Feasibility of MHz High-Intensity Focused Ultrasound (HIFU) to Deliver Agents Locally into Articular Cartilage

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Disclosures:

Introduction: Osteoarthritis (OA) is a major burden worldwide. About 18% of women and 10% of men over 60 years of age suffer from OA [1]. There are disease-modifying drug therapies under development, but unfortunately they are all limited to systemic delivery or injections into the knee [2]. Whereas MHz high-intensity focused ultrasound (HIFU) has been used to treat cancerous pathologies by means of thermal ablation or drug delivery, ultrasound remains under-exploited as a means to deliver drugs for OA therapy.

We have previously demonstrated that low frequency (20 kHz) ultrasound can deliver particles locally into articular cartilage (AC) [3]. However, MHz ultrasound may be preferred, because (a) it generates greater radiation force and acoustic streaming than kHz ultrasound at the same power, which is important for material transport and (b) at higher frequencies there is a lower risk for cavitation-related damage.

In this study, we aimed 1. to demonstrate and quantify the capacity of MHz ultrasound for localized delivery into AC and 2. to assess potentially induced damage in MHz-ultrasound-treated AC.

Methods: Visually normal osteochondral samples (n = 10) from femoral condyles of young bovines were prepared within a week post mortem. Two cylindrical samples (n = 2) [4] were prepared for contrast agent delivery with ultrasound. Paired osteochondral strips, i.e. Samples 3(a,b) - 6(a,b) (n = 8, width = 7 mm), were prepared from femoral condyles for damage assessment (one pair from each joint, one joint from each animal). A custom-made HIFU setup (f = 1.138 MHz, Isptp = 320 W/cm², beam width = 5.9 mm, PRF = 285 Hz, duty cycle = 5.0%, mechanical index i.e. MI = 0.9) was used to deliver 1% w/v phosphotungstic acid (PTA, 2.88 kDa) into AC (base medium was 70% ethanol for Sample 1 and PBS for Sample 2). Samples 1 and 2 were immersed in the contrast agent and the ultrasound focus was at the AC top surface during 2.5 hours of sonication. Samples 3a - 6a, were sonicated in PBS with the same protocol as above, while control Samples 3b - 6b (adjacent tissue) were not exposed to ultrasound.

After sonication, Samples 1 and 2 were imaged with X-ray micro-tomography (XMT) (Nanotom 180 NF, Phoenix X-ray Systems / GE, Fairfield, CT) (75/80 kV and 190/75 μA, 1080/1200 projections, 38/17 μm voxel side length for Samples 1 or 2, respectively). The samples were kept in sealed containers with moistened cotton balls to avoid drying. X-ray attenuation was used to determine the PTA delivery volume and AC thickness (image thresholding). Samples 3(a,b) - 6(a,b) were histologically stained (Masson’s trichrome and Safranin-O) and imaged under light microscope to evaluate potential HIFU-induced damage to AC during sonication.

Results: After HIFU treatment increased PTA delivery (inferred as elevated X-ray attenuation) was observed at the site of the ultrasound focus in Sample 1 up to a maximum depth of 481 ± 76 μm (error estimated as 2 × voxel side length) (Fig. 1A). This depth corresponded to 44% of the cartilage maximum thickness (1100 ± 76 μm) and an average delivery speed of 284 ± 30 μm/h. Similarly, for Sample 2 the maximum delivery depth was 882 ± 34 μm (error estimated as 2 × voxel side length) (Fig. 1B-C). The depth was 51% of cartilage thickness (1720 ± 34 μm) and corresponded to an average delivery speed of 192 ± 14 μm/h. The AC volumes covered by PTA were 3.8 μl and 90 μl for Samples 1 and 2, respectively. The diameter of the PTA volumes were 1.1 mm (n = 2, mean ± S.D., two orthogonal measurements) and 13.9 ± 1.2 mm (n = 2) for Samples 1 and 2, respectively.

In histological analysis, no differences were visually observed between HIFU-treated AC samples (3a - 6a) and reference samples (3b - 6b) in superficial tissue fibrillation, Masson’s trichrome stain contrast and safranin-O-stain contrast (Fig. 2).

Discussion: The results suggest that MHz HIFU is able to deliver agents into AC. As radiation force, acoustic streaming, and cavitation can be present at MI = 0.9, these may explain the delivery mechanism. However, we did not detect HIFU-related damage to the AC surface, i.e. depletion of proteoglycans, depletion of collagen or damage to AC surface architecture. To our knowledge, there are no previously proposed non-destructive techniques for localized delivery of agents into AC. This approach could contribute towards the first localized drug transport technique for OA.

Based on this preliminary data, we conclude that MHz HIFU has potential for localized drug delivery in OA therapy.

Significance: This is the first study to show that high-intensity ultrasound can deliver agents locally into AC with no evident signs of histological damage to AC surface architecture, or collagen and proteoglycan contents.

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References:


Figure 2: Representative histologic sections for osteochondral samples exposed (left, treatment) or not exposed to MHz HIFU (right, control). We did not observe proteoglycan (safranin-O staining) or collagen (Masson’s trichrome staining) depletion or super fibrillation of in treatment samples as compared to control samples. This analysis did not detect HIFU-induced damage to articular cartilage.

AC = articular cartilage