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Regulation of decellularized tissue remodeling via scaffold-mediated lentiviral delivery in anatomically-shaped osteochondral constructs

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ABSTRACT

Cartilage-derived matrix (CDM) has emerged as a promising scaffold material for tissue engineering of cartilage and bone due to its native chondroinductive capacity and its ability to support endochondral ossification. Because it consists of native tissue, CDM can undergo cellular remodeling, which can promote integration with host tissue and enables it to be degraded and replaced by neotissue over time. However, enzymatic degradation of decellularized tissues can occur unpredictably and may not allow sufficient time for mechanically competent tissue to form, especially in the harsh inflammatory environment of a diseased joint. The goal of the current study was to engineer cartilage and bone constructs with the ability to inhibit aberrant inflammatory processes caused by the cytokine interleukin-1 (IL-1), through scaffold-mediated delivery of lentiviral particles containing a doxycycline-inducible IL-1 receptor antagonist (IL-1Ra) transgene on anatomically-shaped CDM constructs. Additionally, scaffold-mediated lentiviral gene delivery was used to facilitate spatial organization of simultaneous chondrogenic and osteogenic differentiation via site-specific transduction of a single mesenchymal stem cell (MSC) population to overexpress either chondrogenic, transforming growth factor-beta 3 (TGF-β3), or osteogenic, bone morphogenetic protein-2 (BMP-2), transgenes. Controlled induction of IL-1Ra expression protected CDM hemispheres from inflammation-mediated degradation, and supported robust bone and cartilage tissue formation even in the presence of IL-1. In the absence of inflammatory stimuli, controlled cellular remodeling was exploited as a mechanism for fusing concentric CDM hemispheres overexpressing BMP-2 and TGF-β3 into a single bi-layered osteochondral construct. Our findings demonstrate that site-specific delivery of inducible and tunable transgenes confers spatial and temporal control over both CDM scaffold remodeling and neotissue composition. Furthermore, these constructs provide a microphysiological *in vitro* joint organoid model with site-specific, tunable, and inducible protein delivery systems for examining the spatiotemporal response to pro-anabolic and/or inflammatory signaling across the osteochondral interface.

1. Introduction

Articular cartilage provides a smooth, low-friction bearing surface for supporting joint loading and movement. However, adult cartilage is avascular and possesses a small resident cell population of chondrocytes. These characteristics limit the capacity of cartilage to self-repair, and untreated defects ultimately progress to the full joint disease of osteoarthritis. While tissue-engineering approaches have attempted to repair defects with functional cartilage replacements, the poor intrinsic ability of cartilage to remodel prevents integration of neocartilage with the host tissue [1]. Strategies have sought to improve tissue integration by anchoring tissue-engineered constructs to the underlying subchondral bone, which possesses a strong propensity to remodel and integrate with the surrounding host tissue [2]. Fabricating osteochondral constructs with both cartilaginous and osseous phases has improved *in vivo*

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outcomes in rat [3] and rabbit [4,5] animal models compared to constructs possessing either phase alone. Furthermore, subchondral bone formation has been shown to be critical for proper cartilage healing [3]. In this regard, the spatial organizing of cartilage and bone production simultaneously within osteochondral constructs has been pursued by varying site-specific scaffold composition [4–11], cell types [9,10,12,13], growth factor presentation [5,11,13,14], and delivery of DNA encoding for tissue-specific transcription factors [3] or growth factors [4,8,15].

One of the major challenges in developing integrated osteochondral constructs is the potential interference or crosstalk of signaling between the cartilaginous and osseous phases. For example, chondrogenic and osteogenic growth factors exert antagonistic effects on one another [16], and co-delivery of genes for transforming growth factor-beta 3 (TGF-β3) and bone morphogenetic protein 2 (BMP-2) reduced calcium deposition compared to either gene individually [15]. Additionally, spatially guided, dual growth factor delivery for osteochondral repair did not improve *in vivo* cartilage repair, but yielded a synergistic effect on subchondral bone formation [14]. Also, despite culturing chondrocytes and bone marrow-derived mesenchymal stem cells (MSCs) in physically separate layers of osteochondral constructs, the presence of chondrocytes inhibited bone formation by MSCs [12,17]. This interference from competing morphogenetic signals on a single cell source such as MSCs, which can undergo both chondrogenesis and osteogenesis [4,5,8,15], has encouraged the implementation of two-part scaffolds [4,9,10,18–20], which are cultured in respective chondrogenic and osteogenic conditions and then later combined into a single osteochondral construct. A limitation of current two-part scaffold approaches is their reliance on external bonding techniques such as sutures [19] or fibrin glue [4,9,18,20] to promote adhesion of the cartilage and bone layers. Ideally, the osteochondral interface would be stabilized through the deposition of newly synthesized matrix from seeded cells as opposed to the implementation of fixation materials, whose degradation rates might not match the rate of neotissue deposition [18].

