ORIGINAL ARTICLE

Dexamethasone Release from Within Engineered Cartilage as a Chondroprotective Strategy Against Interleukin-1 α

Brendan L. Roach, MS^{1,*}, Arta Kelmendi-Doko, MD, MS², Elaine C. Balutis, MD³, Kacey G. Marra, PhD^{2,4,5}, Gerard A. Ateshian, PhD^{1,6} and Clark T. Hung, PhD¹

While significant progress has been made toward engineering functional cartilage constructs with mechanical properties suitable for *in vivo* loading, the impact on these grafts of inflammatory cytokines, chemical factors that are elevated with trauma or osteoarthritis, is poorly understood. Previous work has shown dexamethasone to be a critical compound for cultivating cartilage with functional properties, while also providing chondroprotection from proinflammatory cytokines. This study tested the hypothesis that the incorporation of poly(lactic-co-glycolic acid) (PLGA) (75:25) microspheres that release dexamethasone from within chondrocyte-seeded agarose hydrogel constructs would promote development of constructs with functional properties and protect constructs from the deleterious effects of interleukin-1 α (IL-1 α). After 28 days of growth culture, experimental groups were treated with IL-1 α (10 ng/mL) for 7 days. Reaching native equilibrium moduli and proteoglycan levels, dexamethasone-loaded microsphere constructs exhibited tissue properties similar to microsphere-free control constructs cultured in dexamethasone-supplemented culture media and were insensitive to IL-1 α exposure. These findings are in stark contrast to constructs containing dexamethasone-free microspheres or no microspheres, cultured without dexamethasone, where IL-1 α exposure led to significant tissue degradation. These results support the use of dexamethasone delivery from within engineered cartilage, through biodegradable microspheres, as a strategy to produce mechanically functional tissues that can also combat the deleterious effects of local proinflammatory cytokine exposure.

Introduction

JOINT INJURY, ESPECIALLY in younger patients, dramatically increases the risk of developing osteoarthritis (OA)^{1–3} and has been shown to produce radiological evidence of cartilage degeneration in 50–78% of patients within 20 years of the event.^{4,5} Restoration of damaged cartilage is performed in an effort to arrest or slow the progression of joint degeneration. Living osteochondral allografts represent the gold standard for repair of joint defects greater than 2–3 cm².⁶ As the availability of suitable allografts is insufficient to meet the clinical demand, intensive research efforts are focused on the development of strategies to grow living replacement cartilage tissues. Our laboratory has focused on growing tissues appropriate for repair of

focal defects and entire articular surfaces.^{7–11} The impacts of cytokines that contribute to joint inflammation and pain, however, which are elevated with iatrogenic injury,^{12–14} trauma,^{15,16} or OA,^{17,18} on joint repair are not often considered.

In clinical patients following acute ACL injury,¹⁹ cytokines such as tumor necrosis factor α (TNF- α) and interleukin-1 β (IL-1 β) were significantly elevated from baseline 24 h after injury. At 1 week, while reduced, these cytokines remained elevated above chronic levels. Tsuchida *et al.* analyzed the synovial cytokine profiles of patients undergoing microfracture or autologous cell implantation (ACI) for focal defect repair²⁰ and observed increased concentrations of inflammatory cytokines in the synovial fluid of injured as compared with healthy joints. The increased presence of cytokines in injured joints may contribute to the inferior clinical

¹Department of Biomedical Engineering, Columbia University, New York, New York.

²Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania.

³Department of Orthopedics and Sports Medicine, Mount Sinai Health System, New York, New York.

⁴McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

⁵Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania.

⁶Department of Mechanical Engineering, Columbia University, New York, New York.

*Chondroprotection of tissue-engineered cartilage via internal delivery of dexamethasone. Proceedings of the Orthopaedic Research Society. Las Vegas, NV. Poster No. 345, 2015.

results observed for ACI in some patients,^{21–24} as elevated levels of inflammatory mediators have previously been shown to hamper cartilage regeneration.^{25–27} To this end, data from animal studies and early clinical trials suggest that early inhibition of the intra-articular (IA) inflammatory response (e.g., 4 weeks) and posttraumatic injury of the knee may improve clinical outcomes.²⁸ Morisset *et al.* reported an improvement in cartilage repair following IA injection of an adenoviral vector carrying an IL-1 α gene,²⁹ indicating that modulation of the joint's chemical environment may be beneficial for chondrocyte and tissue health.³⁰

Palliative strategies for managing joint pain include anti-inflammatory analgesics such as nonsteroidal anti-inflammatory drugs alongside IA injections of glucocorticoids or hyaluronan.³¹ IA glucocorticoid injections have proven effective in reducing pain in patients for up to 2 years with no deleterious effects to joint morphology.³² A residence time of only 36–72 h, however, mandates that the concentration of injected solutions and suspensions be high, upwards of 2 mg/mL.³³ Rapid clearance from the joint space results in excess circulating glucocorticoids, which leads to increased susceptibility to viral infections and delayed wound healing^{34,35} systemically, as glucocorticoids are effective in suppressing IL-1 β and TNF production by leukocytes.³⁶

Complementary to its use in IA injections, dexamethasone (dex), a synthetic glucocorticoid, has been reported to inhibit collagen degradation of cultured cartilage explants in response to interleukin-1 α (IL-1 α).³⁷ Work in our laboratory has found that engineered bovine cartilage constructs with native properties can be protected from the deleterious effects of IL-1 α by culture supplementation with dex, in a manner similar to native cartilage explants.^{38–40} Additionally, preexposure to dex has been reported to prevent proteoglycan degradation and restore biosynthesis of cartilage explants exposed to TNF- α and mechanical injury.⁴⁰ Several *in vivo* studies have demonstrated the chondroprotective effects of dex as well. Repeated IA injection of high-dose dex, for instance, was observed to protect cartilage from damage in a lapine posttraumatic model of OA.^{41,42} Malfait *et al.* have demonstrated that dex inhibited proteoglycan degradation induced by IA injection of TNF- α .⁴³ While effective, the continuous administration of drug through IA injection is not recommended, as this increases the risk of infection and patient discomfort, emphasizing the need for sustained release formulations.⁴⁴

Recent work has exploited poly(lactic-co-glycolic acid) (PLGA) systems for the delivery of anti-inflammatory drugs.^{45–53} Higaki *et al.* demonstrated that the continuous administration of betamethasone sodium phosphate through PLGA nanoparticles provided increased inhibition of inflammation in an experimental model of OA when compared with the same dosage of betamethasone sodium phosphate delivered three times through IA injection.⁵⁴ Dang *et al.* demonstrated that dex releasing PLGA microparticles are capable of suppressing the host response to implanted polymer materials in a mouse model. Notably, animals that received a higher drug loading treatment suffered an increased incidence of death within 7–10 days after administration, while a low drug loading group maintained healthy body conditions, but still benefited from the drug's anti-inflammatory properties.⁴⁵ These studies demonstrate that locally delivered, low concentrations of anti-inflammatory steroids are capable of suppressing the local host

response without the systemic impact observed with higher drug concentrations.

In the context of cartilage tissue engineering, our laboratory has broadly employed dex for its proanabolic and anticatabolic effects in cartilage tissue engineering systems,⁵⁵ its proven importance for cultivation of tissues with native properties, and its ability to confer chondroprotection against inflammatory cytokines.^{38,39,56,57} As these factors can impact clinical success of cartilage tissue engineering strategies, we sought to develop a more clinically focused cartilage tissue engineering approach utilizing the FDA-approved steroid dex. An ideal therapy would retain the benefits of dex on engineered cartilage without the requirement for its exogenous supplementation, obviating the systemic⁵⁸ and joint level complications associated with clinical injections of steroids.^{59,60} As such, the current study tested the hypothesis that the incorporation of PLGA microspheres (MS), with the ability to release dex from within chondrocyte-seeded agarose hydrogel constructs, would promote development of constructs with native mechanical properties and protect constructs from the deleterious effects of IL-1 α , laying the foundation for future, clinically focused studies.

