of bone is a growing area of research, which has recently expanded to include elastin-based biomaterials for regenerative cell-matrix interactions. The application of MSCs to bone accelerates regeneration, aids in regenerating non-union cases of

bone injury, and has been suggested to interfere with bone tumor signaling[132]. The major design consideration for these scaffolds is to promote osteogenesis. Therefore, studies have focused on exploiting the mechanical and signaling conditions required for MSCs to initiate bone repair.

Recombinant ELPs modified with extra cross-linking sites to promote fibril formation have been demonstrated to enhance human bmMSC adhesion and proliferation in comparison to purely hydrophobic ELPs[108]. Additionally, the same study found ELP coatings on plastic and glass supported osteogenic differentiation of MSCs and high cell viability even in the absence of osteogenic media[108]. The surfaces of ELP based materials can be tuned at a nanometer scale to explore the in-depth effects of topography on cell behavior. For example, the surfaces of recombinant ELP membranes modified with a HAP motif (found in statherin, which is important for tissue mineralization) can be uniquely patterned to promote rat bmMSC differentiation[106]. The membrane stiffness of 2081 ± 315 kPa is comparable to bone[101], and is likely to have contributed to the differentiation of the cells in combination with the HAP motif[106]. This was reflected by an *in vivo* study, where implantation of bmMSC-seeded ELP-HAP membranes into rat calvarial defect models resulted in enhanced bone regeneration compared to non-bioactive membranes and no-implant control groups[107].

Porcine MSCs grown on recombinant ELP-RGD conjugated hydrogels supported enhanced adhesion, migration and proliferation compared to hydrogels that passively adsorbed the RGD sequence[109]. Hybrid hydrogels consisting of ELPs and other ECM protein fragments may also be beneficial in promoting MSC differentiation. The presence of collagen type I in recombinant ELP coatings encourages high MSC viability[108], and if the binding site of other ECM proteins can be isolated and used to functionalize ELPs, enhanced MSC activity may be achieved.

Tropoelastin has also been explored in terms of bmMSCs. Combining different ratios of tropoelastin and silk (from silkworm, *Bombyx mori*), yields a variety of biomaterials with distinct mechanical and surface properties[120]. The advantages of a silk-tropoelastin biomaterial are numerous. Semicrystalline silk is particularly useful for blending with tropoelastin because it does not require cross-linking to maintain its structure; this is currently an issue with pure tropoelastin studies to date[120]. The β-sheet structures of silk adds stiffness to these hybrid biomaterials, which are beneficial for creating biomaterials with mechanical properties capable of promoting regeneration of tissue types other than elastic tissue[86, 87, 120]. Importantly, the presence of tropoelastin in silk biomaterials is advantageous because it can enhance elasticity depending on the ratio of silk to tropoelastin[120], and increases biocompatibility for several cell types including fibroblasts, myoblasts and MSCs[120, 121, 128].

Hu *et al.*'s study details the effects of modifying the composition of silk-tropoelastin blends on bmMSC adhesion and proliferation *in vitro*[120]. bmMSCs cultured on blends with higher ratios of recombinant human tropoelastin displayed elongated cell morphology (indicating cell adhesion) in comparison to spherical cells on pure silk[120]. Significantly more bmMSCs were seen on blends containing 10% and 25% (molar %) tropoelastin in comparison to pure silk[120], which is consistent with another study that noted an elevation of silk-tropoelastin scaffold biocompatibility with MSCs as the amount of recombinant human tropoelastin increased[128]. Cell proliferation also appeared to be dependent on the amount of tropoelastin in the scaffold[120], which was expected considering the ability of tropoelastin to support cellular activity[21]. Osteogenic markers such as calcium deposition and alkaline phosphatase activity of bmMSCs, increased with the amount of recombinant human tropoelastin in silk-tropoelastin scaffolds in the absence of osteogenic media[121]. This occurred despite a decrease in Young's moduli associated with tropoelastin content (ranging from 27 MPa – 5 MPa for 0% - 50% tropoelastin scaffolds), in contrast to similar experiments[6]. Hu *et al.* also noted an

overall increase in surface roughness of scaffolds as tropoelastin content was elevated that correlated with an increase in osteogenesis, hinting at why MSCs did not favor osteogenesis on pure silk substrates[121]. Therefore, tropoelastin's role in altering scaffold surface topography would be another useful implementation for the design of future bioactive scaffolds.

4.2. Dermal

Experiments and clinical trials have highlighted the beneficial effects of MSCs from a variety of sources in dermal repair[133-136]. Several studies have recognized the importance of delivering MSCs through a scaffold. 18 out of 20 patients with dermatopathies that responded poorly to artificial skin grafts presented successful healing after implantation of a MSC-laden scaffold[137]. Another study showed that adipose MSCs on a decellularized full-thickness ECM dermal scaffold improved neovascularization, compared to scaffold-only treatment groups, when applied in combination with pressure wound therapy[138]. BmMSCs can also reduce scarring by promoting new ECM deposition and diminishing inflammation in murine dermal fibrosis models[139].

