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Tissue-engineered cartilage with inducible and tunable immunomodulatory properties

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ABSTRACT

The pathogenesis of osteoarthritis is mediated in part by inflammatory cytokines including interleukin-1 (IL-1), which promote degradation of articular cartilage and prevent human mesenchymal stem cell (MSC) chondrogenesis. In this study, we combined gene therapy and functional tissue engineering to develop engineered cartilage with immunomodulatory properties that allow chondrogenesis in the presence of pathologic levels of IL-1 by inducing overexpression of IL-1 receptor antagonist (IL-1Ra) in MSCs via scaffold-mediated lentiviral gene delivery. A doxycycline-inducible vector was used to transduce MSCs in monolayer or within 3D woven PCL scaffolds to enable tunable IL-1Ra production. In the presence of IL-1, IL-1Ra-expressing engineered cartilage produced cartilage-specific extracellular matrix, while resisting IL-1-induced upregulation of matrix metalloproteinases and maintaining mechanical properties similar to native articular cartilage. The ability of functional engineered cartilage to deliver tunable anti-inflammatory cytokines to the joint may enhance the long-term success of therapies for cartilage injuries or osteoarthritis.

1. Introduction

Arthritis is the leading cause of disability in the United States and affects an estimated 50 million people, with over half of these patients suffering from osteoarthritis (OA) [1]. Risk factors for OA include age, obesity, altered biomechanics, and joint injury, including focal articular cartilage defects [2,3]. The progressive cartilage degeneration that occurs with OA frequently progresses such that total joint replacement is required, as there are currently no disease-modifying treatments [2]. Because articular cartilage lacks a natural ability to self-repair, tissue engineering strategies may provide solutions for both the repair of focal cartilage defects and the more extensive cartilage degeneration in OA [4,5]. Typically, engineered cartilage constructs are either grown *in vitro* and implanted or formed *in situ* from a combination of cells, biomaterials, and bioactive stimuli [4,6]. We have previously shown that a three-dimensionally (3D) woven, porous, biomimetic scaffold made from poly(ε-caprolactone) (PCL) mimics the nonlinear, anisotropic, compressive, and inhomogeneous mechanical properties of articular cartilage and supports chondrogenesis and extracellular matrix deposition by human mesenchymal stem cells (MSCs) [7–9]. Despite major advances in the control of biomechanical and biochemical properties of engineered tissues, there remains a lack of clinical success with engineered cartilage replacements [5].

One challenge in the ultimate clinical success of these technologies is the potential detrimental influence of the inflammatory environment of the diseased joint [10]. The pathogenesis of OA and post-traumatic arthritis following joint injury is mediated in part by the action of pro-inflammatory cytokines such as interleukin-1 (IL-1), which are found at elevated concentrations in the synovial fluid of OA joints [11–13]. IL-1 promotes catabolic and anti-anabolic signaling in articular chondrocytes by inducing release of other pro-inflammatory factors such as matrix metalloproteinases (MMPs) and nitric oxide (NO) and down-regulating gene

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expression of primary extracellular matrix (ECM) components including type II collagen and aggrecan [11,12]. IL-1 has been shown to inhibit integrative repair of the meniscus *in vitro* by upregulating MMPs and decreasing cellular proliferation [14–16]. More recent *in vitro* evidence shows that IL-1 also prevents MSC chondrogenesis and matrix accumulation in pellet culture [17,18] and within biomaterial scaffolds [19,20]. In this regard, *in vivo* implantation of MSCs for articular cartilage repair can be associated with a loss of chondrogenic potential as well as a shift toward a more hypertrophic phenotype, which may result in endochondral ossification of the implant [21,22]. There is growing evidence that the inflammatory environment within the joint may be in part responsible for this altered MSC differentiation [21,23,24]. Thus, inflammatory signaling mediated by IL-1 within the OA or injured joint may inhibit the development and homeostasis of tissue-engineered cartilage while continuing to advance the degradation of the native tissue.

IL-1 receptor antagonist (IL-1Ra) is a natural inhibitor of IL-1 that competes with IL-1 in binding to the IL-1 receptor [25]. Daily systemic injection of recombinant human IL-1Ra (anakinra) is approved for treatment of rheumatoid arthritis and has been explored in OA treatment, but its efficacy is limited by its short half-life of only a few hours [26]. Intra-articular gene delivery of IL-1Ra, or delivery of cells which have been transduced *ex vivo*, has been studied extensively in animal models and has progressed to clinical trials (reviewed in Ref. [27]). To date, IL-1Ra gene therapy strategies for OA have employed constitutive expression cassettes, which lack regulation of transgene expression. Since IL-1-mediated inflammation may be necessary for early stages of tissue repair such as bone remodeling during fracture healing [28,29], the ability to directly regulate transgene expression could be of great value in the controlled delivery of anti-cytokine therapies. Advances in doxycycline (dox)-inducible expression systems [30,31] that facilitate tunable control of transgene expression via oral administration of a chemical inducer have yet to be explored for the regulation of OA gene therapy.

