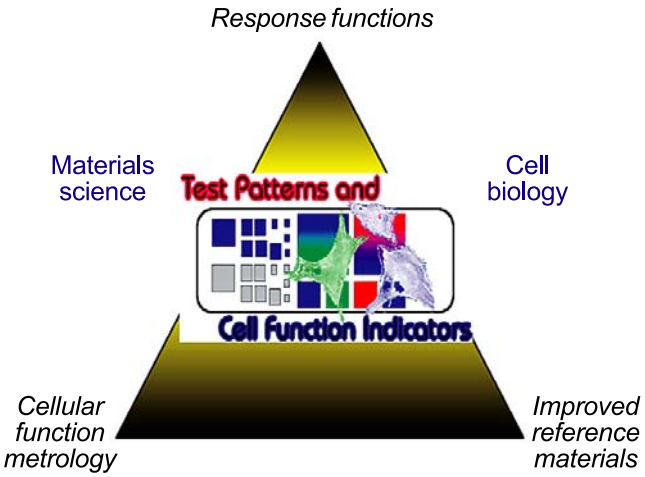
Biomaterials

New materials and devices are radically changing the treatment of injury and disease, yet it is clear that within this rapidly evolving segment of the materials industry, a basic measurement infrastructure does not exist. The Biomaterials Program develops measurement methods, standards, and fundamental scientific understanding at the interface between the materials and biological sciences. For the health care industry, we focus on dental and medical sectors that apply synthetic materials for replacement, restoration, and regeneration of damaged or diseased tissue. Three primary foci exist within this program: biocompatibility, biomaterials characterization, and materials measurements applied to biological systems.

Whether the medical issue involves implanting a hip- or knee-joint prosthesis, a synthetic bone graft, or a tissue engineering scaffold into the human body, one primary issue is biocompatibility. Using our expertise in materials science, we have developed suitable Reference Materials (RM) for investigating biocompatibility and implant suitability. Research has focused on measuring cellular response to powders and bulk materials that are candidates for implants; recently, we produced a realistic wear particle Standard Reference Material (SRM® 2880) for bioactivity testing.

Work on quantitative methods of biomaterials characterization includes assays for adhesion, viability, proliferation, and differentiation of bone cells, 3-dimensional structural/functional imaging of tissue in-growth, and biochemical assays to quantify inflammatory responses to synthetic materials. The focus of this effort is bridging the gap between fundamental knowledge and the product development needs in industry. For example, in collaboration with the Chemical Science and Technology Laboratory, we are developing measurement methodologies and reference materials to assess interactions in complex systems of living cells with synthetic materials. The expected outcome of this work includes reference substrates that induce specific cellular responses, and engineered DNA vectors to act as fluorescent reporters of cellular responses.

Another example of our effort to bridge this gap is our collaboration with the dental industry, which is primarily composed of small manufacturers with limited R&D capability. Collaborations with the American Dental Association Foundation (ADAF) develop improved materials and materials measurements techniques, patent and license these inventions, and, most importantly, provide a technical foundation. Research focuses on improved understanding of the synergistic interaction of the phases of polymer-based composites and the mechanisms of adhesion to dentin and enamel. This approach will ultimately lead to materials with improved durability, toughness, and adhesion to contiguous tooth structure. We also collaborate with the ADAF to develop metrology for the biocompatibility of synthetic bone grafts.



In this era of interdisciplinary research, we provide an added dimension. By taking a physical/mechanical approach to how cells function, respond, and remodel in interaction with synthetic materials, we provide skill sets typically absent in the biomedical community. Mechanical properties issues also arise when considering synthetic bone grafts and tissue engineering scaffolds. Complementing traditional bulk mechanical property measurements, combinatorial approaches are being developed to identify compositions and surface features that affect properties such as biocompatibility and mechanical durability.

Our mechanical property metrology extends further to biological systems that span the range from individual neurons and muscle cells to complete pulmonary arteries. This necessitates the development of unique mechanical testing platforms and application of a materials science approach to understanding integrated properties. Recently, we have developed a bioreactor capable of applying biaxial stresses and allowing monitoring of the stress and strain of a two-dimensional scaffold sheet during tissue growth.

