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Decreased solute adsorption onto cracked surfaces of mechanically injured articular cartilage: Towards the design of cartilage-specific functional contrast agents

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ABSTRACT

Background: Currently available methods for contrast agent-based magnetic resonance imaging (MRI) and computed tomography (CT) of articular cartilage can only detect cartilage degradation after biochemical changes have occurred within the tissue volume. Differential adsorption of solutes to damaged and intact surfaces of cartilage may be used as a potential mechanism for detection of injuries before biochemical changes in the tissue volume occur.
Methods: Adsorption of four fluorescent macromolecules to surfaces of injured and sliced cartilage explants was studied. Solutes included native dextran, dextrans modified with aldehyde groups or a chondroitin sulfate (CS)-binding peptide and the peptide alone.
Results: Adsorption of solutes to fissures was significantly less than to intact surfaces of injured and sliced explants. Moreover, solute adsorption at intact surfaces of injured and sliced explants was less reversible than at surfaces of uninjured explants. Modification of dextrans with aldehyde or the peptide enhanced adsorption with the same level of differential adsorption to cracked and intact surfaces. However, aldehyde–dextran exhibited irreversible adsorption. Equilibration of explants in solutes did not decrease the viability of chondrocytes.
Conclusions and general significance: Studied solutes showed promising potential for detection of surface injuries based on differential interactions with cracked and intact surfaces. Additionally, altered adsorption properties at surfaces of damaged cartilage which visually look healthy can be used to detect micro-damage or biochemical changes in these regions. Studied solutes can be used in in vivo fluorescence imaging methods or conjugated with MRI or CT contrast agents to develop functional imaging agents.

1. Introduction

Articular cartilage is a specialized tissue which covers the sliding surfaces of articulating bones to provide a weight-bearing, smooth surface with low friction [1]. This tissue is highly resistant to compression and shear stresses [2]. However, cartilage exhibits limited self-repair capability due to its avascular nature, low cell density and limited chondrocyte proliferation [2,3]. In primary osteoarthritis, biochemical changes within the cartilage volume can occur over time without any prior condition or external event (like tissue injury) [4], eventually leading to structural changes such as surface fibrillation and fissures. On the other hand, surface damage to articular cartilage can lead to gradual biochemical changes over cartilage volume which ultimately lead to tissue breakdown, more severe cracks, and development of secondary osteoarthritis [5,6]. Development of sensitive and noninvasive imaging tools is therefore essential to detect cartilage injuries and surface irregularities immediately after the insult.

Conventional magnetic resonance imaging (MRI) and X-ray computed tomography (CT) [7–9] only provide anatomical information; hence, signs of cartilage degeneration can be detected when tissue has gone through severe structural and compositional changes [10]. As glycosaminoglycan (GAG) loss is an early hallmark of cartilage degradation [11], contrast enhanced imaging methods like delayed Gadolinium-Enhanced MRI of Cartilage (dGEMRIC) [12] and Contrast Enhanced Computed Tomography (CECT) [13] have been developed to detect cartilage degradation based on alterations in GAG content. In these methods, distribution of anionic [12] or cationic [14] contrast agents are assumed to represent the spatial distribution of negatively charged GAGs within the matrix. The limitation of these techniques is that they are able to detect cartilage degradation only after a significant change in GAG content. Recently, alterations in diffusion rates of contrast agents in cartilage have been suggested for assessment of cartilage

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integrity [15,16]. However, it was shown that solute diffusion is affected only in injured explants with biochemical or structural changes in tissue volume, but solute diffusion was not altered in sliced explants [17]. There is therefore a need for developing new imaging approaches which are able to detect cartilage surface injuries and irregularities well before structural or biochemical changes in the whole tissue volume occur.

We have recently reported that a wide range of fluorescent solutes adsorb onto surfaces of cartilage explants [18] and also that small fluorophores adsorb less significantly at surfaces of fissures compared to adjacent intact surfaces of mechanically injured explants [19]. These findings encourage the conjugation of CT, MRI or fluorescence agents to carrier molecules with differential adsorption onto damaged and intact surfaces of cartilage as a potential approach for development of new functional imaging agents based on solute–surface interactions (as opposed to solute interactions with extracellular matrix components within the cartilage volume in currently available contrast agent-based cartilage imaging methods) for early detection of surface injuries and abnormalities. It is also possible to enhance the contrast agent performance or make it more selective by chemical functionalization of the carrier molecule.

In the present study we therefore aimed to extend previous findings on adsorption of small fluorophores at surfaces of injured cartilage [19] to examine differential adsorption of fluorescently labeled native and modified dextrans to cracked and intact surfaces of mechanically injured cartilage. Dextrans are excellent choices for carrier molecules because they are natural, biodegradable and pharmacologically inert and have been widely used in other medical applications [20–27]. In addition, dextrans are available commercially in different molecular weights and have numerous hydroxyl groups that can be easily used for chemical modification or conjugation to imaging agents [22,23]. A relatively big dextran (70 kDa) was used in this study to examine carrier molecules which have limited penetration into the cartilage and interact mostly with its surfaces. It should be noted that limited contrast agent penetration into cartilage matrix can be beneficial in this application (imaging using surface interactions) since this reduces the interfering signals from within the cartilage volume, resulting in more sensitive detection of surface abnormalities. Dextran was modified by introducing either aldehyde groups or a chondroitin sulfate (CS)-binding peptide to its structure to study if the performance of dextran as a carrier molecule will be improved. It has been shown that aldehyde modification enhances molecular binding to cartilage surfaces [28]. On the other hand, chondroitin sulfate is the main GAG in cartilage matrix [29]. The CS-binding peptide alone was also studied. Findings from this study provide insights for potential novel carrier molecule designs for new contrast agents.

