GLYCOSAMINOGLYCAN PRODUCTION KINETICS IN CARTILAGE TISSUE ENGINEERING - EFFECT OF HYDRODYNAMIC LOADING IN THE CONCENTRIC CYLINDER BIOREACTOR

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INTRODUCTION :

Damaged articular cartilage demonstrates very low capacity for regeneration *in vivo*. Thus, loss of mechanical strength due to mechanical trauma or degenerative diseases like osteoarthritis render the tissue incapable of performing normal physiological functions of weight bearing. Cartilage tissue engineering will soon be an important therapeutic option for the treatment of injured or damaged cartilage [1]. Bioreactors are an important tool for tissue engineering as they allow for well-characterized uniform culture environment.

Precise characterization of structural features of constructs grown in bioreactors with well-defined and controllable environments will directly link tissue growth environment to cartilage architecture. Bioreactors provide us with culture environments in which construct growth conditions can be quantified and well characterized. Our lab focuses mainly on the design and development of bioreactors that define precise quantitative relationships between bioreactor environment and construct growth. A concentric cylinder bioreactor has been developed in our lab for the production of tissue engineered cartilage constructs. This bioreactor provides a homogenous environment for hydrodynamic loading and mass transfer for robust construct development [2].

The deposition of the extracellular matrix (ECM) components such as glycosaminoglycans (GAG) and collagen are enhanced in a bioreactor environment [2]. The properties of the tissue engineered construct that render them implantable are mainly the composition and ultra structure of the matrix components. Glycosaminoglycans such as aggrecan, decorin and cartilage oligomeric matrix protein (COMP) are important constituents of the ECM [3]. The quantity of GAG produced and its architecture in the construct determines the construct's mechanical strength and load bearing capability. Prior observations in the concentric bioreactor suggest that hydrodynamic loading rate and frequency are additional bioprocessing parameters that can be manipulated to affect cartilage development *in vitro* [2]. The goal of this study is to enhance the matrix GAG production kinetics by varying the hydrodynamic environment.

MATERIALS AND METHODS:

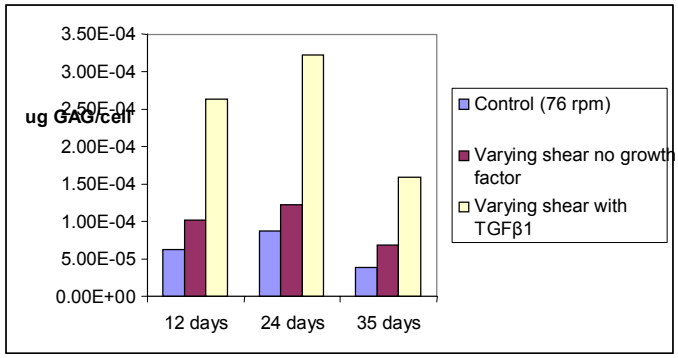
Chondrocytes are aseptically harvested from the femoral patellar grooves of calf knees less than two weeks old. PLLA scaffolds (1 cm diameter, 1.8 mm thick, porosity > 90%) are prepared by salt leaching with sodium chloride. These polymer scaffolds are attached to the inner bob of the concentric cylinder bioreactor. Chondrocyte suspensions (100 million cells per bioreactor) are added to the bioreactor and the scaffolds are seeded under dynamic conditions. Seeding under dynamic conditions ensures uniform seeding in the constructs [2]. To determine the effect of growth factors on chondrocyte proliferation and matrix production, the transforming growth factor β1 (TGF β1) was added at a concentration of 0.2ng/ml. The shear stress on the construct is varied by changing the cup rotation rate during the growth period to increase matrix GAG deposition. Three bioreactors were run for an experiment to compare and contrast the effect of programmed hydrodynamic loading and growth factor on construct GAG production. The programmed hydrodynamic loading was carried out as follows: 0 -12 days 76 rpm, 13-24 days 38 rpm, 25-35 days 19 rpm. The control (bioreactor 1) was run at 76 rpm throughout the growth period. Bioreactor 2 was run with the programmed loading while bioreactor 3 had TGF β1 in the aforementioned concentration with hydrodynamic loading. The bioreactors were run for 35 days.

