A Study in the Stimulation of Chondrocytes seeded in 3D Matrices by Continuous Ultrasound

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Statement of Purpose: The cellular component of the articular cartilage, chondrocytes, have long been recognized as strain-sensitive cells, and this strain-induced biological response of chondrocytes has been exploited to facilitate chondrocyte culture in in vitro systems and examples include the application of hydrostatic pressure, dynamic compression, hydrodynamic shear and application of low-intensity pulsed ultrasound (US). While US has shown to impact cartilage function at the cellular level, there is still a need to better understand the effect of US stimulation of chondrocytes seeded and maintained in 3-D scaffolds, which are better representatives of chondrocytes in-vitro culture. The purpose of this research study is to: (1) evaluate the effect of ultrasound on chondrocytes seeded in 3-D scaffolds, and (2) develop a correlation between ultrasound signal and chondrocyte morphology, biosynthetic activity and cartilage-specific gene expression in ultrasound stimulated chondrocytes.
In the present study a continuous ultrasound wave for predetermined time intervals was employed, as opposed to pulsed-ultrasound used in previous studies, to stimulate chondrocytes seeded in 3-D scaffolds. Both the frequency of application as well the effect of the US signal intensity on the biosynthetic activity of chondrocytes was studied.

Methods: Chondrocytes seeded in 3-D scaffolds were subjected to stimulation by US as follows: 1.5-MHz for 161 seconds, 5.0-MHz for 51 secs and 8.5-MHz for 24 secs and the frequency of US signal application is indicated (1X,2X,4X,8X). Non-US stimulated scaffolds served as the control (C). Both control and US stimulated groups were maintained in culture for 10 days and at the conclusion of the culture period chondrocytes were assayed for the total DNA content, viability by the MTT assay, morphology by SEM analysis, cytoskeletal analysis via immunostaining and cartilage specific gene expression by RT-PCR.

Results: Chondrocytes stimulated by US (1.5, 5.0 and 8.5-MHz) had 1.2 to 1.4 times higher viabilities as determined by the MTT assay (data not included). When compared to control, no increase in hydroxyproline content was observed in cells stimulated with 1.5-MHz US signal, whereas cells stimulated by US at 5.0-MHz and 8.5-MHz had 1.2- times to 1.5 times higher hydroxyproline, respectively, when compared to controls. Dead cells, stained red, were found on most surfaces, however the appearance of dead cells were observed more on control scaffolds and scaffolds stimulated with the 8.5 MHz US signal. We have analyzed the expression of select cartilage specific genes at day-10 in human articular chondrocytes cultured on 3-D scaffolds subjected to US stimulation. At day-10, type-II collagen and aggrecan mRNA was significantly higher in US stimulated samples than in the control group, in addition expression was significantly higher in 5.0 and 8.5 MHz stimulated samples, respectively1. Thus US signal of 5.0 MHz was selected for all future experiments, where the effect of frequency of US stimulation on the mRNA expression levels of collagen type II, aggrecans, cell-surface specific integrins, Sox5/9 and other genes are shown in Figure 1.

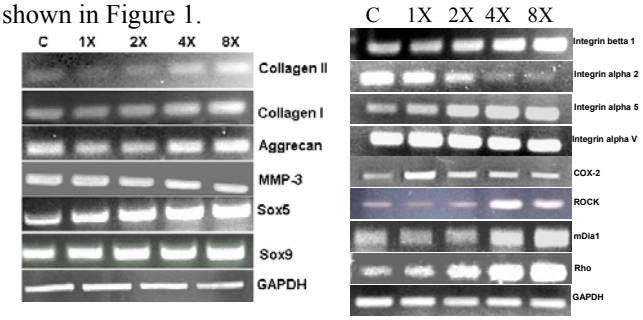

Figure 1. RT-PCR analysis

RT-PCR analysis revealed that US-stimulation treatment increased the cell surface expression of $\alpha 5$ and $\beta 1$ integrins. Other integrins such as $\alpha 2$ and $\beta 3$ are also regulated by US treatment, suggesting that many kinds of integrins are probably involved in the regulation of chondrocytic function in response to US stimuli. Interestingly, at the gene expression level, both Sox5 and Sox9 genes coordinately responded to changes in US stimulation, and generally mirrored the response of collagen type-II transcript to changes in US stimulation.

Conclusions: US stimulation at 5.0 MHz for 51 secs per application was shown to modulate the proliferative capacity, biosynthetic activity and integrin expression of human articular chondrocytes maintained in 3-D matrices. These results demonstrate that US stimulation increases gene expression of integrins and collagen-II but does not impact MMP-3, a key matrix degrading enzyme. Further, the US stimulation regimen adopted here does not result in any significant temperature increase. Thus, changes in chondrocyte metabolism leading to upregulation in the specific production of chondrocytic markers upon US stimulation are not triggered by US-induced temperature changes, but in fact, are specifically induced by the acoustic pressure component of US stimulation.