Materials and Methods

Experimental design

To assess the potential for chondroprotection through internally released dex, PLGA MS, loaded with the steroid, were embedded in the tissue construct during gelation (Fig. 1). Tissues were cultured in chondrogenic medium for 4 weeks before cytokine exposure to allow for sufficient tissue maturation.⁶¹ Following 4 weeks of culture in a chondrogenic medium, a subset of these tissues were treated with supraphysiological levels of inflammatory cytokine for 1 week (Fig. 2). Following 1 week of treatment, tissues were evaluated for their mechanical and biochemical properties.

Microsphere fabrication and preparation

Dex-loaded PLGA MS were fabricated as described previously.⁵¹ Empty MS were also prepared with the same method, omitting the dex. Before the experiment, 8 mg of dex-loaded and empty MS were weighed and sterilized through ultraviolet light. The release of dex was quantified spectrophotometrically at 242 nm. Ten milligrams of MS were placed in a microcentrifuge tube containing 1 mL of phosphate-buffered saline (PBS) ($n=3$). At various time points, samples were centrifuged and releasates were collected and then frozen at -80°C until analysis. The amount of dex in each releasate was quantified by comparison with a standard curve.

Cell culture and sample preparation

Articular cartilage was harvested from stifles of freshly slaughtered 2- to 4-week-old calves. Three joints were used for the experiment and cells were pooled from all joints. Cartilage chunks were digested in 390 U/mL collagenase type IV (Worthington) for 11 h with agitation. Isolated chondrocytes were passaged in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1 ng/mL TGF- β 1, 5 ng/mL FGF-2, and 1% antibiotics/antimycotics.

FIG. 1. Representative schematic of the dex-loaded microsphere strategy employed in this study. PLGA microspheres are embedded alongside chondrocytes in an agarose hydrogel. In this way, dexamethasone is available locally to the cell to optimize the chondroprotective effects of the glucocorticoid against pro-inflammatory cytokines, such as interleukin-1 and tumor necrosis factor alpha. dex, dexamethasone; PLGA, poly(lactic-co-glycolic acid). Color images available online at www.liebertpub.com/tea

Following two passages, cells were prepared for encapsulation in an agarose (type VII; Sigma) hydrogel. For MS-free controls (ctrl, ctrl+IL, ctrl-dex), chondrocytes were seeded at 30×10^6 cells/mL in 2% agarose. For MS-containing samples (dex-loaded [LMS], LMS+IL, and empty microspheres [ULMS]), 1.5 mL of concentrated cell solution was combined with previously prepared MS. This cell/MS suspension was combined with 1.5 mL agarose and cast between glass slides for a final concentration of 2.66 mg MS per milliliter and 30×10^6 cells/mL in a 2% agarose hydrogel. This concentration of MS was selected as it has been previously shown to positively impact biosynthetic activity of human adipose stem cells.⁵¹ Cylindrical constructs (0.40×2.34 mm) were cored and cultured in chondrogenic medium comprised of DMEM with 50 μ g/mL L-proline, 100 μ g/mL sodium pyruvate, 1% ITS+ premix (BD Biosciences), 1% antibiotics/antimycotics, 50 μ g/mL ascorbic acid, and 10 ng/mL TGF- β 3 (R&D Systems) for the first 2 weeks of culture.⁶² MS-loaded tissues effectively received 21.9 ng dex/construct/day. MS-free controls received 100 nM dex at each media change (26.9 ng dex/construct/day). MS-containing samples received no exogenous dex. A subset of MS-free tissues was cultured without dex for the first 28 days of culture to determine

any adverse or beneficial effects of the PLGA on tissue development.

Cytokine insult

Following 28 days in growth culture, a subset of ctrl and LMS samples were subjected to 10 ng/mL IL-1 α . IL-1 α was added at each media change for 7 days. During this period, MS-free samples were starved of dex so as to isolate the potential impact of internally released dex in MS-loaded samples.

Mechanical characterization

A custom unconfined compression device was used to determine the equilibrium compressive modulus (E_Y) of whole samples at days 0, 14, 28, and 35 ($n=5$). An initial 0.02 N tare load was applied, followed by compression to 90% of the unloaded sample thickness, at a strain rate of 0.05%/s. After relaxation, a 2% peak-to-peak sinusoidal strain was applied at 0.01 Hz. E_Y was evaluated from the equilibrium response of stress-relaxation curves. A custom glass-bottom device mounted on the stage of an inverted microscope^{63,64} was used to determine depth-dependent properties of ctrl and LMS samples on day 35. Before testing, samples were halved and maintained in a sterile PBS solution containing calcein-AM (Life Technologies). Following staining, samples were positioned with the cross-section facing the objective and initial measurements of thickness were performed digitally. Initial images of the cross-section were acquired, and the samples were then compressed at nominal 5% strain increments to a final compression of 15%. After each compression cycle, samples were allowed to equilibrate for 15 min, with images being acquired immediately before subsequent compression. Digital image correlation was used to obtain accurate axial and lateral strains,^{63,64} which were combined with incremental compressive loading data to determine depth-dependent mechanical properties.

Biochemical characterization

Samples were thawed, lyophilized, weighted dry, and digested with 0.5 mg/mL proteinase K in 50 mM Tris-buffered saline containing 1 mM EDTA and 1 mM iodoacetamide. DNA content was quantified using a PicoGreen assay (Life Technologies). Glycosaminoglycan (GAG) content was quantified using 1,9-dimethylmethylene blue dye-binding assay⁶⁵ with shark chondroitin-6-sulfate standards (Sigma). The digests

FIG. 2. (a) Schematic of experimental design. ctrl and dex-loaded microsphere (LMS) tissues were treated with interleukin-1 α (10 ng/mL) after 4 weeks of culture. Unloaded microsphere (ULMS) samples, untreated, were cultured as a negative control for the duration of the study. (b) ctrl-dex tissues were cultured for the first 28 days of culture as a microsphere-free, dex-free control. Color images available online at www.liebertpub.com/tea

were further hydrolyzed in 12N HCl at 110°C for 16h for quantification of total collagen content through orthohydroxyproline colorimetric assay.⁶⁶ The collagen and GAG contents were normalized to the construct wet weight. The swelling ratio was also determined from tissue volumes at each time point normalized to average day 0 tissue volumes. Tissue maturity was assessed qualitatively and quantitatively (ImageJ) through histology, before freezing, and day 35 samples were fixed in acid formalin ethanol. Following fixation, samples were then paraffin embedded, sectioned (8 μ m), and stained with Alcian Blue (GAG) and Picrosirius Red (collagen).

Results

Microsphere fabrication and preparation

MS were observed through scanning electron microscopy (Fig. 3a) and determined to have an average diameter of 10 μ m. A burst release of $10.2\% \pm 0.8\%$ of initial dex content was observed after 24h. A linear fit of subsequent release data ($R^2=0.97$) yielded a daily release rate of 250 ng dex per milligram MS (Fig. 3b). After 28 days in culture, $32.8\% \pm 1.1\%$ of dex had been released. The loading capacity was 19.9 ± 1.9 μ g dex per milligram MS. These results have been confirmed through high-performance liquid chromatography.^{50,67}

Bulk mechanical characterization

At day 0, there were no significant differences between MS and ctrl samples (4.8 ± 2.5 kPa vs. 2.7 ± 1.7 kPa). All groups displayed significant growth from day 0 to 28 ($p < 0.05$) (Fig. 4). By day 28, E_y of all groups achieved ~ 200 kPa (Fig. 4a). ULMS and ctrl-dex samples developed similarly to this time point, indicating minimal negative or positive impact from PLGA degradation. Following treatment with IL-1 α , the equilibrium modulus of ctrl+IL tissues dropped significantly ($p < 0.05$). At day 35, ctrl tissues reached 527 ± 74 kPa, whereas ctrl+IL tissues dropped to 194 ± 98 kPa from 297 ± 101 kPa at day 28 (Fig. 4d). LMS tissues reached 387 ± 80 kPa at day 28. Following cytokine exposure, LMS+IL tissues continued to mature to 645 ± 136 kPa, similar to LMS tissues that grew to 717 ± 99 kPa at the same time point. ULMS tissues, those that received neither dex nor IL-1 α , reached 269 ± 49 kPa by day 35. As a result of its inferior mechanical properties at day 35, ULMS tissues were excluded from analysis in Figure 4e and f.

