Imaging and Mechanical Properties of Explanted Tissue Engineered Heart Valves

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I. Introduction

The ideal replacement heart valve would conform to the patient's size, grow with the patient, not be rejected by the patient's immune system, and last for the duration of the patient's life. While no perfect solution yet exists, one method is to make tissue engineered heart valves (TEHV) using autologous cells that have been seeded onto a scaffold in vitro. Nonwoven scaffolds made of a 50:50 mixture of poly(glycolic acid) (PGA) and poly(L-lactic acid) (PLLA) are used for the valves; the resulting samples has functioned properly in growing lambs.

For the TEHVs to work in the clinical aspect, the material properties of the extracellular matrix (ECM) must be determined while in vitro and then in vivo. Additionally, the perfect TEHV would have no scaffold remaining when the tissue has stabilized after implantation. But before the scaffold is absorbed into the tissue, the mechanical properties of the TEHV depends at least in part on the scaffold's properties, including micromechanical interaction and spatio-temporal distributions with the ECM[1].

Another consideration with TEHVs is the collagen gradient after implantation. Mechanical conditioning of the seeded nonwoven 50:50 PGA/PLLA scaffolds by cyclic flexure has been shown to increase collagen concentration as well as the effective stiffness (E); the effects if more pronounced when the cyclic flexure is combined with laminar flow. These tests allowed for a strong linear relationship between E and the

[1] Engelmayer GC Jr, Rabkin E, Sutherland FW, Schoen FJ, Mayer JE Jr, Sacks MS (2005) The independent role of cyclic flexure in the early in vitro development of an engineered heart valve tissue. Biomaterials 26(2):175-187

collagen concentration, suggesting that E is strongly correlated to the collagen concentration. Thus, the E of the ECM ha been defined as the local collagen concentration multiplied by collagen specific stiffness.

A model has been developed to predict the E of the ECM within TEHV samples that have been incubated under static and cyclic flexure conditions. A model to predict the E of the ECM within TEHV samples that have been implanted, as well as the implanted tissue as a whole, needs to be determined.

II. Project

This project will further the work done previously by analyzing TEHVs that have been implanted into ovine specimens and explanted at different times, ranging from one day to six months. The summer research project will involve analyzing these samples to observe collagen movement, degradation of the scaffold, and mechanical properties of the tissue and ECM at different time stamps.

III. Objectives and Methodology

There are several objectives connected with this project. They include:

-Sectioning samples

This will allow for greater detail in the rest of the testing, as the samples will be sliced very thinly and the collagen levels, scaffold amounts, and mechanical properties can be tested at many different layers within the sample.

-Imaging samples

In order to produce a 3D image, every section will have to be put into a computer and then properly aligned. This will be done using various programs on the lab's computers.

-Mechanically testing samples

The entire tissue, as well as the different layers within the valve, need to be tested for mechanical properties such as elasticity and stiffness. Several fixtures in the laboratory allow for direct testing of these characteristics. One such fixture utilizes a high-resolution camera and custom computer program that tracks the amount of bending in a specimen with a given amount of force. Another fixture is able to provide stretching in a biaxial formation, thus more realistically reproducing the stress that would be on a valve when in vivo.

A polyacrylamide gel is used to determine the E of the ECM independently of the tissue. The gel was chosen because it has been shown to have similar stiffness to the ECM.

-Performing immunohistochemsitry (IHC) analyses on samples

IHC is used to determine what type of biomarkers and proteins are within a cell population. The abbreviated procedure for this is to fix a tissue, embed it in a preferred embedding medium, stain the tissue with the appropriate marker for the molecule one desires to identify, and look at the specimen to determine the concentration of the molecule.[2]

-Determining collagen gradients through the samples

A custom Matlab program that calculates the collagen concentrations already exists from previous studies. It works by evaluating grayscale images of cross section of the TEHV that have been stained with pico-sirius red and imaged under flourescence. The intensity within the images provides the program with enough

[2] "Immunohistochemistry Protocol." http://sharmalab.bsd.uchicago.edu/immuno.protocol.html Accessed 17 June, 2009

information to determine the collagen concentration through the entire valve sample. The program and pico-sirius red will be used again with the current samples to determine the collagen concentrations.

IV. Conclusions

The overarching goal of this study is to determine if the method of growing heart valves in vitro in a PGA/PLLA nonwoven scaffold will help the valves perform adequately when placed in vivo. By testing and imaging the samples that have been explanted from ovine specimens, we will be able to determine further if this method should be pursued further or if it should be revised in some way.

V. References

Unless other wise noted, introduction material comes from:

Engelmayr GC Jr, Sacks MS (2008) Prediction of extracellular matrix stiffness in engineered heart valve tissues based on nonwoven scaffolds. Biomech and Model in Mechanobiol (7)4:309-321

Unless other wise noted, information about procedures comes from:

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