Pittsburgh Tissue Engineering Initiative

Annual Progress Report: 2010 Formula Grant

Reporting Period

January 1, 2011 – June 30, 2011

Formula Grant Overview

The Pittsburgh Tissue Engineering Initiative, Inc. received $9,815 in formula funds for the grant award period January 1, 2011 through June 30, 2012. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Acrylic-Bisphosphonate Polymer Mediated Cell Binding to Collagen-Hydroxyapatite Scaffolds - Retention of osteoblasts or progenitor cells is an important characteristic for scaffolds designed to enhance bone healing. We have developed a poly-functional polymer that can be attached to cells and direct their binding to bone or bone substitute materials that contain hydroxyapatite (HA). The polymer has a succinimide functionality that allows covalent attachment to cell surface amine groups and pendant bisphosphonate groups that provide specific attachment to the hydroxyapatite component of bone tissue. In this project we propose to synthesize biomimetic scaffolding made up of electrospun collagen fibers containing hydroxyapatite nanoparticles. The scaffold will be tested for its ability to bind and retain cells and for its degradability under simulated in vivo conditions.

Anticipated Duration of Project

1/1/2011 - 6/30/2012

Project Overview

Scaffold materials are an important part of regenerative medicine. While numerous materials exist for bone repair, few are routinely seeded with cells to enhance remodeling. This project describes the synthesis and testing of a non-woven fabric material made from the components of bone. A fabric composed of micro fibers of collagen with inclusions of nanoparticulate hydroxyapatite will be produced by electrospinning. The ratios of the components used to electrospun the fabrics will be varied to achieve optimized material characteristics of strength, flexibility, porosity, and stability. The fabrics will be analyzed by scanning electron microscopy to determine the porosity of the material. The stability of the material will be measured by the degradability of the material *in vitro* under simulated physiological conditions. The materials should have a degradation profile compatible with bone remodeling activity.

The scaffold material will be tested for its ability to bind to a unique polymer that contains bisphosphonate side groups which bind tightly to hydroxyapatite. The polymer is designed as a bone targeting reagent and has the ability to bind to cells or proteins and bind them to bone materials. The polymer will be assayed for its ability to interact with the scaffold fabrics, and for its ability to bind cells to the fabric. A variant of the polymer which carries a fluorescein moiety will be used in the binding assays. Binding will be followed by fluorescence spectroscopy and by microscopic analysis. The density of cell attachment will be determined by first reacting the polymer with HL60 cells followed by reaction of the cells with the fabrics and counting the bound cells. Mouse osteoblasts cells will be obtained and will be tested for their ability to grow within the scaffolding and for their ability to convert the scaffolding to bone like structures.

Principal Investigator

Richard R. Koepsel, PhD
Research Professor
University of Pittsburgh
450 Technology Drive
Suite 300
Pittsburgh, PA 15217

Other Participating Researchers

Moncy Jose, PhD, Hironobu Murata, PhD, Jill Andersen – employed by University of Pittsburgh

Expected Research Outcomes and Benefits

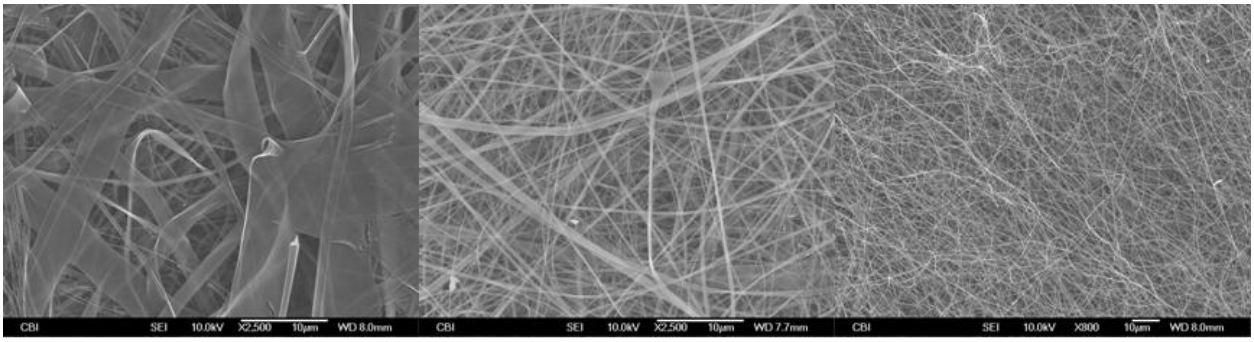
The expected outcome of this research project is the development of a scaffolding material that could be used in repair of bone defects. This material would be seeded with cells via a polymer mediated binding to the scaffolding. The seeded material could then be inserted into a damaged bone and enhance bone healing or accelerate remodeling.

Summary of Research Completed

The first phase of the project was the synthesis of non-woven microfiber fabric scaffolds for testing as binding targets for a novel bone targeting polymer currently under development in the laboratory. As with any electrospinning process the concentration of the fiber forming material is critical to the consistency of the final product. The goal for this project was to spin collagen fibers with diameters in the 0.5 to 2.0 micron range. Optimization of the collagen fibers revealed that a starting concentration of 4% collagen resulted in a preparation of fibers in the desired range (Figure 1). Analysis of the resulting electrospun materials was by scanning electron microscopy (SEM).