Depth-dependent mechanical characterization

MS remained visible in the tissue at day 35 (Fig. 5a). At day 35, strain distributions from both ctrl and LMS sam-

ples displayed tissue inhomogeneity consistent with previous work^{68,69} (Fig. 5b). While LMS tissues displayed a significantly higher ($p < 0.05$) local equilibrium modulus than ctrl in 67% of the thickness of the tissue, tissue inhomogeneity does not appear to be impacted by the local delivery of dex.

Biochemical characterization

Cellularity. The DNA content of all tissues increased throughout time in culture (Fig. 6a). At day 28, ctrl tissues contained 2.04 ± 0.20 million cells per construct. Following IL-1 α treatment, the number of cells in ctrl+IL tissues was significantly lower (1.53 ± 0.19 million) compared to ctrl tissues (1.96 ± 0.23 million cells, $p < 0.05$). Similarly, LMS tissues contained 2.09 ± 0.17 million cells at day 28. Unlike ctrl+IL tissues, the cellularity of LMS+IL tissues was preserved at day 35 (1.92 ± 0.15 million cells) as compared to LMS samples (2.01 ± 0.10 million cells). Uniquely, by day 35, the number of cells in ULMS tissues increased to 3.49 ± 0.52 million cells per construct, a 2.76-fold increase above day 0 values.

GAG incorporation. At day 28 in culture, all tissues contained at least $4.69\% \pm 1.07\%$ GAG per wet weight (GAG/ww) (Fig. 4b). Following IL-1 α treatment, GAG content of ctrl+IL samples ($5.07\% \pm 1.15\%$ GAG/ww) was significantly lower than respective controls ($7.91\% \pm 0.46\%$ GAG/ww, $p < 0.05$) (Fig. 4e). In contrast, LMS+IL tissues continued to grow following the initiation of cytokine treatment, reaching $9.29\% \pm 1.26\%$ GAG/ww by day 35, similar to LMS controls that achieved $10.99\% \pm 1.32\%$ GAG/ww at the same time point. ULMS tissues reached $7.65\% \pm 1.17\%$ GAG/ww by day 35 in culture.

Collagen content. Collagen content of ctrl and LMS tissues reached $1.43\% \pm 0.33\%$ collagen normalized to wet weight (collagen/ww) and $1.53\% \pm 0.34\%$ collagen/ww, respectively, at day 28 (Fig. 4c). ULMS tissues contained $1.86\% \pm 0.18\%$ collagen/ww at the same time point. Following cytokine insult, collagen content significantly decreased in ctrl+IL tissues ($p < 0.05$) when compared with ctrl, LMS+IL (Fig. 4f), and ULMS tissues.

Swelling ratio. All tissues exhibited significant swelling by day 35 in culture (Fig. 6b). ULMS displayed a significantly greater ($p < 0.05$) swelling ratio at day 35 when compared with all other groups, swelling to nearly 2 $\times$ its original volume.

FIG. 3. (a) SEM image. This image demonstrates consistent shape and size of the PLGA microspheres used in this study. Scale bar = 10 μ m. (b) Cumulative release curve of dexamethasone released from 10 mg PLGA microspheres. By 70 days in culture, all dexamethasone had been released from within the microspheres. Dotted line indicates 100% release.

FIG. 4. Mechanical and biochemical development of tissue-engineered cartilage throughout culture. ctrl-dex is provided as a reference for the first 28 days of growth. ULMS tissues exhibited poor growth at day 35 and were not included in other metrics. **(a, d)** Illustrate equilibrium moduli. **(b, e)** Illustrate the GAG content of engineered tissues throughout the study. **(c, f)** Illustrate the collagen content of engineered tissues throughout the study. * Indicates $p < 0.05$ when compared with ctrl+IL at the same time point. ^ Indicates $p < 0.05$ between LMS and ULMS. GAG, glycosaminoglycan; IL, interleukin. Color images available online at www.liebertpub.com/tea

FIG. 5. Cross-section of samples prepared for depth-dependent mechanical testing using a custom designed microscope testing device. **(a)** Transmitted light image of the cross-sectional area of LMS tissues at day 35. The amorphous shape of certain microspheres indicates the degradation of the polymer. Scale bar = 250 μ m. **(b)** Following testing, digital image correlation was performed to determine strain patterns throughout the tissue. A gradient along the horizontal axis indicates higher strain in the central region of the tissue and lower strain at the edges, indicating depth-dependent changes in stiffness. Color images available online at www.liebertpub.com/tea

FIG. 6. Cellular proliferation and tissue swelling throughout the entire culture period. **(a)** Cellular proliferation was experienced in all groups. Samples cultured without dex (ULMS) experienced significant cellular proliferation by day 35 in culture. * Indicates $p < 0.05$ for all samples compared with ctrl+IL at the same time point. ** Indicates $p < 0.05$ for ULMS samples compared to all others at the same time point. **(b)** As expected, all samples swelled to day 35. Samples cultured without dex (ULMS) swelled significantly compared to all other groups at day 35. * Indicates $p < 0.05$ for ULMS samples compared with all other groups at the same time point. ** Indicates $p < 0.05$ for LMS samples compared to ctrl, ctrl+IL at the same time point. ^ Indicates $p < 0.05$ for LMS+IL samples compared with ctrl+IL at the same time point. Color images available online at www.liebertpub.com/tea

Histological/morphological characterization

Histology (Fig. 7a–i), through ImageJ analysis, indicates weaker staining for GAGs in ctrl+IL samples (Fig. 7b) when compared with other groups at day 35. Weaker intercellular staining was observed in ctrl+IL samples (Fig. 7f) at the same time point when compared with other groups. This response is contrasted with LMS+IL samples (Fig. 7d, h), which maintain the intensity of their staining following treatment with IL-1 α .

Discussion

This study demonstrated that dex-loaded PLGA MS embedded in chondrocyte-seeded agarose hydrogels promote development of mechanically functional cartilage tissue in culture. This local dex-delivery system also provides chondroprotection against the deleterious effects of IL-1 α , a ubiquitous cytokine known to play a major role in the innate immune response and articular cartilage degeneration.

FIG. 7. Histological sections from day 35. Alcian Blue staining indicates loss of GAGs following treatment with IL **(b)**. Picrosirius Red displays inferior intercellular staining for collagen in controls treated with IL **(f)**. In LMS tissues treated with IL, the same decrease in proteoglycan and collagen intensity is not observed **(d, h)**. **(a, e)** Correspond to control. **(b, f)** Correspond to control+IL. **(c, g)** Correspond to LMS. **(d, h)** Correspond to LMS+IL. **(i)** Illustrates the gross differences at day 35 between samples cultured +/- LMS and +/- IL. Opacity is conserved between LMS and LMS+IL groups at this time point. Scale bar = 4 mm. Color images available online at www.liebertpub.com/tea

By releasing dex from within engineered cartilage, our therapeutic strategy targets the complicated autocrine control system of chondrolysis, through which cartilage damage is triggered by proinflammatory cytokines produced from leukocytes and the synovium.^{70,71} Compared to strategies that target specific proinflammatory cytokines, such as IL-1 receptor antagonist,^{72,73} dex has been shown to mitigate the deleterious effects of IL, TNF- α , and other cytokines associated with inflammation. Once internalized, dex acts directly and indirectly by activating anti-inflammatory proteins,^{74,75} upregulating their production through glucocorticoid-responsive elements located on DNA,^{76,77} and by interfering with the translocation of other transcriptional factors, such as nuclear factor-kappa B.^{78,79}

As a culture supplement, dex is known to perform a variety of roles, including as a promoter of chondrogenesis in mesenchymal stem cell populations.^{80–82} Additionally, as reiterated in this study, dex is a critical element in the development and maturation of tissue-engineered cartilage. Here, all tissues developed similarly for the first 2 weeks of culture (Fig. 4a), regardless of dex supplementation. Tissues receiving dex achieved an E_Y within the native range⁸³ by the end of culture and were ~2-fold higher than tissues receiving no dex (ULMS) (Fig. 4d). Moreover, dex-loaded MS expedited this tissue development (day 35).