Previous approaches for IL-1Ra gene therapy [27,32–34] have been designed to protect joints from further arthritic degeneration, but are not designed to provide a functional replacement for severely damaged cartilage, which may limit their efficacy in OA treatment. Additionally, injection of virus directly into the joint does not provide control over which cell types are transduced, and it has been shown that most of the transduction occurs in the synovium rather than in articular cartilage [32], although recent studies have shown that adeno-associated virus (AAV)-based transduction of native chondrocytes is possible [35,36]. Biomaterial-mediated gene delivery from a tissue-engineering scaffold may address these issues by providing spatially-defined control of cell transduction *in situ* and localizing production of the therapeutic protein to the engineered repair tissues. We recently established a technique using poly-L-lysine (PLL) to immobilize lentivirus (LV) encoding constitutively expressed morphogenetic transgenes to a 3D woven PCL scaffold [37]. MSCs seeded onto these scaffolds were efficiently engineered to express these transgenes, leading to robust chondrogenic differentiation without the need to supplement cultures with exogenous growth factors. Scaffold-mediated transduction with LV enables spatially-defined and sustained transgene expression that may potentially direct tissue development *in vivo*. The ability to inhibit the catabolic effects of IL-1 on engineered cartilage while maintaining structural and functional properties that match native cartilage may enhance the long-term success of tissue engineering approaches to cartilage repair.

The goal of this study was to combine a gene therapy approach for inducible and tunable anti-cytokine therapy with functional cartilage tissue engineering to create a cartilage construct capable of supporting chondrogenesis in the presence of the catabolic mediator IL-1. We employed scaffold-mediated LV gene delivery of a dox-inducible IL-1Ra expression vector to MSCs to engineer cartilage constructs with tunable IL-1Ra overexpression. We investigated the magnitude and duration of MSC overexpression of IL-1Ra in monolayer cultures as well as in developing cartilage constructs. Furthermore, we characterized the ability of IL-1Ra-expressing constructs to inhibit the detrimental effects of IL-1 on the development of *in vitro* engineered cartilage by analyzing histology, biochemical composition, release of inflammatory factors, and mechanical properties.

2. Materials and methods

2.1. Lentivirus production

The dox-inducible lentiviral vector (TMPrtTA, provided by the Danos Lab) is a single "tet-on" vector that constitutively co-expresses an improved reverse tetracycline-controlled transcriptional activator [30,31,38]. The dox-inducible vector was modified to include an IRES-puromycin expression cassette. IL-1Ra and enhanced green fluorescent protein (eGFP) coding sequences were cloned into the modified TMPrtTA vector [30] or a constitutive lentiviral vector with the EF-1α promoter (Addgene 12250) [39] (Fig. 1A). HEK293T/17 (ATCC CRL-11268, Manassas, VA) were co-transfected with the appropriate expression transfer vector (20 μg), packaging plasmid (psPAX2, Addgene 12259, 15 μg), and envelope plasmid (pMD2.G, Addgene 12260, 6 μg) via calcium phosphate precipitation to produce VSV-G pseudotyped LV as previously described [40]. LV was concentrated ~80-fold via centrifugation in 100 kDa MWCO filters (Millipore, Cork, Ireland) and frozen at −80 °C. Biological titration of eGFP LV was performed in HeLa (ATCC CCL-2, Manassas, VA) using the Accuri flow cytometer (BD Biosciences, Franklin Lakes, NJ), to obtain the functional titer in transducing units/mL as previously described [40].

2.2. MSC isolation and expansion

MSCs were isolated from human bone marrow waste from three adult bone marrow transplant donors at Duke University Medical Center. Non-adherent cells were removed by the end of the first passage. MSCs from each donor were combined in equal numbers into a superlot and expanded for 4 passages prior to use in experiments. Expansion medium consisted of DMEM-LG (Gibco, Life Technologies, Carlsbad, CA), 10% lot-selected FBS (Hyclone, Thermo Scientific, Waltham, MA), 1% pen/strep (Gibco), and 10 ng/mL basic fibroblast growth factor (bFGF) (Roche, Basel, Switzerland).

2.3. LV transduction and optimization of doxycycline concentration in monolayer culture

MSCs were plated on tissue culture plastic 1 day prior to transduction (100 cells/mm^2). For transduction, MSC culture medium was replaced with concentrated constitutive or inducible IL-1Ra LV that was resuspended in MSC expansion medium to a final biological titer of 7.6×10^5 transducing units/mL with 4 μg/mL polybrene (Sigma–Aldrich, St. Louis, MO). Medium was replaced after 16 h, and this day was defined as day 0. Control MSCs were not transduced. MSCs were treated with various concentrations of dox (Sigma–Aldrich). Conditioned medium was collected and frozen at −20 °C and replaced every 3 days. MSCs were passaged into new wells every 6 days at the same density as initial plating and cultured for 30 days following transduction. Conditioned medium was assayed for IL-1Ra production via human IL-1Ra ELISA, according to the manufacturer's protocol (R&D Systems, Cat. No. DY280).

2.4. 3D woven PCL scaffold production

Scaffolds were woven from multifilament PCL yarns (EMS-Griltech, Domat, Switzerland) using a custom-built weaving machine, as previously described [7]. For this study, 7 layers of yarns were axially oriented in alternating *x* and *y* directions with a third set of fibers passing through the thickness of the structure (*z*-direction). Total void space within the scaffold was ~61% with interconnected rectangular pores measuring approximately 350 μm × 250 μm × 100 μm. Scaffolds were treated with 4 N NaOH for 16 h to increase surface hydrophilicity, rinsed in DI H_2O, and dried. Scaffolds were subsequently heat set for 10 min at 57 °C in DI H_2O. Dried scaffolds were then punched using a trephine to obtain uniform 5 mm disks. For scanning electron microscopy, disks were mounted, sputter-coated with gold, and imaged with a scanning electron microscope (FEI XL30 SEM-FEG, Eindhoven, Netherlands). For tissue engineering experiments, disks were ethylene-oxide sterilized in 24 well ultra-low attachment plates (Corning, Corning, NY) prior to use.

2.5. MSC transduction via immobilization of LV on 3D woven PCL scaffolds

Scaffold disks were incubated with 0.002% PLL (Sigma–Aldrich) overnight to facilitate non-covalent association of viral particles with the scaffold surface via