Instrumented Bioreactors

Objective

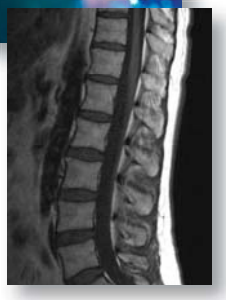
Our goal is to develop novel bioreactors that combine mechanical stimulation with integrated sensing to enable real-time optimization of the durability, permeability, and transport properties of engineered tissues during growth. Improved control of the processing environment will enable the synthesis of more robust and reproducible tissue engineered materials, promoting increased acceptance, certification, and commercialization of tissue engineered products.

Impact and Customers

- Tissue engineering will potentially benefit over 20 million Americans, especially those suffering from osteoarthritis. Over 700,000 cartilage repair surgeries are performed annually. Engineered tissue offers great promise for cartilage regeneration, providing a long-term solution for osteoarthritis sufferers.

- Currently, engineered tissues have insufficient mechanical durability for load-bearing applications, such as cartilage replacement. The development of robust tissue is critical for these applications.

- Over 250 companies worldwide are developing engineered tissues for skin, cartilage, and bone. Spin-off applications include as alternatives to animal testing for pharmaceuticals and consumer products. Engineered tissues are also being exploited for individual patient therapies (theragnostics) and for screening the effects of chemical and biological warfare agents.



Approach

We are applying the principles of intelligent manufacturing to the field of tissue engineering. We are embedding sensing techniques into a custom bioreactor platform to enable real-time monitoring of tissue integrity during growth. Our present design builds on earlier bioreactors constructed at NIST that provided biaxial mechanical stimulation, with optical microscopy used to periodically monitor cell growth. The next generation of bioreactors include the ability to monitor the quality of the tissue as it is growing by measuring biological and histological parameters on line.

We are collaborating with Kristi Anseth (University of Colorado), a leading tissue engineering researcher, to develop a new bioreactor for cell-seeded hydrogel scaffold constructs. Ultrasonic sensors have been incorporated to monitor extracellular matrix content, and electrochemical sensors have been developed to measure metabolic activity. Dr. Anseth plans to use this bioreactor to investigate the effects of polymer chemistry on scaffold durability, helping her advance hydrogel-based cartilage replacement.

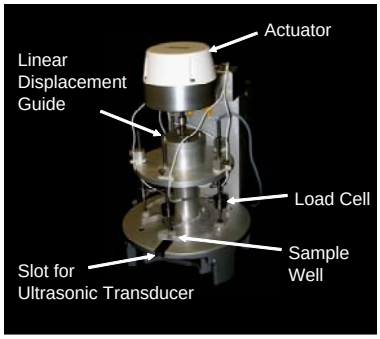
BIOMATERIALS

Accomplishments

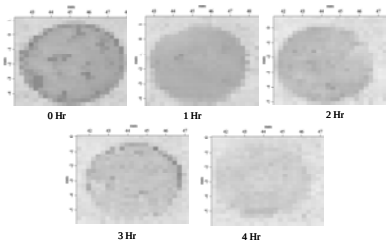
We have designed and constructed a new custom bioreactor providing compressive mechanical stimulation, real-time mechanical characterization of the scaffold, optical inspection, and integrated ultrasonic imaging for determining extracellular matrix (ECM) content.

Our bioreactor houses five cubic hydrogel specimens (5 millimeters on a side) in wells filled with nutritive solution. Platens contact the specimens from above and apply compressive mechanical loads. The linear actuator can apply controlled stress or strain to the specimens, depending on the desired stimulation. A specimen rotates into position over a 30 MHz ultrasonic transducer to perform the ECM measurement through the specimen thickness. Optical flats are machined on the sides of the wells to allow imaging with a video microscope.

To measure the mechanical properties of the engineered tissue, the platen compresses the specimen while video images are acquired. Image correlation software is then used to calculate the strain in the sample, while stress is calculated from the force measured by the load cell.



Instrumented bioreactor

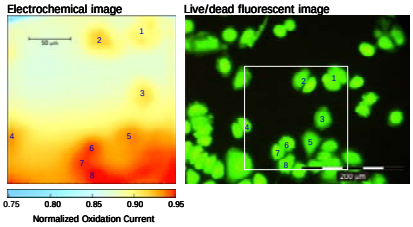


Scans of increasingly degraded cartilage

To calibrate the ultrasonic sensor, we prepared reference samples with varying concentrations of the components of the extracellular matrix. We extracted bovine cartilage specimens suitable for ultrasonic, mechanical, and chemical analysis, selectively degraded the cartilage extracellular matrix, and determined the degree of degradation and the relative percentage of cartilage components. The images above illustrate the correlation between the ultrasonic scan and the extent of collagen cross-linking.

Development of high-quality specimens is critical to demonstrate the functionality of our new bioreactor. Cell-seeded hydrogel scaffolds are our target demonstration material. To prepare specimens, we first synthesized and characterized a polymeric (PEG-CAP-DM and PEG-DM) scaffold and encapsulated adult human mesenchymal stem cells (hMSCs) in it to prove cell viability and differentiation. We demonstrated that hMSCs could live and begin to differentiate in these scaffold systems. In addition, the scaffolds could be degraded chemically to allow room for the cells to grow their own extracellular matrix.

An additional sensing technology is also being prepared for integration into the bioreactor. Scanning electrochemical microscopy (SECM) has been used to image metabolic activity of cells in culture. The instrument consists of a 5 micrometer Pt microelectrode, a Pt wire counter electrode, and a Ag/AgCl reference electrode. $FcCH_2OH$ was used as a redox mediator. We have shown that this technique can be an early indicator of cell apoptosis at the cellular level rather than at the whole culture level as with traditional assays. Future work will integrate SECM into the bioreactor design to provide an additional measure of cell viability.



SECM of adrenal cells

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Publications

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Quinn TP, Oreskovic TL, Landis FA, Washburn, NR, *Material model measurements and predictions for a random pore poly(epsilon-caprolactone) scaffold*, J Biomed Mat Res B 82(1): 205 (2007)

Waters KR, Applications of High Frequency Ultrasound to Diagnostic and Regenerative Medicine, Ultrasonic and Advanced Methods for Nondestructive Testing and Materials Characterization, Chen CH, Hackensack, NJ (2007)