While delivery of dex from within constructs cultivates tissues with E_Y in the range of native values, the internal delivery of dex from homogeneously distributed MS was not observed to modulate the axial inhomogeneity of tissue properties reported in chondrocyte-seeded agarose constructs of similar dimensions.⁶⁸ This inhomogeneity can be described as a U-shape, with stiffer regions of tissue located at the periphery.^{84,85} (Fig. 5b), and is thought to reflect nutrient gradients associated with free-swelling culture. This finding suggests that dex is not responsible for the nonuniform tissue development observed in our current study. Recent work suggests that other media components, such as insulin and glucose, may play a role in this phenomenon.^{86,87} The high binding affinity of active TGF- β 3, an ~25 kDa molecule (compared with 400 Da for dex), to agarose and cartilage matrix has also been implicated in contributing some part to the development of tissue heterogeneity.⁸⁸

The E_Y values observed here are interestingly in tandem with cellular density and swelling ratio. With respect to cellularity, tissues that received no dex throughout the study (ULMS) proliferated to a cellular density ~2.75-fold higher than those that did (ctrl, LMS) (Fig. 6a). The antiproliferative effects of dex seen here are consistent with work in 2D chondrocyte cultures.⁸⁹ While a higher cellularity yielded near-native GAG levels (~7% GAG/ww, data not shown) and GAG levels comparable to those in ctrl tissues, E_Y remained significantly lower at the final time point (Fig. 4d), despite minimal variation in collagen content across tissue groups. Furthermore, ULMS tissues experience greater swelling and inferior mechanical properties compared to LMS, attributed to the absence of dex. We speculate that dex influences the biosynthetic behavior of chondrocytes that dictates the structural organization of the collagen network and collagen–proteoglycan interactions that serve to combat tissue swelling.^{6,90,91}

In addition to the physical demands of the joint, its harsh inflammatory environment will likely impact the perfor-

mance of engineered cartilage. The presence of IL-1 β upregulates the expression of aggrecanases, the primary proteases identified in pathological aggrecan degradation in human OA,⁹² and has a predominant effect on the GAG content of engineered cartilage. Previous studies have shown that GAG content of IL-1-treated engineered cartilage is depleted ~4-fold as compared with controls.³⁸ It is also known, however, that dex supplementation reduces sulfated GAG loss and rescues proteoglycan synthesis in cartilage explants exposed to inflammatory cytokines.⁴⁰ In the study presented here, LMS and ctrl tissues were treated with IL-1 α beginning at day 28 in culture. The time point selected for cytokine insult

FIG. 8. GAG content from preliminary study using adult human chondrocytes. Chondrocytes were isolated from the femoral articular cartilage of three donors. Following casting at 40 million cells per milliliter in 2% agarose hydrogels, tissues were cultured as described previously. At day 28, samples were divided and a portion was treated with 0.1 ng/mL IL-1. (a) As expected, there was no change in collagen content among treatment groups following cytokine insult. (b) It was observed, however, that GAG levels in LMS+IL tissues increased from ~5% at day 28 to ~8% GAG/ww at day 35, a value within the range of native tissues and significantly higher than all other groups ($p < 0.05$). At the same time, ctrl tissues experienced a significant decrease in GAG content ($p < 0.05$) from ~6% GAG/ww at day 28 to ~4% GAG/ww at day 35. This biochemical profile suggests that the chondroprotective effect of dex-loaded microspheres observed in juvenile bovine chondrocyte constructs is applicable to adult human chondrocyte constructs, as well, providing motivation for further investigation into the role that dex-loaded microspheres may play clinically. * Indicates $p < 0.05$ when compared with all other tissues. ** Indicates $p < 0.05$ when compared with ctrl, LMS, LMS+IL, and ULMS+IL. ^ Indicates $p < 0.05$ when compared with ctrl+IL, LMS+IL, ULMS, and ULMS+IL. # Indicates $p < 0.05$ when compared with ctrl, LMS, LMS+IL, and ULMS. GAG/ww, GAG per wet weight. Color images available online at www.liebertpub.com/tea

was informed by work in our laboratory and others suggesting that construct maturity plays an important role in the tissue's response to cytokine and *in vivo* outcomes,^{38,61,93} with delayed exposure to insult being beneficial to tissue health. Following 7-day treatment of IL-1 α , ctrl+IL tissues contained ~36% less GAG than their respective controls (Fig. 4e), resulting in an inferior tissue with equilibrium moduli measuring ~2.75-fold lower than ctrl tissues. LMS tissues, however, did not experience a similar depletion of GAGs. In fact, LMS tissues exposed to cytokine continued to elaborate their extracellular matrix beyond day 28, increasing GAG content nearly 2-fold by day 35 (Fig. 4d). The continued biosynthetic activity of LMS tissues in the presence of a supraphysiological dose of IL-1 α (10 ng/mL vs. 0.288 ng/mL⁹⁴ in moderate OA joints), compared with the significant decline in synthesis by ctrl+IL tissues, is encouraging in the context of using dex-loaded MS as a strategy to promote cartilage repair *in vivo*.⁶¹

Recent work demonstrates the successful translation of this dex microsphere strategy to engineered constructs seeded with adult human chondrocytes⁹⁵ from three donors (Fig. 8), supporting the need for continued research efforts pursuing dex-loaded MS. Similar to constructs derived from juvenile bovine chondrocytes, coencapsulation of adult human chondrocytes and dex-loaded MS were able to promote cartilaginous tissue development similar to exogenous dex supplementation of the culture media and afforded chondroprotection against IL-1 α .

While simplistic, the use of a single proinflammatory cytokine, IL-1 α , is a well-established culture model for OA^{38,40,71,72,96–98} proven to provide valuable insight on the impact of cytokines on articular cartilage. The use of IL-1, specifically, exploits the cytokine's importance in inflammation.⁹⁹ IL-1, along with TNF- α , is responsible for the majority of degradation of the extracellular matrix *in vivo*¹⁰⁰ and both cytokines share a common set of pathways, including the activation of nuclear factor-kappa B (NF- κ B).¹⁰¹ The utilization of dex is therefore unique, as glucocorticoids have been shown to physically interact with the p65 subunit of NF- κ B and transrepress its function.¹⁰² While anti-inflammatory cytokines such as IL-4 and IL-13 are known to directly regulate IL-1 function in the joint,^{103–105} the use of dex in the context of cartilage tissue engineering is critical for functional maturation of the tissue (as shown in this work) and has been demonstrated to confer chondroprotection against both IL-1 α and TNF- α .⁴⁰

This study demonstrates the ability of dex-loaded MS to cultivate tissues that are mechanically viable (within native levels of compressive modulus) while simultaneously providing chondroprotection. In addition to the benefits of dex-loaded MS in culture, a controlled release approach to steroid administration is particularly advantageous in the context of current therapies for early intervention in OA. While it has been reported that IA injections lead to improved outcomes at 2-year follow-ups,³² the inherently short duration of therapeutic action for corticosteroids¹⁰⁶ and dex (6–8 days¹⁰⁷), in particular, the result of rapid systemic absorption suggests that more frequent injections may obtain ideal joint pain outcomes. The accumulated availability of glucocorticoids through repeated injections, however, has been shown to lead to gross morphological cartilage changes¹⁰⁸ alongside lowered viability and biosynthetic activity

of chondrocytes.¹⁰⁹ Successively elevated systemic levels of glucocorticoid also impact the hypothalamus–pituitary–adrenal (HPA) axis,¹¹⁰ inhibiting the immune system's ability to respond to infection. The dual impact of rapid systemic absorption, both inside and outside the joint, suggests that sustained delivery of low levels of glucocorticoids may address these concerns while improving patient outcomes.