The degradation properties not only of fixation materials but also of the scaffold itself are crucial in defining the success of osteochondral constructs upon implantation [6]. While synthetic scaffolds can offer highly tunable degradation properties, their degradation is often decoupled from cellular remodeling processes and instead is mediated by hydrolysis [3,21,22]. Overly rapid degradation can lead to premature loss of scaffold properties before mechanically competent tissue has formed, resulting in implant failure [23]. Conversely, the persistence of synthetic polymers beyond the remodeling process can induce a foreign body response [24] and fibrotic tissue deposition [3,14,25,26], which inhibit subchondral bone remodeling [3,14,26] and cause osseous wall resorption and defect widening [6]. Additionally, non-degradable hydrogels sequester newly synthesized matrix to the pericellular region [27,28], and implants delivering TGF-β3 and BMP-2 only observed tissue elaboration in degradable scaffolds [27], which highlights the importance of scaffold degradation in osteochondral repair. While the incorporation of enzymatically-cleavable degradation sites within synthetic polymers have associated scaffold degradation with the remodeling process [28,29], there is still concern over the degradation products of synthetic scaffolds, which stimulate macrophages to secrete inflammatory cytokines, catabolic enzymes, and cytotoxic factors [22]. In contrast, scaffolds derived from the extracellular matrix of native tissues retain the capacity to be remodeled by cells, and their degradation products promote a constructive remodeling M2 phenotype in macrophages [30,31], which has been correlated with more favorable outcomes in preclinical animal models [32].

Interestingly, in the absence of exogenous growth factors, degradation products of the cartilage extracellular matrix possess the capacity to stimulate chondrogenic differentiation of MSCs in a dose-dependent manner [33]. Furthermore, even in the presence of TGF-β inhibitors, cartilage-derived matrix (CDM) activates chondrogenic genes and exhibits a potentiated response with TGF-β3 supplementation, suggesting that the mechanisms by which CDM induces chondrogenic differentiation are independent of and synergistic with exogenous growth factors [34]. Not only has CDM been used extensively as a biomaterial for cartilage regeneration [35], but it also has been incorporated into biphasic constructs for *in vivo* osteochondral repair [36–38]. Within biphasic constructs, CDM was only used in the cartilage portion, while the osseous phase was fabricated with materials that mimicked the composition of bone to promote osteogenesis through the intramembranous ossification pathway [36–38]. However, intramembranous ossification generates excessive mineralization that inhibits vascularization of the subchondral bone [12] producing a necrotic core [39], which could be problematic for larger implants. In contrast, endochondral ossification recapitulates the developmental process of bone formation, and utilizes a cartilaginous substrate for generating mature mineralized tissue with a vascular network [40]. These benefits highlight the spatial regulation of endochondral ossification as a promising strategy for fabricating osteochondral constructs [9,12,15]. CDM has been used as a template for endochondral bone formation [41]. Therefore, the ability of CDM to support both cartilage [35] and bone [41] regeneration empowers CDM to serve as a single, compositionally homogenous substrate for engineering osteochondral constructs. Additionally, CDM can be remodeled *in vivo* [42]. However, the *in vivo* degradation profile of CDM is uneven, random, and irregular over time [43], which contributes to the failure of CDM constructs in long-term, high load bearing, osteochondral repair [44]. Attempts to delay the degradation of CDM have included the use of chemical crosslinking agents, which confer resistance to enzymatic degradation [45,46]; however, chemically-crosslinked, tissue-derived scaffolds can evoke chronic foreign body response, fibrotic encapsulation, and ultimately poor outcomes *in vivo* [32]. Moreover, chemical crosslinking treatments negatively impact the ability of CDM to participate in cell-matrix interactions, and diminish the chondroinductive capacity of CDM [47]. Therefore, there is a need to be able to tailor scaffold degradation without modifying the intrinsic properties of CDM via chemical crosslinking.

The overarching goal of this study was to spatially and temporally control both scaffold degradation properties as well as simultaneous cartilage and bone formation within anatomically-shaped CDM scaffolds. As opposed to implementing chemical crosslinking techniques, the current study utilized dehydrothermal treatment, which is a physical crosslinking method that increases the compressive modulus of CDM scaffolds [48] and prevents cell mediated contraction [49], while still preserving cell-matrix interactions [47] and enzymatic remodeling [50]. In order to regulate scaffold degradation, we examined the hypothesis that IL-1Ra production can be used to control the degradation of MSC-seeded CDM scaffolds treated with IL-1 at levels found in the OA joint [51]. Specifically, interleukin-1 (IL-1) receptor antagonist (IL-1Ra) has been shown to decrease inflammatory mediators and catabolic proteases in an *in vivo* model of primary OA [52] and to influence macrophage polarization towards a constructive remodeling M2 phenotype *in vitro* [53]. Furthermore, viral gene delivery of IL-1Ra has been shown to slow the rate of cartilage loss *in vivo* [54] and prevented IL-1-mediated atrophy of tissue-engineered cartilage [55]. Therefore, controlled IL-1Ra production may inhibit cell-based catabolic processes and thus reduce the CDM degradation rate. To spatially dictate protein secretion, scaffold-mediated gene delivery