Fundamental to the Biomaterials program is recognition of the need for an integrated systems approach. Collaborations among and between project teams are critical to progress against the ambitious goals of this program.

Table of Contents

Biomaterials

Ceramics

Materials Reliability

Metallurgy

Polymers

Controlled Size and Shape UHMWPE Particle SRM for Bioactivity Testing

The average life of an artificial human joint replacement is about ten to fifteen years. Failures of these joints have been traced to adverse human bioactivity towards wear particles generated inside the body from joint movements. The detailed mechanism of this bioreactivity is well understood, especially the size and shape influence. Conclusions from several industrial workshops held at NIST in the last several years have suggested a strong need for a realistic wear particle reference material for bioactivity testing. We have responded to this need by producing a controlled size and shape UHMWPE wear particle Standard Reference Material, SRM 2880, to be made available in September 2004 for bioactivity testing.

Stephen M. Hsu

To generate wear particles of controlled size and shape, random particle generators such as cryogenic grinding and wear testing cannot be used. Instead, we tested the idea of using surface texturing to provide controlled abrasion. As a preliminary test, we textured a stainless steel surface using abrasives to produce linear and cross-hatched grooves. Subsequent rubbing of ultrahigh molecular weight polyethylene (UHMWPE) pins against the surface produced promising results, but the particle size and shape distributions were too wide. We then used a semiconductor microfabrication technique to produce specific cutting edges of controlled dimensions using silicon covered with a thin film of chromium for wear resistance. This process, Figure 1, successfully generated suitable particles in distilled water.

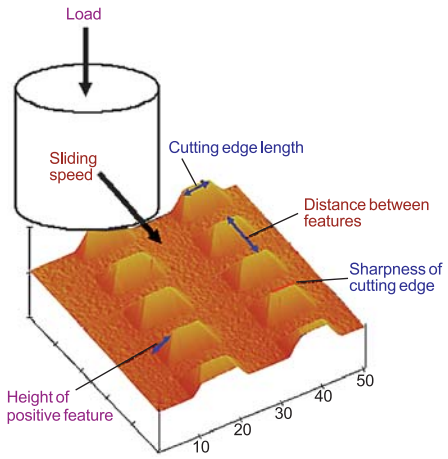


Figure 1: *Polyethylene pins rubbing on a textured surface to generate wear particles.*

Different surface texture patterns were designed to generate particles of various sizes and shapes. Prof. Paul Wooley of Wayne State University carried out air pouch bioactivity tests in mice and found that elongated particles were more highly reactive than small round particles. The results, published in a biomaterials journal, generated considerable interest in the scientific community.

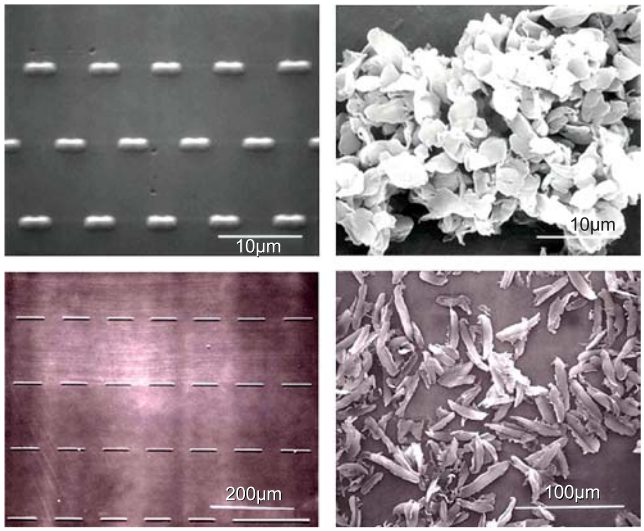


Figure 2: *Surface texture features and the resulting particles.*

Using this method, different size and shape particles were produced in large quantity under sterile conditions. Two different types of particles were produced: round particles and elongated particles with three different sizes. The particles were packaged in sealed amber ampoules under an inert atmosphere, to avoid oxidation, and were certified in terms of average diameter, aspect ratio, and the minimum number of particles per ampoule. To avoid potential cell culture influence, no dispersant was used in the SRM, but a dispersant that can be used to provide a more accurate particle count per ampoule was recommended.