As the aberrant expression of elastin at wound sites leads to a lack of elasticity and function in regenerated tissue[85], a biomaterial that could provide elasticity to skin during healing, and perhaps long-term post-implantation, would be important to utilize in a dermal substitute scaffold[87]. Therefore, scaffolds of this type would be especially beneficial for patients with chronic wounds such as ulcers[140] or full thickness wounds such as severe burns[86].

Electrospun tropoelastin scaffolds have similar elasticity to skin[87], thus, it is conceivable that they could provide adipose MSCs with sufficient mechanical cues to induce differentiation without requiring growth factors, similar to Engler *et al.*'s experiment[6]. Machula *et al.* showed that adipose MSCs are capable of adhering to a pure recombinant human tropoelastin

scaffold[140]. Preliminary *in vitro* studies showed that adipose MSCs formed *de novo* ECM when attached to the scaffold, and implantation of these cell laden scaffolds into mice resulted in the formation of neovasculature, stratum corneum and epithelium growth[140]. These results are in line with previous studies which have shown that adipose MSCs are capable of differentiating into keratinocytes[141], epithelial cells[142], endothelial cells[142-144] or perivascular cells[145]. This agrees with Machula *et al.*'s study that adipose MSCs contributed to the faster wound closure and thicker regenerated epithelium that was observed *in vivo* with the seeded MSC scaffold in comparison to unaided wound healing[140]. However, given that previous studies investigating electrospun recombinant human tropoelastin scaffolds have already found that tropoelastin alone encouraged dermal regeneration[87], the combination of adipose MSCs and tropoelastin warrants further investigation, especially in comparison to scaffolds without cells.

4.3. Cartilage

Cartilage is a tissue often associated with age-related diseases because it naturally degenerates over time; in particular, the low cell density hinders the ability of cartilage to regenerate. Results from osteoarthritis studies injecting intra-articular MSC suspensions to promote cartilage regeneration have varied from no repair, found through X-ray analysis of four patients treated with bmMSCs[146], to cartilage regeneration and improved range of movement in a single patient study[147]. Even though cell-free scaffolds based on natural polymers, for example, hyaluronan[148] and collagen[149], and synthetic polymers, such as polycaprolactone[150], have yielded encouraging results by alleviating the effects of degraded cartilage, the combination of cells and scaffolds has been the main focus of regenerative studies to date[151].

ELP hydrogels can be designed to mimic the shear moduli of other collagen and hyaluronan hydrogels; this is achieved by engineering the inverse temperature transition of ELPs alone such that the scaffold greatly stiffens upon coacervation[152]. Chondrocytic-associated genes (such as SOX9 and collagen II) of adipose MSCs encapsulated in recombinant ELP hydrogels were upregulated in low oxygen tension conditions *in vitro*[153]. This gene upregulation was reflected by increased collagen II deposition found through immunohistochemical staining[153]. Of note is that these changes appeared independently of chondrocytic factors, dexamethasone and TGF-β1, indicating that the ELP scaffold provided the adipose MSCs appropriate cues to initiate the chondrocytic process[153]. Recombinant silk-elastinlike hydrogels consist of repeating sequences from silk and mammalian elastin[154]. One study showed that these hydrogels, in combination with TGF-β3, also upregulated chondrocytic genes such as SOX9 and collagen X in MSCs and gave rise to newly deposited collagen II[154]. These studies display the benefits of encapsulating MSCs in elastin-based biomaterials to present a chondrogenic environment, paving the way for novel, viable strategies for regenerating cartilage.

5. Conclusions

The strong evidence for the beneficial relationship between tissue elasticity and MSC activity justifies the combination of elastin-based biomaterials with MSCs. The successes highlighted by this review demonstrate the value in exploring this synergistic relationship across a range of mechanically diverse tissues such as skin and bone. Although the applications of elastin-based biomaterials and MSCs have focused on bone, skin and cartilage, further investigation will open opportunities for the regeneration of other tissues and improved clinical efficacies using this approach.