Low dose, sustained delivery of glucocorticoids, as presented in this work, provides a forward thinking strategy for improved cartilage repair and healing. The MS used in this study have been shown to release drug for up to 70 days (Fig. 2b), maintaining a consistent level of drug release throughout culture and ensuring local availability of the steroid beyond the 5 weeks observed in this study. Daily release of the drug is measured at only ~19 ng per construct. This amount is 35 $\times$ lower than the amount used in standard tissue culture³⁸ and 11 $\times$ lower than commonly used triamcinolone acetonide IA injection dosages.^{97,107,111,112} Additionally, this strategy has been shown to minimize the potential effects on the HPA axis, having been employed *in vivo* and shown to promote tissue formation with minimal systemic impact.⁵²

We further anticipate a strategy of chondroprotection where a glucocorticoid, administered from the locale of the injury/repair site in a manner that provides its sustained release at much lower levels than clinical injections, can also impact tissue healing and repair of the engineered graft upon implantation.^{41,42} While future plans are aimed at further optimization of the dex microsphere strategy, including the testing of other proinflammatory cytokines such as TNF- α and the evaluation of tissue integrative repair in a cartilage explant ring model,^{113–115} the efficacy of our approach on cartilage repair will necessarily have to be evaluated *in vivo*.

Conclusion

In this study, we have demonstrated that the internal release of dexamethasone, an anti-inflammatory and FDA-approved synthetic glucocorticoid, can promote the development of mechanically functional engineered cartilage and simultaneously provide protection against a supraphysiological dosage of the proinflammatory cytokine IL-1 α . As such, this study presents a strategy for delivery of dexamethasone from within engineered cartilage as a potential strategy for improving the success of cartilage repair *in vivo*.

Acknowledgments

The authors would like to thank the Musculoskeletal Transplant Foundation Established Investigator Grant (C.T.H.), National Institutes of Health (AR060361 [C.T.H., G.A.A.], AR068133 [C.T.H.], 5T32AR059038 [B.L.R.]), and National Science Foundation Graduate Research Fellowship Program under Grant No. DGE-11-44155 (B.L.R.).

Disclosure Statement

No competing financial interests exist.

References

1. Roos, H., Adalberth, T., Dahlberg, L., and Lohmander, L.S. Osteoarthritis of the knee after injury to the anterior

- cruciate ligament or meniscus: the influence of time and age. *Osteoarthritis Cartilage* **3**, 261, 1995.
2. Gelber, A.C., Hochberg, M.C., Mead, L.A., Wang, N.-Y., Wigley, F.M., and Klag, M.J. Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. *Ann Intern Med* **133**, 321, 2000.
 3. Roos, E.M. Joint injury causes knee osteoarthritis in young adults. *Curr Opin Rheumatol* **17**, 195, 2005.
 4. Porat von, A. High prevalence of osteoarthritis 14 years after an anterior cruciate ligament tear in male soccer players: a study of radiographic and patient relevant outcomes. *Ann Rheum Dis* **63**, 269, 2004.
 5. Lohmander, L.S., Englund, P.M., Dahl, L.L., and Roos, E.M. The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis. *Am J Sports Med* **35**, 1756, 2007.
 6. Williams, R.J., Peterson, L., and Cole, B. Cartilage repair strategies. In: Williams, R.J., III, ed. Totowa, NJ: Humana Press, 2007, pp. 37–53.
 7. Ateshian, G.A., and Hung, C.T. Patellofemoral joint biomechanics and tissue engineering. *Clin Orthop Relat Res* **81**, 2005.
 8. Hung, C.T., Mauck, R.L., Wang, C.C.B., Lima, E.G., and Ateshian, G.A. A paradigm for functional tissue engineering of articular cartilage via applied physiologic deformational loading. *Ann Biomed Eng* **32**, 35, 2004.
 9. Hung, C.T., Lima, E.G., Mauck, R.L., Taki, E., LeRoux, M.A., Lu, H.H., *et al.* Anatomically shaped osteochondral constructs for articular cartilage repair. *J Biomech* **36**, 1853, 2003.
 10. Ng, K.W., Bian, L., O'Connor, C.J., Jayabalan, P.S., Stoker, A.M., Kuroki, K., *et al.* Passaged adult chondrocytes can form engineered cartilage with functional mechanical properties: a canine model. *Tissue Eng Part A* **16**, 13, 2010.
 11. Roach, B.L., Hung, C.T., Cook, J.L., Ateshian, G.A., and Tan, A.R. Fabrication of tissue engineered osteochondral grafts for restoring the articular surface of diarthrodial joints. *Methods* **84**, 103, 2015.
 12. Amin, A.K., Huntley, J.S., Bush, P.G., Simpson, A.H.R.W., and Hall, A.C. Chondrocyte death in mechanically injured articular cartilage—the influence of extracellular calcium. *J Orthop Res* **27**, 778, 2009.
 13. Amin, A.K., Huntley, J.S., Simpson, A.H.R.W., and Hall, A.C. Increasing the osmolality of joint irrigation solutions may avoid injury to cartilage: a pilot study. *Clin Orthop Relat Res* **468**, 875, 2010.
 14. Houston, D.A., Amin, A.K., White, T.O., Smith, I.D.M., and Hall, A.C. Chondrocyte death after drilling and articular screw insertion in a bovine model. *Osteoarthritis Cartilage* **21**, 721, 2013.
 15. Lotz, M.K., and Kraus, V.B. New developments in osteoarthritis. Posttraumatic osteoarthritis: pathogenesis and pharmacological treatment options. *Arthritis Res Ther* **12**, 211, 2010.
 16. Patwari, P.R., Cook, M.N., DiMicco, M.A., Blake, S.M., James, I.E., Kumar, S., *et al.* Proteoglycan degradation after injurious compression of bovine and human articular cartilage in vitro: interaction with exogenous cytokines. *Arthritis Rheum* **48**, 1292, 2003.
 17. D'Lima, D., Hermida, J., Hashimoto, S., Colwell, C., and Lotz, M. Caspase inhibitors reduce severity of cartilage lesions in experimental osteoarthritis. *Arthritis Rheum* **54**, 1814, 2006.
 18. Goldring, S.R., and Goldring, M.B. The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clin Orthop Relat Res* **427 Suppl**, S27, 2004.
 19. Irie, K., Uchiyama, E., and Iwasa, H. Intraarticular inflammatory cytokines in acute anterior cruciate ligament injured knee. *Knee* **10**, 93, 2003.
 20. Tsuchida, A.I., Beekhuizen, M., 't Hart, M.C., Radstake, T.R.D.J., Dhert, W.J.A., Saris, D.B.F., *et al.* Cytokine profiles in the joint depend on pathology, but are different between synovial fluid, cartilage tissue and cultured chondrocytes. *Arthritis Res Ther* **16**, 441, 2014.
 21. Vanlauwe, J., Vanlauwe, J., Saris, D.B., Saris, D.B., Victor, J., Victor, J., *et al.* Five-year outcome of characterized chondrocyte implantation versus microfracture for symptomatic cartilage defects of the knee: early treatment matters. *Am J Sports Med* **39**, 2566, 2011.
 22. Filardo, G., Vannini, F., Marcacci, M., Andriolo, L., Ferruzzi, A., Giannini, S., *et al.* Matrix-assisted autologous chondrocyte transplantation for cartilage regeneration in osteoarthritic knees: results and failures at midterm follow-up. *Am J Sports Med* **41**, 95, 2013.
 23. Saris, D.B.F., Dhert, W.J.A., and Verbout, A.J. Joint homeostasis. *J Bone Joint Surg* **85**, 1067, 2003.
 24. Ozsoy, M.H., Aydogdu, S., Taskiran, D., Sezak, M., Hayran, M., Oztop, F., *et al.* The effects of early or late treatment of osteochondral defects on joint homeostasis: an experimental study in rabbits. *Knee Surg Sports Traumatol Arthrosc* **17**, 578, 2009.
 25. Yang, K.G.A., Saris, D.B.F., Verbout, A.J., Creemers, L.B., and Dhert, W.J.A. The effect of synovial fluid from injured knee joints on in vitro chondrogenesis. *Tissue Eng* **12**, 2957, 2006.
 26. Beekhuizen, M., van Osch, G.J.V.M., Bot, A.G.J., Hoekstra, M.C.L., Saris, D.B.F., Dhert, W.J.A., *et al.* Inhibition of oncostatin M in osteoarthritic synovial fluid enhances GAG production in osteoarthritic cartilage repair. *Eur Cells Mater* **26**, 80, 2013.
 27. Rodrigo, J.J., Steadman, J.R., Syftestad, G., Benton, H., and Silliman, J. Effects of human knee synovial fluid on chondrogenesis in vitro. *Am J Knee Surg* **8**, 124, 1995.
 28. Olson, S.A., Horne, P., Furman, B., Huebner, J., Al-Rashid, M., Kraus, V.B., *et al.* The role of cytokines in posttraumatic arthritis. *J Am Acad Orthop Surg* **22**, 29, 2014.
 29. Morisset, S., Frisbie, D.D., Robbins, P.D., Nixon, A.J., and McIlwraith, C.W. IL-1 α /IGF-1 gene therapy modulates repair of microfractured chondral defects. *Clin Orthop Relat Res* **462**, 221, 2007.
 30. Elsaid, K.A., Zhang, L., Shaman, Z., Patel, C., Schmidt, T.A., and Jay, G.D. The impact of early intra-articular administration of interleukin-1 receptor antagonist on lubricin metabolism and cartilage degeneration in an anterior cruciate ligament transection model. *Osteoarthritis Cartilage* **23**, 114, 2015.
 31. Zhang, W., Moskowitz, R.W., Nuki, G., Abramson, S., Altman, R.D., Arden, N., *et al.* OARS recommendations for the management of hip and knee osteoarthritis, part II: OARS evidence-based, expert consensus guidelines. *Osteoarthritis Cartilage* **16**, 137, 2008.
 32. Raynauld, J.P., Buckland Wright, C., Ward, R., Choquette, D., Haraoui, B., Martel Pelletier, J., *et al.* Safety and efficacy of long-term intraarticular steroid injections in osteoarthritis of the knee: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* **48**, 370, 2003.