2. Methods

2.1. Solutes

Solutes included 70 kDa dextran (Dex), aldehyde–dextran (Dex–CHO) and a chondroitin sulfate (CS)-binding peptide (peptide). Additionally, dextran conjugated with the CS-binding peptide (Dex–peptide) was also included in some experiments. All solutes were labeled with fluorescein isothiocyanate (FITC). FITC was selected as fluorescent label because it has been shown that compared to positively charged fluorophores (tetramethylrhodamine isothiocyanate (TRITC) and carboxytetramethylrhodamine (TAMRA)), negatively charged FITC affects the adsorption of solutes onto cartilage surfaces less significantly [18]. In addition, our previous study [19] showed that TRITC and TAMRA do not exhibit superior sensitivity in distinguishing cracked from intact surfaces compared to FITC. 70 kDa dextran already conjugated with FITC was obtained from Sigma. It was used directly or oxidized by sodium periodate (Sigma) with sodium periodate/glucose unit molar ratio of 1 to obtain FITC conjugated Dex–CHO [30]. A CS-binding peptide (sequence: YKTNFRRYYRF, MW: 2115) conjugated to FITC was obtained from CanPeptide Inc. The amino acid sequence of the peptide was based on a previous study [31]. The same peptide without FITC label (MW: 1613) was obtained from CanPeptide Inc. and was conjugated to FITC labeled Dex–CHO by established protocols [20,32] at peptide/aldehyde molar ratio of 0.09.

2.2. Solute characterization

Absorbance spectra of Dex (0.22 mg/ml) and Dex–CHO (0.16 mg/ml) were obtained using a UV–visible spectrophotometer (Evolution 300, Thermo Scientific) to confirm the aldehyde modification of dextran. The degree of aldehyde modification was determined by hydroxylamine titration assay [33]. Raman spectra of Dex (0.03 mg/ml) and Dex–peptide (0.04 mg/ml) were obtained using a DXR Raman microscope (Thermo Scientific) with 532 nm excitation laser to confirm the presence of peptide in the structure of Dex–peptide.

2.3. Cartilage explants

Visually healthy osteochondral cores, 5 mm in diameter, were drilled from the distal femur of skeletally mature adult bovine knees and their cartilage was trimmed to the 3 mm diameter (Fig. 1A). Trimmed samples were then randomly assigned into three groups (injured, uninjured controls, and sliced positive controls) and incubated in chondrocyte culture medium (high-glucose DMEM; 0.1 mM nonessential amino acids; 10 mM HEPES; 10% fetal bovine serum; and 1% penicillin–streptomycin–amphotericin) (Life Technologies) for 1–2 days until experimental use. All the experiments were performed in a 37 °C, 5% CO_2 environment.

2.4. Mechanical injury and positive control slicing

Osteochondral cores were injured by single radially unconfined axial compression at 0.7 s^{-1} strain rate and 14 MPa peak stress as described previously [34]. For comparison, positive sliced controls were created by slicing the articular surface of uninjured explants to a depth of approximately 200 μm along the diameter using a vibrotome (VT1200, Leica Microsystems). Injured and sliced explants were used in experiments within 2 h of injury or slicing.

2.5. Equilibration and desorption baths

Solute adsorption to cartilage surfaces was examined after equilibration with the solute or after equilibration and subsequent desorption processes. Uninjured (control), injured or sliced osteochondral explants were equilibrated with culture medium containing Dex (0.28 mg/ml), Dex–CHO (0.28 mg/ml), Dex–peptide (0.18 mg/ml) or peptide (0.0085 mg/ml) for 20–22 h. For desorption experiments, explants were then removed from equilibration baths, rinsed with blank culture medium and transferred to blank culture medium baths for another 20–22 h. Osteochondral cores from at least three different joints were mixed and 6 explants were used randomly for each solute of each group (uninjured, injured or sliced) for adsorption or desorption experiments. In the case of Dex–peptide, only solute adsorption to surfaces of uninjured ($n = 4$) and injured explants ($n = 6$) after equilibration process was examined.

2.6. Long-term desorption

To examine the reversibility of adsorption and bulk uptake (absorption) of solutes, an independent experiment was performed in which uninjured explants were equilibrated with culture medium containing Dex–CHO (0.28 mg/ml), Dex–peptide (0.18 mg/ml) or peptide (0.0085 mg/ml) for 24 h. Explants were removed from equilibration baths for fluorescence microscopy or they were transferred

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