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References

1. W. Teng, J. Cappello and X. Wu, *Biomacromolecules*, 2009, **10**, 3028-3036.
2. A. J. Friedenstein, K. V. Petrakova, A. I. Kurolesova and G. P. Frolova, *Transplantation*, 1968, **6**, 230-247.
3. M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, D. Prockop and E. Horwitz, *Cytotherapy*, 2006, **8**, 315-317.
4. J. E. Grove, E. Bruscia and D. S. Krause, *Stem cells*, 2004, **22**, 487-500.
5. M. F. Pittenger, A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig and D. R. Marshak, *Science*, 1999, **284**, 143-147.
6. A. J. Engler, S. Sen, H. L. Sweeney and D. E. Discher, *Cell*, 2006, **126**, 677-689.
7. R. K. Das and O. F. Zouani, *Biomaterials*, 2014, **35**, 5278-5293.
8. E. Cukierman, R. Pankov, D. R. Stevens and K. M. Yamada, *Science*, 2001, **294**, 1708-1712.
9. N. Huebsch, P. R. Arany, A. S. Mao, D. Shvartsman, O. A. Ali, S. A. Bencherif, J. Rivera-Feliciano and D. J. Mooney, *Nature materials*, 2010, **9**, 518-526.
10. L. S. Wang, J. E. Chung, P. P. Chan and M. Kurisawa, *Biomaterials*, 2010, **31**, 1148-1157.
11. W. J. Polacheck, A. E. German, A. Mammoto, D. E. Ingber and R. D. Kamm, *Proceedings of the National Academy of Sciences of the United States of America*, 2014, **111**, 2447-2452.
12. D. E. Ingber, *Progress in biophysics and molecular biology*, 2008, **97**, 163-179.
13. E. A. Klein, L. Yin, D. Kothapalli, P. Castagnino, F. J. Byfield, T. Xu, I. Levental, E. Hawthorne, P. A. Janmey and R. K. Assoian, *Current biology*, 2009, **19**, 1511-1518.
14. J. P. Winer, P. A. Janmey, M. E. McCormick and M. Funaki, *Tissue engineering. Part A*, 2009, **15**, 147-154.
15. J. S. Choi and B. A. Harley, *Biomaterials*, 2012, **33**, 4460-4468.
16. Y. Lei, S. Gojgini, J. Lam and T. Segura, *Biomaterials*, 2011, **32**, 39-47.
17. P. M. Gilbert, K. L. Havenstrite, K. E. Magnusson, A. Sacco, N. A. Leonardi, P. Kraft, N. K. Nguyen, S. Thrun, M. P. Lutolf and H. M. Blau, *Science*, 2010, **329**, 1078-1081.
18. L. A. Flanagan, L. M. Rebaza, S. Derzic, P. H. Schwartz and E. S. Monuki, *Journal of neuroscience research*, 2006, **83**, 845-856.
19. J. F. Almine, S. G. Wise and A. S. Weiss, *Birth defects research. Part C, Embryo today : reviews*, 2012, **96**, 248-257.
20. N. Ono, W. Ono, T. Mizoguchi, T. Nagasawa, P. S. Frenette and H. M. Kronenberg, *Developmental cell*, 2014, **29**, 330-339.
21. P. Lee, D. V. Bax, M. M. Bilek and A. S. Weiss, *The Journal of biological chemistry*, 2014, **289**, 1467-1477.
22. M. Vicente-Manzanares, D. J. Webb and A. R. Horwitz, *Journal of cell science*, 2005, **118**, 4917-4919.
23. J. Jokinen, E. Dadu, P. Nykvist, J. Kapyla, D. J. White, J. Ivaska, P. Vehvilainen, H. Reunanen, H. Larjava, L. Hakkinen and J. Heino, *The Journal of biological chemistry*, 2004, **279**, 31956-31963.
24. D. V. Bax, U. R. Rodgers, M. M. Bilek and A. S. Weiss, *The Journal of biological chemistry*, 2009, **284**, 28616-28623.

