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Tailoring hydrogel surface properties to modulate cellular response to shear loading ☆

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ABSTRACT

Biological tissues at articulating surfaces, such as articular cartilage, typically have remarkable low-friction properties that limit tissue shear during movement. However, these frictional properties change with trauma, aging, and disease, resulting in an altered mechanical state within the tissues. Yet, it remains unclear how these surface changes affect the behaviour of embedded cells when the tissue is mechanically loaded. Here, we developed a cytocompatible, bilayered hydrogel system that permits control of surface frictional properties without affecting other bulk physicochemical characteristics such as compressive modulus, mass swelling ratio, and water content. This hydrogel system was applied to investigate the effect of variations in surface friction on the biological response of human articular chondrocytes to shear loading. Shear strain in these hydrogels during dynamic shear loading was significantly higher in high-friction hydrogels than in low-friction hydrogels. Chondrogenesis was promoted following dynamic shear stimulation in chondrocyte-encapsulated low-friction hydrogel constructs, whereas matrix synthesis was impaired in high-friction constructs, which instead exhibited increased catabolism. Our findings demonstrate that the surface friction of tissue-engineered cartilage may act as a potent regulator of cellular homeostasis by governing the magnitude of shear deformation during mechanical loading, suggesting a similar relationship may also exist for native articular cartilage.

Statement of Significance

Excessive mechanical loading is believed to be a major risk factor inducing pathogenesis of articular cartilage and other load-bearing tissues. Yet, the mechanisms leading to increased transmission of mechanical stimuli to cells embedded in the tissue remain largely unexplored. Here, we demonstrate that the tribological properties of loadbearing tissues regulate cellular behaviour by governing the magnitude of mechanical deformation arising from physiological tissue function. Based on these findings, we propose that changes to articular surface friction as they occur with trauma, aging, or disease, may initiate tissue pathology by increasing the magnitude of mechanical stress on embedded cells beyond a physiological level.

1. Introduction

Hydrogels are highly-hydrated three-dimensional (3D) matrices formed of crosslinked polymeric networks which show promise in a variety of biomedical applications including medical devices, drug delivery, and as scaffolds for tissue engineering and regenerative medicine [1]. Hydrogels allow for cell encapsulation with high viability [2], can be readily tuned to mimic key features of

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the native extracellular matrix (ECM) [3,4], permit control of mechanical and physicochemical properties [5], and facilitate physiological cell differentiation and function [6]. Additionally, their surface friction can be tailored by manipulating the gel's chemical structure, hydrophilicity, crosslinking density, water content, elasticity, or charge density [7]. As such, they offer key advantages over traditional, two-dimensional cell culture methods which frequently fail to adequately mimic the extracellular microenvironment associated with physiological tissue and disease-associated processes [8]. As a result, the use of hydrogels as engineered microenvironments to study cellular behaviour in a more physiologically relevant manner has been increasing steadily [9]. Previous studies using hydrogels as ECM mimics emphasized the role of the structural and mechanical properties of the cellular microenvironment, in particular ECM stiffness, which has been shown to affect cell motility [10], stem cell differentiation [11–13], tumour progression [14], cellular reprogramming [15], and other biological phenomena. However, it remains largely unclear how these and other physicochemical ECM properties impact cellular behaviour when the tissue is loaded mechanically.

Most mammalian cell types including chondrocytes, the cells within cartilage, respond to mechanical stimuli through mechanotransduction – a process by which cells convert physical forces to biochemical signals [16]. While mechanical stress within a certain, physiological range induces anabolic processes and thus contributes to cartilage ECM maintenance and remodelling [17,18], excessive stress is considered to be a main driving factor for catabolic processes initiating joint pathology and degeneration in diseases such as osteoarthritis (OA) [19,20]. To prevent excessive tissue strains during joint ambulation, healthy synovial joints exhibit remarkably effective lubrication [21–25] leading to a coefficient of friction (COF) as low as 0.001–0.025 for cartilage-on-cartilage – a value lower than any other known bearing [26–29]. Given these extremely low COFs, shear strains resulting from normal joint articulation are minor and may have a rather protective function on cartilage homeostasis, inducing extracellular matrix production and turnover [30,31]. Acute injury, changes in biomechanics, or inflammatory events, however, result in a reduced lubricating function and increased frictional forces between articulating surfaces [32–35]. This may in turn be involved in the initiation of posttraumatic cartilage degeneration by increasing the magnitude of loading-induced tissue strain beyond a physiological level. Nevertheless, although earlier studies suggested an increase in shear strain following superficial cartilage damage and depleted lubrication [36–38], surprisingly little is known about the functional relationship between the frictional properties of native or tissue-engineered cartilage and chondrocyte behaviour upon mechanical stimulation.