A unit package of SRM 2880 consists of two sets of ampoules: one containing round particles and the other containing elongated particles. This SRM is scheduled to be available for distribution (**https://srmors.nist.gov/**) in September 2004.

Contributors and Collaborators

Y. Liang, P.P. Kavuri, H-W. Fang (Ceramics Division, NIST); J. Tesk (Polymers Division, NIST); D. Schroder (Biomet); C. Merrit (FDA); P. Wooley (Wayne State University); J. Sangers (U of Maryland)

Biomaterials Metrology: Pediatric Pulmonary Hypertension

Pulmonary hypertension is a potentially fatal complication of congenital heart defects in children who live at high altitudes. Discovering the pathophysiology and expression of the disease may open the way to finding new, more effective diagnostics and treatments. Our contribution to this goal is to provide data on the mechanical properties of the pulmonary artery and its constituents in healthy and diseased tissue.

Elizabeth S. Drexler

Background

Children born with heart defects (~ 1.0 %) have an increased risk of developing pulmonary hypertension at the high altitudes of the Rocky Mountain region. The mechanisms for developing this potentially fatal complication are not clearly understood. It is known that the heart becomes enlarged while trying to maintain a constant flow volume. A cyclic response whereby the heart increases pressure to maintain flow volume and the pulmonary arteries (PAs) remodel to compensate for the increased stress on the walls will ultimately result in morbidity or mortality if not treated.

Technical Strategy

The goal of the project is to determine the critical factors in the development of pulmonary hypertension in children so that we can prevent or mitigate the effects. The strategy is to characterize the fluid dynamics of the system, and identify how, where, why, and over what period the tissue of the artery remodels. The "how" will be answered through measurement of the mechanical properties; the "where" through the histology of the artery; the "why" through study of the biochemical signals that cause the tissue to remodel; and the time frame by using input from all the preceding. Our contribution will be to measure and compare the mechanical properties and histology of PAs using animal models. Our collaborators will perform the clinical and biochemical studies. Tests are conducted within 24 hours of excising the arteries, so that the cells are still viable and responsive. A bubble test is used to obtain stress–strain data, and an acoustic microscope is used to obtain speed of sound and attenuation from the PAs of control, monocrotaline-treated, and hypoxic rats. An additional population of hypoxic rats (knockouts) that are missing the endothelin B receptor (responsible for the expression of vasodilators) has been tested.

Accomplishments

Data from the PAs of 10 monocrotaline-treated rats were reduced and analyzed in FY04. Additionally, the PAs from 12 hypoxic knock-out rats, six hypoxic normal rats, and 12 control rats were tested and analyzed. Figure 1 shows the data from six rats each for the control and hypoxic knockout conditions. It is clear that the PAs from hypoxic rats demonstrated less strain at a given stress than did the controls. The PAs from the monocrotaline-treated rats did not display overtly different stress–strain behavior as compared with the controls.

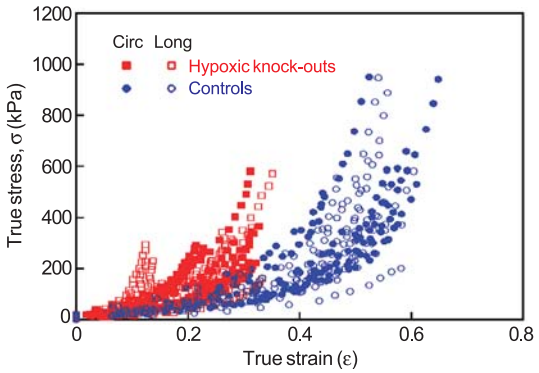


Figure 1: *Comparison of hypoxic and control data from the proximal arteries of rats.*

We believe that monocrotaline may not be an effective agent for inducing hypertension in the extrapulmonary arteries. The PAs from the hypoxic normal rats also exhibited stiffer behavior than did the controls, but not to the extent of the knock-out hypoxics. The histology indicates that the adventitial layer is thicker in the hypoxic PAs due to collagen deposition, but no obvious differences were observed between the PAs of the controls and the monocrotaline-treated rats. Fixturing has been developed and preliminary results from the acoustic microscope have been generated and are being analyzed. Studies continue to determine the differences in the remodeling of the arterial walls and how those disparities influence the mechanical properties. Analysis of the data in light of general material thickening is being pursued.