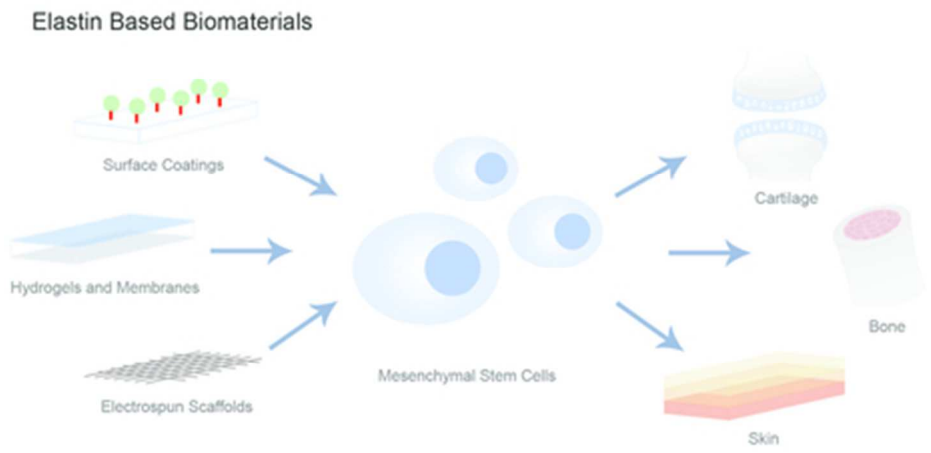
25. M. Schaff, C. Tang, E. Maurer, C. Bourdon, N. Receveur, A. Eckly, B. Hechler, C. Arnold, A. de Arcangelis, B. Nieswandt, C. V. Denis, O. Lefebvre, E. Georges-Labouesse, C. Gachet, F. Lanza and P. H. Mangin, *Circulation*, 2013, **128**, 541-552.
26. W. J. Seeto, Y. Tian and E. A. Lipke, *Acta biomaterialia*, 2013, **9**, 8279-8289.
27. J. A. Burdick and K. S. Anseth, *Biomaterials*, 2002, **23**, 4315-4323.
28. N. S. Hwang, S. Varghese, H. Li and J. Elisseeff, *Cell and tissue research*, 2011, **344**, 499-509.
29. A. S. Rowlands, P. A. George and J. J. Cooper-White, *American journal of physiology. Cell physiology*, 2008, **295**, C1037-1044.
30. E. Altrock, C. A. Muth, G. Klein, J. P. Spatz and C. Lee-Thedieck, *Biomaterials*, 2012, **33**, 3107-3118.
31. M. Arnold, E. A. Cavalcanti-Adam, R. Glass, J. Blümmel, W. Eck, M. Kantlehner, H. Kessler and J. P. Spatz, *ChemPhysChem*, 2004, **5**, 383-388.
32. A. A. Abdeen, J. B. Weiss, J. Lee and K. A. Kilian, *Tissue engineering. Part A*, 2014, **20**, 2737-2745.
33. F. P. Seib, M. Prewitz, C. Werner and M. Bornhauser, *Biochemical and biophysical research communications*, 2009, **389**, 663-667.
34. A. Mammoto, K. M. Connor, T. Mammoto, C. W. Yung, D. Huh, C. M. Aderman, G. Mostoslavsky, L. E. Smith and D. E. Ingber, *Nature*, 2009, **457**, 1103-1108.
35. L. Chen, E. E. Tredget, P. Y. Wu and Y. Wu, *PloS one*, 2008, **3**, e1886.
36. H. Yagi, A. Soto-Gutierrez, B. Parekkadan, Y. Kitagawa, R. G. Tompkins, N. Kobayashi and M. L. Yarmush, *Cell transplantation*, 2010, **19**, 667-679.
37. A. Hoeben, B. Landuyt, M. S. Highley, H. Wildiers, A. T. Van Oosterom and E. A. De Bruijn, *Pharmacological reviews*, 2004, **56**, 549-580.
38. J. Doorn, G. Moll, K. Le Blanc, C. van Blitterswijk and J. de Boer, *Tissue engineering. Part B, Reviews*, 2012, **18**, 101-115.
39. F. Djouad, P. Plence, C. Bony, P. Tropel, F. Apparailly, J. Sany, D. Noel and C. Jorgensen, *Blood*, 2003, **102**, 3837-3844.
40. X. X. Jiang, Y. Zhang, B. Liu, S. X. Zhang, Y. Wu, X. D. Yu and N. Mao, *Blood*, 2005, **105**, 4120-4126.
41. D. M. Patel, J. Shah and A. S. Srivastava, *Stem cells international*, 2013, **2013**, 496218.
42. D. J. Waugh and C. Wilson, *Clinical cancer research*, 2008, **14**, 6735-6741.
43. K. Middleton, J. Jones, Z. Lwin and J. I. Coward, *Critical reviews in oncology/hematology*, 2014, **89**, 129-139.
44. V. Falanga, S. Iwamoto, M. Chartier, T. Yufit, J. Butmarc, N. Kouttab, D. Shrayer and P. Carson, *Tissue engineering*, 2007, **13**, 1299-1312.
45. A. Stoff, A. A. Rivera, N. Sanjib Banerjee, S. T. Moore, T. Michael Numnum, A. Espinosa-de-Los-Monteros, D. F. Richter, G. P. Siegal, L. T. Chow, D. Feldman, L. O. Vasconez, J. Michael Mathis, M. A. Stoff-Khalili and D. T. Curiel, *Experimental dermatology*, 2009, **18**, 362-369.
46. F. Lu, H. Mizuno, C. A. Uysal, X. Cai, R. Ogawa and H. Hyakusoku, *Plastic and reconstructive surgery*, 2008, **121**, 50-58.
47. H. T. de Freitas, M. G. Rebel, B. P. Coelho, V. G. da Silva, G. G. Galaxe-Almeida and A. Giraldi-Guimaraes, *Journal of the neurological sciences*, 2015, **348**, 166-173.
48. Y. S. Yoon, J. S. Park, T. Tkebuchava, C. Luedeman and D. W. Losordo, *Circulation*, 2004, **109**, 3154-3157.
49. M. Breitbach, T. Bostani, W. Roell, Y. Xia, O. Dewald, J. M. Nygren, J. W. Fries, K. Tiemann, H. Bohlen, J. Hescheler, A. Welz, W. Bloch, S. E. Jacobsen and B. K. Fleischmann, *Blood*, 2007, **110**, 1362-1369.