In this work, we aimed to harness the adaptability of hydrogel materials to investigate the role of variations in surface friction on the biological response of articular chondrocytes to physiologically relevant mechanical stimulation. We, therefore, established and characterized a poly(ethylene glycol) diacrylate (PEG)-based hydrogel system that allows for tailoring of the COF to model the frictional properties of healthy, damaged, and osteoarthritic cartilage. Thin sheets of these hydrogels were then co-polymerized with chondrocyte-laden, photocrosslinkable alginate methacrylate (ALMA), which supports a chondrogenic cell phenotype [39], to form bilayered hydrogel constructs with tuneable surface friction. Constructs containing human articular chondrocytes were finally subjected to dynamic shear loading in a customized mechanical stimulation bioreactor to investigate the effects of variations in surface friction on the phenotypic expressions of encapsulated chondrocytes.

2. Materials and methods

2.1. Synthesis of alginate methacrylate (ALMA)

Medium viscosity alginate (MW ~260 kDa) was modified to include photocrosslinkable groups by reaction with methacrylic anhydride (MAAh) (both Sigma-Aldrich, St Louis, MO, USA). Alginate was dissolved in distilled water at 2% and reacted with a 10-fold molar excess of MAAh over total alginate hydroxyl groups for 24 h on ice and under constant stirring [40]. The pH was regularly adjusted to 8 using 5 M NaOH. After the reaction period, insoluble MAAh was removed by centrifugation, followed by dialysis against deionized water using a 12 kDa MWCO cellulose dialysis membrane (Sigma-Aldrich) for 5–7 days. The pH of the dialyzed polymer solutions was adjusted to 7.4, after which they were lyophilized and stored at −20 °C protected from light and moisture.

2.2. Chondrocyte isolation and expansion culture

Articular cartilage was obtained with institutional ethics approval from consenting patients undergoing total knee replacement surgeries for osteoarthritis (donors: two female + one male, age 62–80 years). Chondrocytes were isolated from areas of macroscopically normal full-thickness cartilage, as described elsewhere [41]. Cells were propagated on tissue culture plastic (3000 cells/cm^2) in low-D-glucose chondrocyte basal medium (Dulbecco's modified Eagle's medium (DMEM) with 2 mM GlutaMAX™, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.1 mM nonessential amino acids, 50 U/mL penicillin, 50 µg/mL streptomycin, 0.5 µg/mL amphotericin B (Fungizone®) (all Invitrogen, CA, USA), 0.4 mM L-proline and 0.1 mM L-ascorbic acid (both Sigma-Aldrich)) supplemented with 10% foetal bovine serum (FBS) (Hyclone, Logan, UT, USA).

Unless stated otherwise, all cells and cell/hydrogel constructs were maintained at 37 °C in a humidified 5% CO_2/95% air CO_2 incubator with the medium refreshed every 3–4 days.

2.3. Preparation of thin hydrogel sheets with controlled frictional properties

To tailor the frictional properties of poly(ethylene glycol) diacrylate-based hydrogels (PEG, average M_n = 700 g/mol), various concentrations of negatively charged 4-styrene sulfonic acid (SSA) (both Sigma-Aldrich) were added to the PEG precursor solution and covalently incorporated into the PEG network during photo-polymerization. In order to retain alike total molarities and comparable polymer network densities in all PEG-based hydrogels, the uncharged compound methyl methacrylate (MMA) was added to hydrogels with a lower SSA content (PEG-40S) (Table 1). Just like SSA, MMA allows photocrosslinking via its vinyl group, resulting in comparable crosslinking densities.

Briefly, 2% w/v ALMA or PEG/SSA with or without MMA were dissolved in phosphate-buffered saline (PBS) containing 0.2% w/v Irgacure 2959 (1-(4-(2-hydroxyethoxy)-phenyl)-2-hydroxy-2-methyl-1-propane-1-one, BASF, Ludwigshafen, Germany) and sterile filtered using a 0.2 µm syringe filter. Hydrogels were photocrosslinked between two parallel glass slides separated with a 300 µm spacer by exposure to 365 nm light at an intensity of ~2.5 mW/cm^2 in a CL-1000 crosslinker (UVP, Upland, CA, USA) for 30 min. The glass slides were separated after solidification, leaving a thin hydrogel layer behind on one of the slides. Hydrogels were washed briefly in sterile PBS to remove unreacted compounds.