

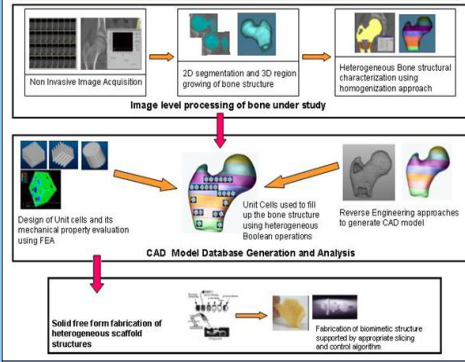
Abstract

Our objective was to study the culture of fibroblasts on PCL scaffolds. The motivation for this study is the potential for implantable scaffolds to aid in healing or regeneration of tissues, perhaps including bone. Tissue culture of cells alone does not promote the regeneration of formed tissues such as bone. We employ scaffolds because scaffolds provide 3-D shape and support for regenerating tissue. Tissues may have heterogeneous structures; therefore, it is desirable to incorporate this heterogeneity into tissue scaffolds. Tissue scaffolds must meet the requirements of proper anatomic shape, suitable mechanical properties, biocompatibility, and porosity and interconnectivity attributes which enable nutrient transfer and cell migration.

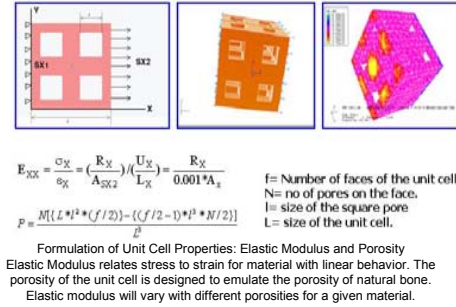
- CT-Scan data is processed by the software program MIMICS to yield a scheme of the mechanical properties of the areas of interest within a paired, intact bone.
- Unit cells with matching properties are positioned within the schematized representation of the CT-Scan data.
- An STL file is produced which represent the unit mechanical properties. This file is delivered to a free form fabricator, such as a PCL extruder. (Free-form fabrication allows the creation of complex scaffold geometries.)
- At the present time, research is being conducted on scaffolds made of PCL and scaffolds made of alginate.
- PCL scaffolds are employed in experiments in which fibroblasts are seeded on PCL scaffolds.
- The scaffolds with seeded cells are then incubated and later assayed for cell replication and viability.

Modeling and Design of Bone and Scaffolding

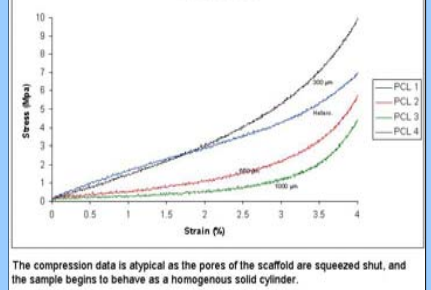
Overview of Generating a Biomimetic Bone Structure



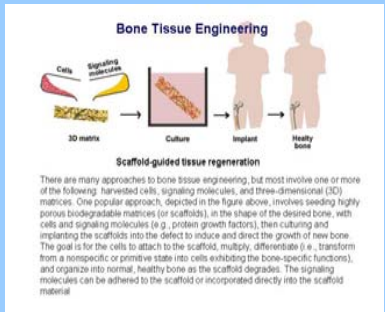
Mechanical Properties - Finite Element Analysis



Mechanical Properties of PCL Scaffolds from an Instron Mechanical Tester

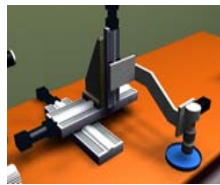


Background



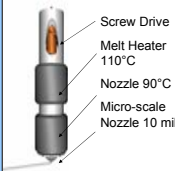
Scaffold Fabrication

PCL Extruder



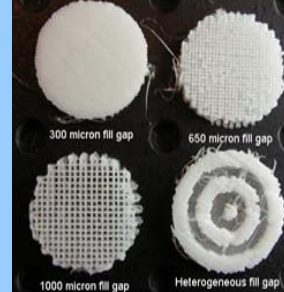
The movement of the PCL extruder is controlled by a computer using a tool path derived from an STL file. (The STL file is created, using MIMICS, from reconstructed CT data.)

Extruder Nozzle

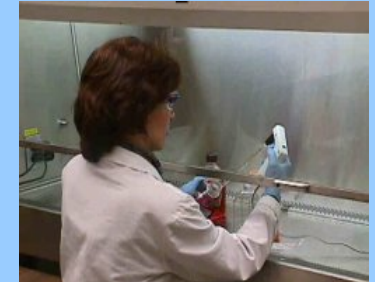


- Pellets of PCL are heated and extruded into a filament by a rotating screw.
- This extrusion head is moved by a 3D positioning system with 10 micron locational resolution.
- The molten PCL (melting temp 60 degrees C), solidifies rapidly, and is rigid prior to the laying down of subsequent layers.
- Extrusion into a coolant increases the rate of solidification allowing for smaller nozzles.

Different Fill Gaps



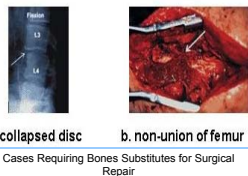
Using Sterile Technique in Tissue Lab



Working under sterile conditions at a laminar flow bench. The bench provides positive pressure which helps exclude airborne microorganisms. Everything brought into the hood must first be sprayed with alcohol and wiped down. Ultraviolet light bathes the interior when operator is not using bench.

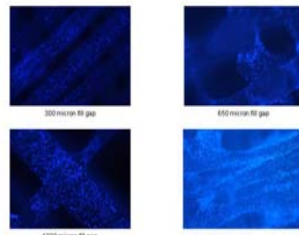
Motivation

Existing techniques for bone grafting are not adequate in many cases of nonunion of fractures, collapsed discs and other various traumas. Research into alternative methods and procedures are an effort to remedy this unmet need.



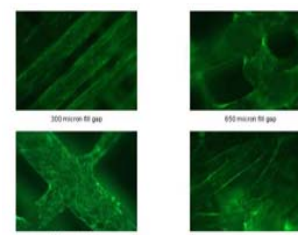
Cell Growth on Scaffolds

Nuclear Staining



Nuclear staining is accomplished by incubation with bis-benzamide and visualization is by fluorescence microscopy.

Membrane Staining



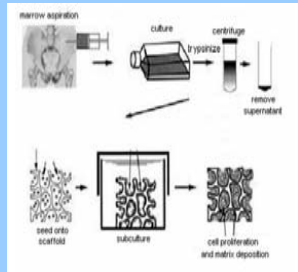
Membrane staining is achieved by incubation with ricin communis agglutinin 1 (a lectin stain) and visualization is by fluorescence microscopy.

Acknowledgements

Support from NSF 022770—Dr. Mary Poats (Program Manager)
 Dr. Wei Sun, MEM Dept., Drexel University
 Connie Gomez and Andrew Darling, CATE, Drexel University
 Joanne Ferroni, Drexel RETAIN Coordinator

Recent Investigation

These investigators used stromal marrow cells from patients to seed hydroxapatite blocks. They found that the bone-regenerating ability of the marrow cells did not vary with the age of the patients (16-76 years).



Yoshikawa, T., Ohgushi, H., Ichijima, K., and Takakura, Y., "Bone Regeneration by Grafting of Cultured Human Bone" Tissue Engineering, Vol. 10:688-698:2004