50. X. Tian, J. Fan, M. Yu, Y. Zhao, Y. Fang, S. Bai, W. Hou and H. Tong, *PloS one*, 2014, **9**, e108105.
51. A. Yamawaki-Ogata, X. Fu, R. Hashizume, K. L. Fujimoto, Y. Araki, H. Oshima, Y. Narita and A. Usui, *European journal of cardio-thoracic surgery*, 2014, **45**, e156-165.
52. C. Baldock, A. F. Oberhauser, L. Ma, D. Lammie, V. Siegler, S. M. Mithieux, Y. Tu, J. Y. Chow, F. Suleman, M. Malfois, S. Rogers, L. Guo, T. C. Irving, T. J. Wess and A. S. Weiss, *Proceedings of the National Academy of Sciences of the United States of America*, 2011, **108**, 4322-4327.
53. C. A. Hoeve and P. J. Flory, *Biopolymers*, 1974, **13**, 677-686.
54. B. Bochicchio, A. Pepe and A. M. Tamburro, *Chirality*, 2008, **20**, 985-994.
55. M. Miao, J. T. Cirulis, S. Lee and F. W. Keeley, *Biochemistry*, 2005, **44**, 14367-14375.
56. A. M. Tamburro, A. Pepe and B. Bochicchio, *Biochemistry*, 2006, **45**, 9518-9530.
57. J. Djajamuliadi, T. F. Kagawa, K. Ohgo and K. K. Kumashiro, *Matrix biology*, 2009, **28**, 92-100.
58. L. B. Dyksterhuis and A. S. Weiss, *Biochemical and biophysical research communications*, 2010, **396**, 870-873.
59. S. G. Wise, S. M. Mithieux, M. J. Raftery and A. S. Weiss, *Journal of structural biology*, 2005, **149**, 273-281.
60. Y. Fung, *Mechanical properties of living tissues*, Springer-Verlag, New York, 1993.
61. S. M. Mithieux, S. G. Wise and A. S. Weiss, *Advanced drug delivery reviews*, 2013, **65**, 421-428.
62. S. A. Jensen, B. Vrhovski and A. S. Weiss, *The Journal of biological chemistry*, 2000, **275**, 28449-28454.
63. G. C. Yeo, F. W. Keeley and A. S. Weiss, *Advances in colloid and interface science*, 2011, **167**, 94-103.
64. U. R. Rodgers and A. S. Weiss, *Pathologie-biologie*, 2005, **53**, 390-398.
65. T. J. Broekelmann, B. A. Kozel, H. Ishibashi, C. C. Werneck, F. W. Keeley, L. Zhang and R. P. Mecham, *The Journal of biological chemistry*, 2005, **280**, 40939-40947.
66. R. P. Mecham, A. Hinek, R. Entwistle, D. S. Wrenn, G. L. Griffin and R. M. Senior, *Biochemistry*, 1989, **28**, 3716-3722.
67. R. M. Senior, G. L. Griffin, R. P. Mecham, D. S. Wrenn, K. U. Prasad and D. W. Urry, *The Journal of cell biology*, 1984, **99**, 870-874.
68. A. Hinek and M. Rabinovitch, *The Journal of cell biology*, 1994, **126**, 563-574.
69. S. K. Karnik, B. S. Brooke, A. Bayes-Genis, L. Sorensen, J. D. Wythe, R. S. Schwartz, M. T. Keating and D. Y. Li, *Development*, 2003, **130**, 411-423.
70. S. K. Karnik, J. D. Wythe, L. Sorensen, B. S. Brooke, L. D. Urness and D. Y. Li, *Matrix biology*, 2003, **22**, 409-425.
71. R. O. Hynes, *Cell*, 2002, **110**, 673-687.
72. E. F. Plow, T. A. Haas, L. Zhang, J. Loftus and J. W. Smith, *The Journal of biological chemistry*, 2000, **275**, 21785-21788.
73. N. Perrimon and M. Bernfield, *Nature*, 2000, **404**, 725-728.
74. C. Fornieri, M. Baccarani-Contri, D. Quaglino, Jr. and I. Pasquali-Ronchetti, *The Journal of cell biology*, 1987, **105**, 1463-1469.
75. M. Baccarani-Contri, D. Vincenzi, F. Cicchetti, G. Mori and I. Pasquali-Ronchetti, *European journal of cell biology*, 1990, **53**, 305-312.
76. Y. Tu and A. S. Weiss, *Biomacromolecules*, 2008, **9**, 1739-1744.
77. Y. Tu and A. S. Weiss, *Matrix biology*, 2010, **29**, 152-159.
78. B. Vrhovski and A. S. Weiss, *European journal of biochemistry / FEBS*, 1998, **258**, 1-18.