The next step in the process was the incorporation of hydroxyapatite crystals into the collagen fiber mat. This was achieved by adding various concentrations of nano-crystalline HA into the collagen solution. The goal was to incorporate enough HA into the fibers that the crystals would extend beyond the boundaries of the collagen fibers without disrupting the integrity of the fibers.

This would result in materials that had both the composition and structure of healing bone. It was found that the minimum concentration of HA that fit the criteria was 15% HA by weight in the 4% collagen solution. Figure 2 shows SEM pictures of a fiber mat that was electrospun from a 4% collagen solution that contained 15% HA. The nano-particulate HA particles agglomerated into clumps, many of which protrude from the surface of the fibers (Figure 2 inset). While many of the agglomerated HA clumps are larger than the diameter of the collagen fibers, few seem to be disrupting the fibers. Additionally few of the HA clumps appear to be at the ends of fibers suggesting that the clumps are in the middle of the fibers and are integral to the fiber structure.



Collagen -8% Collagen -5% Collagen 4%

Figure 1. Optimization of collagen concentrations by electrospinning

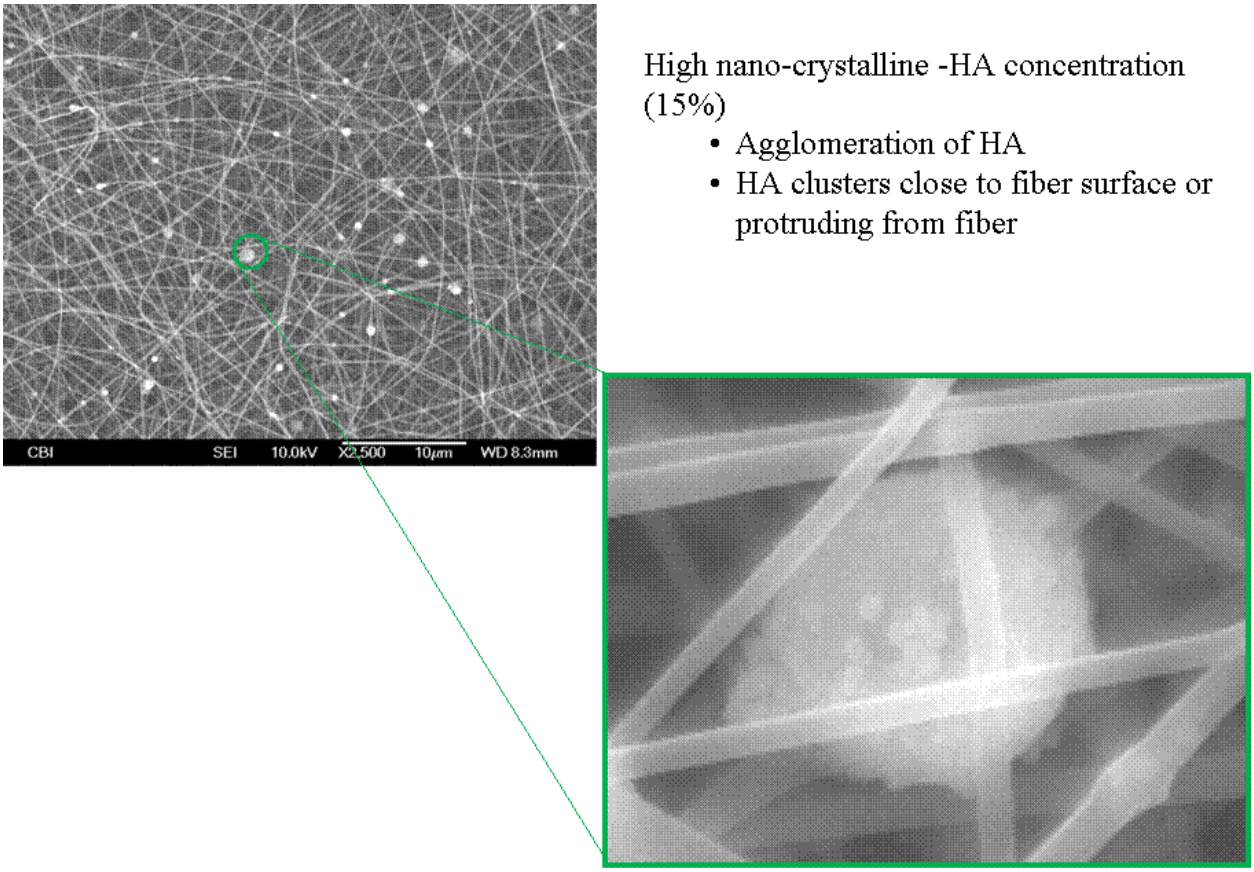


Figure 2. Electrospun collagen Hydroxyapatite fibers