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Enhanced articular cartilage by human mesenchymal stem cells in enzymatically mediated transiently RGDS-functionalized collagen-mimetic hydrogels

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ABSTRACT

Recapitulation of the articular cartilage microenvironment for regenerative medicine applications faces significant challenges due to the complex and dynamic biochemical and biomechanical nature of native tissue. Towards the goal of biomaterial designs that enable the temporal presentation of bioactive sequences, recombinant bacterial collagens such as Streptococcal collagen-like 2 (Scl2) proteins can be employed to incorporate multiple specific bioactive and biodegradable peptide motifs into a single construct. Here, we first modified the backbone of Scl2 with glycosaminoglycan-binding peptides and cross-linked the modified Scl2 into hydrogels via matrix metalloproteinase 7 (MMP7)-cleavable or non-cleavable scrambled peptides. The cross-linkers were further functionalized with a tethered RGDS peptide creating a system whereby the release from an MMP7-cleavable hydrogel could be compared to a system where release is not possible. The release of the RGDS peptide from the degradable hydrogels led to significantly enhanced expression of collagen type II (3.9-fold increase), aggrecan (7.6-fold increase), and SOX9 (5.2-fold increase) by human mesenchymal stem cells (hMSCs) undergoing chondrogenesis, as well as greater extracellular matrix accumulation compared to non-degradable hydrogels (collagen type II; 3.2-fold increase, aggrecan; 4-fold increase, SOX9; 2.8-fold increase). Hydrogels containing a low concentration of the RGDS peptide displayed significantly decreased collagen type I and X gene expression profiles, suggesting a major advantage over either hydrogels functionalized with a higher RGDS peptide concentration, or non-degradable hydrogels, in promoting an articular cartilage phenotype. These highly versatile Scl2 hydrogels can be further manipulated to improve specific elements of the chondrogenic response by hMSCs, through the introduction of additional bioactive and/or biodegradable motifs. As such, these hydrogels have the possibility to be used for other applications in tissue engineering.

Statement of Significance

Recapitulating aspects of the native tissue biochemical microenvironment faces significant challenges in regenerative medicine and tissue engineering due to the complex and dynamic nature of the tissue. The ability to take advantage of, mimic, and modulate cell-mediated processes within novel naturally-derived hydrogels is of great interest in the field of biomaterials to generate constructs that more closely resemble the biochemical microenvironment and functions of native biological tissues such as articular cartilage. Towards this goal, the temporal presentation of bioactive sequences such as RGDS on the chondrogenic differentiation of human mesenchymal stem cells is considered important as it has been shown to influence the chondrogenic phenotype. Here, a novel and versatile platform to recreate a high

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degree of biological complexity is proposed, which could also be applicable to other tissue engineering and regenerative medicine applications.

1. Introduction

Articular cartilage is a highly complex connective tissue that covers the surface of bones in synovial joints [1]. The unique spatial organization of the components of cartilage extracellular matrix is fundamental to its ability to carry out its biomechanical functions [2,3]. Trauma to articular cartilage and/or disease of the joint can stimulate catabolic responses that disturb tissue homeostasis and can lead to progressive degeneration [4]. This is aggravated by the aneural and avascular nature of articular cartilage, combined with the limited ability of resident cells to migrate to sites of injury, which contribute to a restricted capacity for self-repair and regeneration [5]. Current clinical treatments for articular cartilage ailments, such as non-steroidal anti-inflammatory drugs [6], viscosupplementation [4], mosaicplasty [7], autologous chondrocyte implantation [8], microfracture [9], and periosteal transplantation [10], generally provide short-term pain relief and recovery of joint mobility to patients, but long-term benefits often remain elusive [3]. The repair tissue formed as a result of the surgical interventions listed here often does not exhibit the same biochemical composition as native tissue, leading to inferior biomechanical properties. Repair tissue is typically rapidly degenerated, ultimately leading to the failure of the intervention [4], thus requiring additional treatment and eventually total joint arthroplasty [11].

To overcome the limitations of current repair strategies, increasing efforts are aimed at the development of biomaterial scaffolds tailored to promote chondrogenesis, notably by providing instructive microenvironments that are reminiscent of aspects of the native pericellular matrix (PCM) [12,13]. Cell-matrix interactions are dynamic; thus, biomaterials that present temporal changes to the presentation of bioactive cues, i.e., by harnessing the remodeling in response of resident cells, may allow improved control over complex processes such as chondrogenic differentiation [14].