79. A. Trott, *Annals of emergency medicine*, 1988, **17**, 1279-1283.
80. P. Maurice, S. Blaise, S. Gayral, L. Debelle, M. Laffargue, W. Hornebeck and L. Duca, *Trends in cardiovascular medicine*, 2013, **23**, 211-221.
81. I. Perrotta, E. Russo, C. Camastra, G. Filice, G. Di Mizio, F. Colosimo, P. Ricci, S. Tripepi, A. Amorosi, F. Triumbari and G. Donato, *Histopathology*, 2011, **59**, 504-513.
82. G. S. Schultz and A. Wysocki, *Wound repair and regeneration*, 2009, **17**, 153-162.
83. F. Antonicelli, G. Bellon, L. Debelle and W. Hornebeck, *Current topics in developmental biology*, 2007, **79**, 99-155.
84. C. J. Reddel, A. S. Weiss and J. K. Burgess, *Pulmonary pharmacology & therapeutics*, 2012, **25**, 144-153.
85. T. P. Amadeu, A. S. Braune, L. C. Porto, A. Desmouliere and A. M. Costa, *Wound repair and regeneration*, 2004, **12**, 169-174.
86. J. Rnjak, S. G. Wise, S. M. Mithieux and A. S. Weiss, *Tissue engineering. Part B, Reviews*, 2011, **17**, 81-91.
87. J. Rnjak-Kovacina, S. G. Wise, Z. Li, P. K. Maitz, C. J. Young, Y. Wang and A. S. Weiss, *Acta Biomaterialia*, 2012, **8**, 3714-3722.
88. G. C. Yeo, B. Aghaei-Ghareh-Bolagh, E. P. Brackenreg, M. A. Hiob, P. Lee and A. S. Weiss, *Advanced Healthcare Materials*, 2015, n/a-n/a.
89. L. Duca, N. Floquet, A. J. Alix, B. Haye and L. Debelle, *Critical reviews in oncology/hematology*, 2004, **49**, 235-244.
90. S. M. Mithieux and A. S. Weiss, in *Advances in Protein Chemistry*, eds. A. D. P. David and M. S. John, Academic Press, 2005, vol. Volume 70, pp. 437-461.
91. M. I. Chung, M. Miao, R. J. Stahl, E. Chan, J. Parkinson and F. W. Keeley, *Matrix biology : journal of the International Society for Matrix Biology*, 2006, **25**, 492-504.
92. R. P. Mecham and J. A. Foster, *Biochimica et biophysica acta*, 1979, **577**, 147-158.
93. B. A. Cox, B. C. Starcher and D. W. Urry, *Biochimica et biophysica acta*, 1973, **317**, 209-213.
94. Z. Indik, W. R. Abrams, U. Kucich, C. W. Gibson, R. P. Mecham and J. Rosenbloom, *Archives of Biochemistry and Biophysics*, 1990, **280**, 80-86.
95. S. L. Martin, B. Vrhovski and A. S. Weiss, *Gene*, 1995, **154**, 159-166.
96. P. J. Stone, S. M. Morris, S. Griffin, S. Mithieux and A. S. Weiss, *American journal of respiratory cell and molecular biology*, 2001, **24**, 733-739.
97. D. W. Urry, M. M. Long, B. A. Cox, T. Ohnishi, L. W. Mitchell and M. Jacobs, *Biochimica et biophysica acta*, 1974, **371**, 597-602.
98. D. W. Urry, L. W. Mitchell and T. Ohnishi, *Biochimica et Biophysica Acta*, 1975, **393**, 296-306.
99. A. Girotti, J. Reguera, J. C. Rodriguez-Cabello, F. J. Arias, M. Alonso and A. Matestera, *Journal of materials science. Materials in Medicine*, 2004, **15**, 479-484.
100. S. A. Wood, J. E. Lemons, K. U. Prasad and D. W. Urry, *Journal of Biomedical Materials Research*, 1986, **20**, 315-335.
101. E. Tejeda-Montes, K. H. Smith, M. Poch, M. J. Lopez-Bosque, L. Martin, M. Alonso, E. Engel and A. Mata, *Acta Biomaterialia*, 2012, **8**, 998-1009.
102. T. Lee, A. Cooper, R. Apkarian and V. Conticello, *Advanced Materials*, 2000, **12**, 1105-1110.
103. D. Kaufmann, A. Fiedler, A. Junger, J. Auernheimer, H. Kessler and R. Weberskirch, *Macromolecular Bioscience*, 2008, **8**, 577-588.
104. P. L. Benitez, J. A. Sweet, H. Fink, K. P. Chennazhi, S. V. Nair, A. Enejder and S. C. Heilshorn, *Advanced Healthcare Materials*, 2013, **2**, 114-118.
105. J. C. Liu and D. A. Tirrell, *Biomacromolecules*, 2008, **9**, 2984-2988.