33. MacMahon, P.J., Eustace, S.J., and Kavanagh, E.C. Injectable corticosteroid and local anesthetic preparations: a review for radiologists. *Radiology* **252**, 647, 2009.
34. Glaser, R., and Kiecolt-Glaser, J.K. Stress-induced immune dysfunction: implications for health. *Nat Rev Immunol* **5**, 243, 2005.
35. Singh, A., Zelazowska, E.B., Petrides, J.S., Raybourne, R.B., Sternberg, E.M., Gold, P.W., *et al.* Lymphocyte subset responses to exercise and glucocorticoid suppression in healthy men. *Med Sci Sports Exerc* **28**, 822, 1996.
36. DeRijk, R., Michelson, D., Karp, B., Petrides, J., Galliven, E., Deuster, P., *et al.* Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1 beta (IL-1 beta), IL-6, and tumor necrosis factor-alpha (TNF alpha) production in humans: high sensitivity of TNF alpha and resistance of IL-6. *J Clin Endocrinol Metab* **82**, 2182, 1997.
37. Saito, S., Katoh, M., Masumoto, M., Matsumoto, S., and Masuho, Y. Dexamethasone inhibits collagen degradation induced by the combination of interleukin-1 and plasminogen in cartilage explant culture. *Biol Pharm Bull* **22**, 727, 1999.
38. Lima, E.G., Tan, A.R., Tai, T., Bian, L., Stoker, A.M., Ateshian, G.A., *et al.* Differences in interleukin-1 response between engineered and native cartilage. *Tissue Eng Part A* **14**, 1721, 2008.
39. Lima, E.G., Tan, A.R., Ateshian, G.A., Cook, J.L., and Hung, C.T. Physiologic Deformational loading does not counteract the catabolic effects of interleukin-1 in long-term culture of chondrocyte-seeded agarose constructs. *J Biomech* **41**, 3253, 2008.
40. Lu, Y.C.S., Lu, Y.C.S., Evans, C.H., Evans, C.H., Grodzinsky, A.J., and Grodzinsky, A.J. Effects of short-term glucocorticoid treatment on changes in cartilage matrix degradation and chondrocyte gene expression induced by mechanical injury and inflammatory cytokines. *Arthritis Res Ther* **13**, R142, 2011.
41. Huebner, K.D., Shrive, N.G., and Frank, C.B. Dexamethasone inhibits inflammation and cartilage damage in a new model of post-traumatic osteoarthritis. *J Orthop Res* **32**, 566, 2014.
42. Huebner, K.D., Shrive, N.G., and Frank, C.B. New surgical model of post-traumatic osteoarthritis: isolated intra-articular bone injury in the rabbit. *J Orthop Res* **31**, 914, 2013.
43. Malfait, A.M., Tortorella, M., Thompson, J., Hills, R., Meyer, D.M., Jaffee, B.D., *et al.* Intra-articular injection of tumor necrosis factor-alpha in the rat: an acute and reversible in vivo model of cartilage proteoglycan degradation. *Osteoarthritis Cartilage* **17**, 627, 2009.
44. Gerwin, N., Hops, C., and Lucke, A. Intraarticular drug delivery in osteoarthritis. *Adv Drug Deliv Rev* **58**, 226, 2006.
45. Dang, T.T., Bratlie, K.M., Bogatyrev, S.R., Chen, X.Y., Langer, R., and Anderson, D.G. Spatiotemporal effects of a controlled-release anti-inflammatory drug on the cellular dynamics of host response. *Biomaterials* **32**, 4464, 2011.
46. Galeska, I., Kim, T.-K., Patil, S.D., Bhardwaj, U., Chattopadhyay, D., Papadimitrakopoulos, F., *et al.* Controlled release of dexamethasone from PLGA microspheres embedded within polyacid-containing PVA hydrogels. *AAPS J* **7**, E231, 2005.
47. Tang, G., Yang, Y., Sun, A., Song, T., Zhao, Y., Yuan, X., *et al.* Controlled release of dexamethasone from porous PLGA scaffolds under cyclic loading. *Sci China Chem* **53**, 594, 2010.
48. Bae, S.E., Choi, D.H., Han, D.K., and Park, K. Effect of temporally controlled release of dexamethasone on in vivo chondrogenic differentiation of mesenchymal stromal cells. *J Control Release* **143**, 23, 2010.
49. Hickey, T., Kreutzer, D., Burgess, D.J., and Moussy, F. Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices. *Biomaterials* **23**, 1649, 2002.
50. Hickey, T., Kreutzer, D., Burgess, D.J., and Moussy, F. In vivo evaluation of a dexamethasone/PLGA microsphere system designed to suppress the inflammatory tissue response to implantable medical devices. *J Biomed Mater Res* **61**, 180, 2002.
51. Rubin, J.P., Rubin, J.P., Rubin, J.P., DeFail, A., DeFail, A., Rajendran, N., *et al.* Encapsulation of adipogenic factors to promote differentiation of adipose-derived stem cells. *J Drug Target* **17**, 207, 2009.
52. Kelmendi-Doko, A., Marra, K.G., Vidic, N., Tan, H., and Rubin, J.P. Adipogenic factor-loaded microspheres increase retention of transplanted adipose tissue. *Tissue Eng Part A* **20**, 2283, 2014.
53. Defail, A.J., Edington, H.D., Edington, H.D., Matthews, S., Matthews, S., Lee, W.C., *et al.* Controlled release of bioactive doxorubicin from microspheres embedded within gelatin scaffolds. *J Biomed Mater Res A* **79**, 954, 2006.
54. Higaki, M., Ishihara, T., Izumo, N., Takatsu, M., and Mizushima, Y. Treatment of experimental arthritis with poly(D, L-lactic/glycolic acid) nanoparticles encapsulating betamethasone sodium phosphate. *Ann Rheum Dis* **64**, 1132, 2005.
55. Florine, E.M., Miller, R.E., Porter, R.M., Evans, C.H., Kurz, B., and Grodzinsky, A.J. Effects of dexamethasone on mesenchymal stromal cell chondrogenesis and aggrecanase activity: comparison of agarose and self-assembling peptide scaffolds. *Cartilage* **4**, 63, 2013.
56. Dvorak, L.D., Cook, J.L., Kreeger, J.M., Kuroki, K., and Tomlinson, J.L. Effects of carprofen and dexamethasone on canine chondrocytes in a three-dimensional culture model of osteoarthritis. *Am J Vet Res* **63**, 1363, 2002.
57. Kuroki, K., Stoker, A.M., and Cook, J.L. Effects of proinflammatory cytokines on canine articular chondrocytes in a three-dimensional culture. *Am J Vet Res* **66**, 1187, 2005.
58. Neustadt, D.H. Intra-articular injections for osteoarthritis of the knee. *Cleve Clin J Med* **73**, 897, 2006.
59. Backes, J.R., Bentley, J.C., Politi, J.R., and Chambers, B.T. Dexamethasone reduces length of hospitalization and improves postoperative pain and nausea after total joint arthroplasty: a prospective, randomized controlled trial. *J Arthroplasty* **28(8 Suppl)**, 11, 2013.
60. Ikeuchi, M., Kamimoto, Y., Izumi, M., Fukunaga, K., Aso, K., Sugimura, N., *et al.* Effects of dexamethasone on local infiltration analgesia in total knee arthroplasty: a randomized controlled trial. *Knee Surg Sports Traumatol Arthrosc* **22**, 1638, 2013.
61. Miot, S., Brehm, W., Dickinson, S., Sims, T., Wixmerten, A., Longinotti, C., *et al.* Influence of in vitro maturation of engineered cartilage on the outcome of osteochondral repair in a goat model. *Eur Cells Mater* **23**, 222, 2012.
62. Bian, L., Ng, K.W., Mauck, R.L., Byers, B.A., Tuan, R.S., Ateshian, G.A., *et al.* The beneficial effect of delayed compressive loading on tissue-engineered cartilage constructs