The total GAG present in the construct was measured by the colorimetric reaction of the sulfated GAGs with dimethylmethylene blue (DMMB) using a spectrophotometer [4].

RESULTS:

At the end of the growth period, the GAG produced was higher in the bioreactors with varying shear as compared to the control (Fig1). Bioreactor 3 showed a higher GAG production by at least approximately 1.5 times when compared with Bioreactor 2 (Fig 1). As hypothesized, the specific GAG produced increased with the varied hydrodynamic loading with bioreactor 3 exhibiting highest specific GAG amongst all the three bioreactors. To understand the kinetics of GAG production, new GAG synthesized per day was evaluated. The bioreactors with hydrodynamic loading showed a prolonged increase in GAG production and increased kinetics of GAG production when compared with the control. Bioreactor 3 exhibited highest initial rates of GAG production and also the prolonged production rates indicating that the growth factor supplementation and the varied shear stress on the construct mutually aided in increased GAG production in the

construct. This was in agreement with a previous study where addition of TGF β1 increased GAG production in the construct.



1: Specific production of GAG

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DISCUSSION:

Studies of *in vitro* chondrogenesis demonstrate that constructs typically contain less GAG than native tissue primarily because GAG biosynthesis appears to rapidly decrease in culture. In our studies, a decrease is observed in GAG production during the later stages of growth. Therefore, an issue with cartilage tissue engineering is identification of nutrient and loading conditions leading to prolonged GAG production and deposition in the constructs by the chondrocytes. Sustaining the production of GAG is an important challenge in cartilage tissue engineering. The present results indicate that varying the construct hydrodynamic loading enhances chondrocyte specific production rates. Keeping the cell culture environment dynamic enhances the production of GAG production per cell. This agrees with previous results that a highly dynamic environment enhances matrix deposition.

Growth factors regulate cartilage development and ECM maintenance *in vivo*[5]. In cartilage, TGF β1 stimulates ECM production. Addition of this growth factor to the culture media is known to enhance matrix production in hydrogels [6]. Growth factor enhanced matrix GAG deposition in the bioreactor by a factor of at least 2 without greatly affecting the cell number. An additive effect of the growth factor and the varying shear is observed in the GAG production per cell. At this stage it is difficult to attribute the increased GAG production to an individual factor. One explanation for this observation is the possibility that the growth factor and the hydrodynamic loading mutually assist in the increased matrix production. This is in fact a scenario similar to that observed *in* vivo, where both physical and biochemical forces are acting in tandem. These results indicate that it takes more than a single biochemical/mechanical cue to enhance GAG production in the cartilage constructs *in vitro*.

The appropriate bioreactor environment should be one that enables incorporation of both mechanical and biochemical modifications uniformly to all constructs. Identification of factors regulating tissue morphogenesis and designing suitable bioreactors is important to develop constructs suitable for implantation.

REFERENCES :

1. Temenoff, J. S.; Mikos, A. G. "Review: Tissue Engineering for Regeneration of Articular Cartilage". *Biomaterials* **2000,** *21*, 431-440.
2. Saini S., and Wick T.M., 2002, "Concentric Cylinder Bioreactor for Production of Tissue Engineered Cartilage: Effect of Seeding Density and Hydrodynamic Loading on Construct Development "
3. Kuettner, K. E.; Aydelotte, M. B.; Thonar, E. J. "Articular Cartilage Matrix and Structure: a Minireview." *J. Rheumatol. Suppl* **1991,** *27*, 46-48.
4. Farndale, R. W.; Sayers, C. A.; Barrett, A. J. A Direct Spectrophotometric Microassay for Sulfated Glycosaminoglycans in Cartilage Cultures. Connect. Tissue Res. 1982, 9, 247-248.
5. Hill D., Logan A.,1992 "Peptide Growth Factors and their interactions during chondrogenesis", Prog. Growth Factor Res, 4: 45 -68
6. Elisseeff J., McIntosh w., Fu K., Blunk T., Langer R., 2001, "Controlled release of IGF-I and TGF-β1 in a photopolymerizing hydrogel for cartilage tissue engineering", J. Ortho. Res, 19 1098-1114