106. E. Tejeda-Montes, K. H. Smith, E. Rebollo, R. Gomez, M. Alonso, J. C. Rodriguez-Cabello, E. Engel and A. Mata, *Acta Biomaterialia*, 2014, **10**, 134-141.
107. E. Tejeda-Montes, A. Klymov, M. R. Nejadnik, M. Alonso, J. C. Rodriguez-Cabello, X. F. Walboomers and A. Mata, *Biomaterials*, 2014, **35**, 8339-8347.
108. B. Celebi, M. Cloutier, R. B. Rabelo, D. Mantovani and A. Bandiera, *Macromolecular Bioscience*, 2012, **12**, 1546-1554.
109. S. Ravi, V. R. Krishnamurthy, J. M. Caves, C. A. Haller and E. L. Chaikof, *Acta biomaterialia*, 2012, **8**, 627-635.
110. X. Punet, R. Mauchauffe, M. I. Giannotti, J. C. Rodriguez-Cabello, F. Sanz, E. Engel, M. A. Mateos-Timoneda and J. A. Planell, *Biomacromolecules*, 2013, **14**, 2690-2702.
111. K. A. Woodhouse, P. Klement, V. Chen, M. B. Gorbet, F. W. Keeley, R. Stahl, J. D. Fromstein and C. M. Bellingham, *Biomaterials*, 2004, **25**, 4543-4553.
112. A. Waterhouse, Y. Yin, S. G. Wise, D. V. Bax, D. R. McKenzie, M. M. Bilek, A. S. Weiss and M. K. Ng, *Biomaterials*, 2010, **31**, 8332-8340.
113. Y. Yin, S. G. Wise, N. J. Nosworthy, A. Waterhouse, D. V. Bax, H. Youssef, M. J. Byrom, M. M. Bilek, D. R. McKenzie, A. S. Weiss and M. K. Ng, *Biomaterials*, 2009, **30**, 1675-1681.
114. N. Annabi, S. M. Mithieux, E. A. Boughton, A. J. Ruys, A. S. Weiss and F. Dehghani, *Biomaterials*, 2009, **30**, 4550-4557.
115. S. M. Mithieux, J. E. Rasko and A. S. Weiss, *Biomaterials*, 2004, **25**, 4921-4927.
116. S. M. Mithieux, Y. Tu, E. Korkmaz, F. Braet and A. S. Weiss, *Biomaterials*, 2009, **30**, 431-435.
117. N. Annabi, S. M. Mithieux, P. Zorlutuna, G. Camci-Unal, A. S. Weiss and A. Khademhosseini, *Biomaterials*, 2013, **34**, 5496-5505.
118. A. Fathi, S. M. Mithieux, H. Wei, W. Chrzanowski, P. Valtchev, A. S. Weiss and F. Dehghani, *Biomaterials*, 2014, **35**, 5425-5435.
119. N. Annabi, S. M. Mithieux, A. S. Weiss and F. Dehghani, *Biomaterials*, 2010, **31**, 1655-1665.
120. X. Hu, X. Wang, J. Rnjak, A. S. Weiss and D. L. Kaplan, *Biomaterials*, 2010, **31**, 8121-8131.
121. X. Hu, S.-H. Park, E. S. Gil, X.-X. Xia, A. S. Weiss and D. L. Kaplan, *Biomaterials*, 2011, **32**, 8979-8989.
122. X. Hu, M. D. Tang-Schomer, W. Huang, X.-X. Xia, A. S. Weiss and D. L. Kaplan, *Advanced functional materials*, 2013, **23**, 3875-3884.
123. S. G. Wise, G. C. Yeo, M. A. Hiob, J. Rnjak-Kovacina, D. L. Kaplan, M. K. Ng and A. S. Weiss, *Acta biomaterialia*, 2014, **10**, 1532-1541.
124. L. Nivison-Smith, J. Rnjak and A. S. Weiss, *Acta biomaterialia*, 2010, **6**, 354-359.
125. M. J. McClure, S. A. Sell, D. G. Simpson, B. H. Walpoth and G. L. Bowlin, *Acta Biomaterialia*, 2010, **6**, 2422-2433.
126. X. Zhang, V. Thomas and Y. K. Vohra, *Journal of materials science. Materials in medicine*, 2010, **21**, 541-549.
127. J. Han, P. Lazarovici, C. Pomerantz, X. Chen, Y. Wei and P. I. Lelkes, *Biomacromolecules*, 2011, **12**, 399-408.
128. H. Liu, S. G. Wise, J. Rnjak-Kovacina, D. L. Kaplan, M. M. Bilek, A. S. Weiss, J. Fei and S. Bao, *Biomaterials*, 2014, **35**, 5138-5147.
129. R. Hu, W. Ling, W. Xu and D. Han, *PloS one*, 2014, **9**, e92676.
130. X. Hu, K. Shmelev, L. Sun, E. S. Gil, S. H. Park, P. Cebe and D. L. Kaplan, *Biomacromolecules*, 2011, **12**, 1686-1696.
131. R. R. Sharma, K. Pollock, A. Hubel and D. McKenna, *Transfusion*, 2014, **54**, 1418-1437.