- cultured with TGF- β 3. Osteoarthritis Cartilage **15**, 1025, 2007.
63. Wang, C., Hung, C.T., and Mow, V. Analysis of the effects of depth-dependent aggregate modulus on articular cartilage stress-relaxation behavior in compression. *J Biomech* **34**, 10, 2001.
 64. Chahine, N.O., Chahine, N.O., Wang, C.C., Hung, C.T., *et al.* Anisotropic strain-dependent material properties of bovine articular cartilage in the transitional range from tension to compression. *J Biomech* **37**, 1251, 2004.
 65. Farndale, R.W., Buttle, D.J., and Barrett, A.J. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim Biophys Acta* **883**, 173, 1986.
 66. Stegemann, H., and Stadler, K. Determination of hydroxyproline. *Clin Chim Acta* **18**, 267, 1967.
 67. Lamiabie, P.D., Vistelle, R., Millart, H., Sulmont, P.V., Fay, R., Caron, J., *et al.* High-performance liquid chromatographic determination of dexamethasone in human plasma. *J Chromatogr* **378**, 486, 1986.
 68. Kelly, T.A., Ng, K.W., Wang, C.C., Ateshian, G.A., and Hung, C.T. Spatial and temporal development of chondrocyte-seeded agarose constructs in free-swelling and dynamically loaded cultures. *J Biomech* **39**, 1489, 2006.
 69. Kelly, T.-A.N., Roach, B.L., Weidner, Z.D., Mackenzie-Smith, C.R., O'Connell, G.D., Lima, E.G., *et al.* Tissue-engineered articular cartilage exhibits tension-compression nonlinearity reminiscent of the native cartilage. *J Biomech* **46**, 1784, 2013.
 70. Shinmei, M., Masuda, K., Kikuchi, T., Shimomura, Y., and Okada, Y. Production of cytokines by chondrocytes and its role in proteoglycan degradation. *J Rheumatol Suppl* **27**, 89, 1991.
 71. Shinmei, M., Masuda, K., Kikuchi, T., and Shimomura, Y. The role of cytokines in chondrocyte mediated cartilage degradation. *J Rheumatol Suppl* **18**, 32, 1989.
 72. Kimmerling, K.A., Furman, B.D., Furman, B.D., Mangiapani, D.S., Moverman, M.A., Sinclair, S.M., *et al.* Sustained intra-articular delivery of IL-1RA from a thermally-responsive elastin-like polypeptide as a therapy for post-traumatic arthritis. *Eur Cells Mater* **29**, 124, 2015.
 73. Olson, S.A., Furman, B.D., Kraus, V.B., Huebner, J.L., and Guilak, F. Therapeutic opportunities to prevent post-traumatic arthritis: lessons from the natural history of arthritis after articular fracture. *J Orthop Res* **33**, 1266, 2015.
 74. Hafezi-Moghadam, A., Simoncini, T., Yang, Z., Limbourg, F.P., Plumier J.-C., Rebsamen, M.C., *et al.* Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. *Nat Med* **8**, 473, 2002.
 75. Cato, A.C.B., Nestl, A., and Mink, S. Rapid actions of steroid receptors in cellular signaling pathways. *Sci STKE* **2002**, re9, 2002.
 76. Hebbar, P.B., and Archer, T.K. Chromatin remodeling by nuclear receptors. *Chromosoma* **111**, 495, 2003.
 77. Nagaich, A.K., Rayasam, G.V., Martinez, E.D., Becker M, Qiu, Y., Johnson, T.A., *et al.* Subnuclear trafficking and gene targeting by steroid receptors. *Ann N Y Acad Sci* **1024**, 213, 2004.
 78. McKay, L.I., and Cidlowski, J.A. Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev* **20**, 435, 1999.
 79. De Bosscher, K., Vanden Berghe, W., and Haegeman, G. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev* **24**, 488, 2003.
 80. Mackay, A.M., Beck, S.C., Murphy, J.M., Barry, F.P., Chichester, C.O., and Pittenger, M.F. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng* **4**, 415, 1998.
 81. Sekiya, I., Vuorio, J.T., Larson, B.L., and Prockop, D.J. In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. *Proc Natl Acad Sci U S A* **99**, 4397, 2002.
 82. Wang, Y., Kim, U.-J., Blasioli, D.J., Kim, H.-J., and Kaplan, D.L. In vitro cartilage tissue engineering with 3D porous aqueous-derived silk scaffolds and mesenchymal stem cells. *Biomaterials* **26**, 7082, 2005.
 83. Kiviranta, P., Lammintausta, E., Toyra, J., Kiviranta, I., and Jurvelin, J.S. Indentation diagnostics of cartilage degeneration. *Osteoarthritis Cartilage* **16**, 796, 2008.
 84. Kelly, T.A., Kelly, T.A., Ng, K.W., Ateshian, G.A., Hung, C.T. Analysis of radial variations in material properties and matrix composition of chondrocyte-seeded agarose hydrogel constructs. *Osteoarthritis Cartilage* **17**, 73, 2009.
 85. Nims, R.J., Cigan, A.D., Albro, M.B., Vunjak-Novakovic, G., Hung, C.T., and Ateshian, G.A. Matrix production in large engineered cartilage constructs is enhanced by nutrient channels and excess media supply. *Tissue Eng Part C Methods* **21**, 747, 2015.
 86. Cigan, A.D., Nims, R.J., Albro, M.B., Esau, J.D., Dreyer, M.P., Vunjak-Novakovic, G., *et al.* Insulin, ascorbate, and glucose have a much greater influence than transferrin and selenous acid on the in vitro growth of engineered cartilage in chondrogenic media. *Tissue Eng Part A* **19**, 1941, 2013.
 87. Nims, R.J., Cigan, A.D., Albro, M.B., Hung, C.T., and Ateshian, G.A. Synthesis rates and binding kinetics of matrix products in engineered cartilage constructs using chondrocyte-seeded agarose gels. *J Biomech* **47**, 2165, 2014.
 88. Albro, M.B., Nims, R.J., Durney, K.M., Cigan, A.D., Shim, J.J., Vunjak-Novakovic, G., *et al.* Heterogeneous engineered cartilage growth results from gradients of media-supplemented active TGF- β and is ameliorated by the alternative supplementation of latent TGF- β . *Biomaterials* **77**, 173, 2016.
 89. Hainque, B., Dominice, J., Jaffray, P., Ronot, X., and Adolphe, M. Effects of dexamethasone on the growth of cultured rabbit articular chondrocytes—relation with the nuclear glucocorticoid-receptor complex. *Ann Rheum Dis* **46**, 146, 1987.
 90. Olson, S.A., and Guilak, F. (eds.). (2015). *Post-Traumatic Arthritis* (1st ed., pp. 29–40). Boston, MA: Springer US. <http://doi.org/10.1007/978-1-4899-7606-2>
 91. Bastiaansen-Jenniskens, Y.M., Koevoet, W., de Bart, A.C.W., van der Linden, J.C., Zuurmond, A.-M., Weinans, H., *et al.* Contribution of collagen network features to functional properties of engineered cartilage. *Osteoarthritis Cartilage* **16**, 359, 2008.
 92. Song, R.H., Tortorella, M.D., Malfait, A.M., Alston, J.T., Yang, Z., Amer, E.C., *et al.* Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. *Arthritis Rheum* **56**, 575, 2007.
 93. Francioli, S., Cavallo, C., Grigolo, B., Martin, I., and Barbero, A. Engineered cartilage maturation regulates

