Sodium and T1-rho MR Analysis of Bioengineered Cartilage

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Many experimental approaches are taken when it comes to articular cartilage tissue engineering. There are likely more methods and approaches than in other tissue engineering endeavors however all are challenged by the same underlying dilemma of maintaining the cartilage phenotype while making mechanically sound replacement material. The ultimate goal of generating a suitable cartilage like biomaterial that bears all the necessary characteristics of natural cartilage and can ultimately integrate into damaged regions or restore defects. These in vitro methods include monolayer, pellet, and bioreactor cultures, and sometimes include culture in biodegradable scaffolds to more rapidly achieve a three dimensional cartilage-like mass. Maintaining the chondrocytes’ articular cartilage phenotype is of paramount concern in this research. In these studies a method utilizing a self-aggregating high-density suspension culture was used and differs with most others in that it does not use a scaffold or foreign matrix. Our self-aggregating suspension culture (SASC) model is one such example of cartilage tissue engineering where chondrocytes are grown in culture dishes that are coated with poly (2-hydroxyethyl methacrylate). We recently determined that if chondrocytes were grown in this way, at high densities, they coalesce and rapidly form a mass and their phenotype is maintained for long periods of time and that their “cartilage-likeness” is improved by the application of a mechanical load during culture. We have demonstrated the usefulness of this culture technique in several studies with different chondrocyte sources including human, equine, and porcine. In this study we used this SACS model and neonatal porcine chondrocytes to generate a “cartilage like tissue equivalent” (CLTE) and tested its biochemical and molecular properties using imaging.

Sodium MRI provides a nondestructive means to assay the fixed charge density (FCD) and hence the PG content of the engineering tissue samples. Sodium MRI as a quantitative method of computing FCD and PG content has been validated in ex vivo bovine cartilage by correlating FCD as measured by sodium MRI with an independent measurement of PG content by spectrophotometric assay. T1ρ MRI can be used as a secondary measure of biochemical content of the CLTE samples where the T1ρ parameter describes the spin-lattice relaxation in the rotating frame. The nature of the interaction of proteoglycan with the surrounding fluids in cartilage results in this approach being a sensitive and reliable approach to measure this constitutive cartilage macromolecule. T1ρ has been observed to linearly correlate with PG content in controlled degradation experiments as well as in cytokine-induced animal models of cartilage degeneration, and osteoarthritic human specimens.
Using imaging along with biomechanical and molecular we can more fully assess the characteristics of engineered cartilage-like biomass and in doing so determine the presence of necessary constitutive cartilage components. Since the goal of all cartilage tissue engineering studies is to produce a biomaterial that is closest to natural cartilage that will eventually be implanted in vivo cartilage defects, the need to assess the cartilage in these cases would require a non-invasive approach. Performing first in vitro experiments using these various approaches prepares the way and basis for using these imaging approaches as non-invasive measures of biomaterial that would be implanted in vivo. Together with Ravinder Reddy at the University of Pennsylvania’s MMRRCC we were able to use our bioengineered material and assess the proteoglycan content using sodium and T1\(_p\) methodologies.

Shown on the left is an study using an 8-week bioengineered cartilage grown using the SASC method. In this example the average sodium concentration ranged from 260 to 278 mM and the average T1\(_p\) relaxation times from 105-107 ms (data not shown). Both closely parallel the values for natural cartilage indicating similar proteoglycan content. Using Fourier transform Infrared (FT-IR) imaging (together with Nancy Pleshko) in other experiments we demonstrated that the biomass that is generated using the SASC model bear a sticking similarity to natural cartilage in components and in some organizational aspects.

This presentation will discuss various methods of tissue engineering cartilage and the imaging approaches that may be used to assess the extracellular matrix components of “neotissue” formed in using these approaches. A focus will be on our SASC model and the use of sodium and T1\(_p\) methodologies with some discussion of FT-IR. Exploring new imaging modalities as they are presented in tissue engineering applications sets the stage for the use of these non-invasive techniques in clinical settings.

References