Hydrogels are three-dimensional (3D) aqueous-based matrices that have been widely explored as scaffolds to encapsulate cells. Many attempts have been made to recapitulate aspects of the complex and dynamic cell-extracellular matrix (ECM) interactions present in articular cartilage and other tissues by incorporating specific bioactive and/or biodegradable components into hydrogel systems [15–17]. Biodegradable hydrogels in particular, have been extensively studied in cartilage repair applications to create space for newly deposited matrix [14]. Hydrogel degradation and ECM accumulation rates that are closely linked have been suggested to be fundamental to optimal tissue repair [13,14].

Hydrogel biodegradability is often implemented through the incorporation of hydrolytically or enzymatically cleavable cross-linkers [14,18]. Hydrolytic degradation of hydrogels can be partially tunable and has been shown to stimulate cell proliferation and ECM accumulation in cartilage tissue engineering [19]. However, the rate of degradation in such gels is more dependent on the macromer composition than on cell behavior and generally does not comply with cellular function. In contrast, enzymatically degradable hydrogels respond to changes in protease secretion by encapsulated cells, allowing for cell-mediated control over hydrogel degradation kinetics. Matrix metalloproteinases (MMPs) and other enzymes including plasmin [14] are commonly exploited for this purpose as they are involved in native tissue remodeling [20–25]. The use of such enzymatic-degradation systems has also been shown to lead to improved cartilage ECM accumulation and elaboration [14,17,18,21].

Scl2 proteins have recently been the subject of a number of studies as a potential alternative to mammalian collagens for tissue engineering applications [21,26–32]. Scl2 proteins consist of a characteristic repeating $(Gly–Xaa–Yaa)_n$ sequence arranged in a triple helical conformation, but lack the bioactive sites that mediate cell responses in mammalian collagens [33]. In contrast to mammalian collagens, Scl2 proteins are non-immunogenic, non-cytotoxic, and can be recombinantly produced in high yields with minimal batch to batch variation [32]. Additionally, the backbone of Scl2 helices can easily be altered to incorporate bioactive and/or biodegradable components via tethering or site-directed mutagenesis, in order to modulate cellular behavior [21,34]. Previously, Scl2 proteins have been used to generate poly(ethylene glycol) (PEG)-Scl2 hybrid hydrogels functionalized with an integrin-binding sequence (GFPGER) to interact with smooth muscle and endothelial cells for vascular grafts [31]. Our group has recently developed Scl2-based scaffolds functionalized with glycosaminoglycan (GAG)-binding peptides and/or cross-linked by enzymatically-cleavable peptides, designed to drive the chondrogenic differentiation of hMSCs and degradation of the hydrogels [21,34,35].

Human mesenchymal stem cells (hMSCs) have been shown to benefit from the presence of fibronectin in the early stages of chondrogenic differentiation [18,22–24]. Fibronectin gene expression levels are also up-regulated during these early stages of chondrogenic differentiation [24,25]. The interaction of hMSCs with this extracellular protein via integrin-adhesive ligands is thought to affect cell-signaling and aid condensation and differentiation into chondrocytes [26]. More specifically, the arginine–glycine–aspartic acid (RGD) cell-adhesive sequence present on fibronectin has been shown to play an important role in initiating hMSC chondrogenesis [18]. However, in the later stages of chondrogenesis, fibronectin gene expression levels are down-regulated [26,27], allowing complete differentiation of hMSCs towards chondrocytes. The RGD motif is often used to maintain hMSC viability in hydrogels that do not offer other inherent cell-adhesive motifs [18,28,29]. However, studies have demonstrated that the persistence of the RGD moiety can delay or even alter the chondrogenic differentiation of hMSCs, often leading to hypertrophy, as clearly demonstrated in a previous study [18]. Concentration and temporal presentation of this RGD moiety are thus important design criteria for the development of hydrogels that promote chondrogenic differentiation.

In this work, we designed MMP7-cleavable hydrogels based on Scl2 functionalized within the backbone with GAG-binding peptides, using concepts from our previous work and presenting RGDS moieties that can also be released by the action of MMP7. We first modified the backbone of Scl2 to incorporate heparin (H), hyaluronic acid (HA), and chondroitin sulfate (CS)-binding sequences via site-directed mutagenesis (Fig. 1). Recent studies have shown the selected GAG-binding peptides to bind specifically and non-covalently to heparin, HA, and CS, respectively [36]. The inclusion of the HA-binding and CS-binding peptides was verified in our previous work [21] and was shown to enhance hMSC chondrogenesis. Heparin is present in articular cartilage and is known to encourage the recruitment of, and to form stable complexes with, growth factors such as TGF-β thus further aiding chondrogenesis in long-term