- cytokine production and interleukin-1 β response. *Clin Orthop Relat Res* **469**, 2773, 2011.
94. McNulty, A.L., McNulty, A.L., Rothfus, N.E., Rothfus, N.E., Leddy, H.A., Leddy, H.A., *et al.* Synovial fluid concentrations and relative potency of interleukin-1 alpha and beta in cartilage and meniscus degradation. *J Orthop Res* **31**, 1039, 2013.
 95. O'Connell, G.D., Tan, A.R., Cui, V., Bulinski, J.C., Cook, J.L., Attur, M., *et al.* Human chondrocyte migration behaviour to guide the development of engineered cartilage. *J Tissue Eng Regen Med* 2015. [Epub ahead of print]; DOI: 10.1002/term.1988.
 96. Kumar, A., Bendele, A.M., Blanks, R.C., and Bodick, N. Sustained efficacy of a single intra-articular dose of FX006 in a rat model of repeated localized knee arthritis. *Osteoarthritis Cartilage* **23**, 151, 2015.
 97. Wilusz, R.E., Weinberg, J.B., Guilak, F., and McNulty, A.L. Inhibition of integrative repair of the meniscus following acute exposure to interleukin-1 in vitro. *J Orthop Res* **26**, 504, 2008.
 98. McNulty, A.L., Moutos, F.T., Weinberg, J.B., and Guilak, F. Enhanced integrative repair of the porcine meniscus in vitro by inhibition of interleukin-1 or tumor necrosis factor α . *Arthritis Rheum* **56**, 3033, 2007.
 99. Rhen, T., and Cidlowski, J.A. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N Engl J Med* **353**, 1711, 2005.
 100. Wojdasiewicz, P., Poniatowski, Ł.A., and Szukiewicz, D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm* **2014**, 561459, 2014.
 101. Kawai, T., and Akira, S. TLR signaling. *Cell Death Differ* **13**, 816, 2006.
 102. McKay, L.I., and Cidlowski, J.A. Cross-talk between nuclear factor-kappa B and the steroid hormone receptors: mechanisms of mutual antagonism. *Mol Endocrinol* **12**, 45, 1998.
 103. Joosten, L.A., Lubberts, E., Helsen, M.M., Saxne, T., Roo, C.J.C.-D., Heinegård, D., *et al.* Protection against cartilage and bone destruction by systemic interleukin-4 treatment in established murine type II collagen-induced arthritis. *Arthritis Res* **1**, 81, 1999.
 104. Cleaver, C.S. Interleukin 13 blocks the release of collagen from bovine nasal cartilage treated with proinflammatory cytokines. *Ann Rheum Dis* **60**, 150, 2001.
 105. Joosten, L., Lubberts, E., and Durez, P. Role of interleukin-4 and interleukin-10 in murine collagen-induced arthritis. Protective effect of interleukin-4 and interleukin-10 treatment on cartilage destruction. *Arthritis Rheum* **40**, 249, 1997.
 106. Goodman, L.S. Goodman and Gilman's the Pharmacological Basis of Therapeutics. New York: McGraw-Hill, 1996, pp. 1587–1612.
 107. Wernecke, C., Braun, H.J., and Dragoo, J.L. The effect of intra-articular corticosteroids on articular cartilage: a systematic review. *Orthop J Sports Med* **3**, 2325967115581163, 2015.
 108. Breuner, C.W., and Orchinik, M. Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J. Endocrinol* **175**, 99, 2002.
 109. Song, Y.W., Zhang, T., and Wang, W.B. Glucocorticoid could influence extracellular matrix synthesis through Sox9 via p38 MAPK pathway. *Rheumatol Int* **32**, 3669, 2012.
 110. Habib, G.S. Systemic effects of intra-articular corticosteroids. *Clin Rheumatol* **28**, 749, 2009.
 111. Shepherd, D.E., and Seedhom, B.B. Thickness of human articular cartilage in joints of the lower limb. *Ann Rheum Dis* **58**, 27, 1999.
 112. Parma, H., Gilmore, R.S., and Palfrey, A.J. The area of the articular surface of the distal end of the human femur. *J Anat* **157**, 233, 1988.
 113. Theodoropoulos, J.S., De Croos, J.N.A., Park, S.S., Pilliar, R., and Kandel, R.A. Integration of tissue-engineered cartilage with host cartilage: an in vitro model. *Clin Orthop Relat Res* **469**, 2785, 2011.
 114. Gilbert, S.J., Singhrao, S.K., Khan, I.M., Gonzalez, L.G., Thomson, B.M., Burdon, D., *et al.* Enhanced tissue integration during cartilage repair in vitro can be achieved by inhibiting chondrocyte death at the wound edge. *Tissue Eng Part A* **15**, 1739, 2009.
 115. Hunter, C.J., and Levenston, M.E. Maturation and integration of tissue-engineered cartilages within an in vitro defect repair model. *Tissue Eng* **10**, 736, 2004.

Address correspondence to:

Clark T. Hung, PhD

Department of Biomedical Engineering

Columbia University

1210 Amsterdam Avenue

351 Engineering Terrace

New York, NY 10027

E-mail: cth6@columbia.edu

Received: January 21, 2016

Accepted: February 23, 2016

Online Publication Date: March 30, 2016