132. W. L. Grayson, B. A. Bunnell, E. Martin, T. Frazier, B. P. Hung and J. M. Gimble, *Nature reviews. Endocrinology*, 2015.
133. C. Fathke, L. Wilson, J. Hutter, V. Kapoor, A. Smith, A. Hocking and F. Isik, *Stem cells (Dayton, Ohio)*, 2004, **22**, 812-822.
134. P. Liu, Z. Deng, S. Han, T. Liu, N. Wen, W. Lu, X. Geng, S. Huang and Y. Jin, *Artificial organs*, 2008, **32**, 925-931.
135. M. Kim, I. Kim, S. K. Lee, S. I. Bang and S. Y. Lim, *Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al.]*, 2011, **37**, 750-759.
136. C. Shrestha, L. Zhao, K. Chen, H. He and Z. Mo, *International Journal of Endocrinology*, 2013, **2013**, 592454.
137. T. Yoshikawa, H. Mitsuno, I. Nonaka, Y. Sen, K. Kawanishi, Y. Inada, Y. Takakura, K. Okuchi and A. Nonomura, *Plastic and reconstructive surgery*, 2008, **121**, 860-877.
138. I. Sahin, S. Ozturk, M. Deveci, A. U. Ural, O. Onguru and S. Isik, *Journal of plastic, reconstructive & aesthetic surgery : JPRAS*, 2014, **67**, 107-114.
139. Y. Wu, S. Huang, J. Enhe, K. Ma, S. Yang, T. Sun and X. Fu, *International wound journal*, 2014, **11**, 701-710.
140. H. Machula, B. Ensley and R. Kellar, *Advances in wound care*, 2014, **3**, 367-375.
141. Y. Du, D. S. Roh, M. L. Funderburgh, M. M. Mann, K. G. Marra, J. P. Rubin, X. Li and J. L. Funderburgh, *Molecular vision*, 2010, **16**, 2680-2689.
142. C. Nie, D. Yang, J. Xu, Z. Si, X. Jin and J. Zhang, *Cell transplantation*, 2011, **20**, 205-216.
143. Y. Cao, Z. Sun, L. Liao, Y. Meng, Q. Han and R. C. Zhao, *Biochemical and biophysical research communications*, 2005, **332**, 370-379.
144. C. Auxenfans, C. Lequeux, E. Perrusel, A. Mojallal, B. Kinikoglu and O. Damour, *Journal of tissue engineering and regenerative medicine*, 2012, **6**, 512-518.
145. A. C. Zannettino, S. Paton, A. Arthur, F. Khor, S. Itescu, J. M. Gimble and S. Gronthos, *Journal of cellular physiology*, 2008, **214**, 413-421.
146. F. Davatchi, B. S. Abdollahi, M. Mohyeddin, F. Shahram and B. Nikbin, *International journal of rheumatic diseases*, 2011, **14**, 211-215.
147. C. J. Centeno, D. Busse, J. Kisiday, C. Keohan, M. Freeman and D. Karli, *Pain physician*, 2008, **11**, 343-353.
148. M. Marcacci, M. Berruto, D. Brocchetta, A. Delcogliano, D. Ghinelli, A. Gobbi, E. Kon, L. Pederzini, D. Rosa, G. L. Sacchetti, G. Stefani and S. Zanasi, *Clinical orthopaedics and related research*, 2005, 96-105.
149. T. Efe, C. Theisen, S. Fuchs-Winkelmann, T. Stein, A. Getgood, M. B. Rominger, J. R. Paletta and M. D. Schofer, *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA*, 2012, **20**, 1915-1922.
150. S. Martinez-Diaz, N. Garcia-Giralt, M. Lebourg, J. A. Gomez-Tejedor, G. Vila, E. Caceres, P. Benito, M. M. Pradas, X. Nogues, J. L. Ribelles and J. C. Monllau, *The American journal of sports medicine*, 2010, **38**, 509-519.
151. E. Kon, A. Roffi, G. Filardo, G. Tesei and M. Marcacci, *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association*, 2015.
152. H. Betre, L. A. Setton, D. E. Meyer and A. Chilkoti, *Biomacromolecules*, 2002, **3**, 910-916.
153. H. Betre, S. R. Ong, F. Guilak, A. Chilkoti, B. Fermor and L. A. Setton, *Biomaterials*, 2006, **27**, 91-99.
154. M. Haider, J. Cappello, H. Ghandehari and K. W. Leong, *Pharmaceutical research*, 2008, **25**, 692-699.



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