Contributors and Collaborators

Chris McCowan, Tim Quinn, Andy Slifka, Kendall Waters, Joyce Wright (Materials Reliability Division, MSEL, NIST), D. Vecchia, J. Splett (Statistical Engineering Division, ITL, NIST); R. Shandas (University of Colorado); K. Colvin, D. Ivy (University of Colorado Health Sciences Center and Children's Hospital of Denver)

Biomaterials Metrology: Mechanical Response of Tissue Engineering Constructs

Tissue engineering offers the hope that diseased or injured structures within the body can be replaced by tissues grown on scaffolds. To be effective, test methods for the mechanical properties of the polymeric scaffold with and without tissue ingrowth must be developed and the properties themselves measured.

Timothy P. Quinn and Tammy L. Oreskovic

Background

A proposed method for treating disease and injury in the human body is to replace the diseased tissue with tissue that is grown outside the body. Typically, healthy tissue is cultured to grow into a synthetic or natural scaffold. The scaffold supports the tissue and coaxes it to grow into the proper geometrical shape. The scaffold can be cultured with cells *in vitro*, or a bare (or initially seeded) scaffold can be used. In both cases, the mechanical properties of the scaffold or scaffold/tissue construct must be known in order to ensure that it does not fail mechanically after it is implanted. A typical approach would be for the implant designer to use a finite element model to predict the response of the implant to *in vivo* loads. The mechanical quality of the scaffolds must be assured before they are implanted as well.

Mechanical Models

In collaboration with the Polymers Division, an unseeded poly(e-caprolactone) (PCL) scaffold was mechanically tested in compression, and the resulting stress–strain curves were fitted with empirical models. The pore structure of the scaffold was varied by changing the randomly distributed pore size. The models are hyperbolic in form: $\sigma = E\frac{\varepsilon}{1+A\varepsilon}$. The hyperbolic model was compared to the secant modulus used for rigid cellular plastics as recommended by ASTM (Table 1).

Table 1. ***Summary of results of model parameters and pore size***

Pore Size Area (mm^2)	E (MPa)	A	Secant Modulus (MPa)
0.0014	89 ± 40	19 ± 10	30 ± 5*
0.0039	122 ± 60	21 ± 13	38 ± 6
0.0150	144 ± 58	31 ± 18	36 ± 5

* *Significantly different ($p < 0.05$) from the secant modulus of the other two pore size samples. No other parameters showed significance.*

Optical coherence microscopy (OCM) was used to scan the interior of the scaffold samples, and the pores were measured automatically with image analysis software. The OCM images were also used to form the basis of finite element (FE) models that were used to predict the initial elastic modulus of the scaffold (E). Each 4 μm pixel of the OCM image was represented with a brick element and assigned the Young's modulus of bulk PCL, and a value of 0 for a pore. A compressive strain was imposed on the model, and the resulting stresses were calculated. The elastic constants of scaffold were then calculated by use of Hooke's law for a linear-elastic, isotropic material.

The stress–strain curve of the porous PCL cannot easily be approximated with a line up to 10 % strain. However, the hyperbolic model has the drawback of having relatively large standard deviations in its fitted parameters *E* and *A* but is the best fit for the non-linear stress–strain curve. The secant modulus offers a much lower variability and could be used for quality control. The results from the FE model are within one standard deviation of the experimental values of *E* (the initial modulus).

Novel Bioreactors

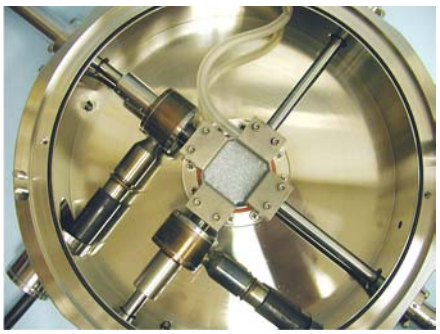


Figure 1: *The copper-colored load cells measure the applied force in this biaxial bioreactor. The sample is held in the center of the fixture and can be imaged from below to measure strain. The sample is bathed in nutrients (not shown), and the nutrient is also pumped across the surface of the scaffold through the grips.*

A bioreactor capable of applying biaxial stresses (Figure 1) has been developed. The applied stress and strain of the two-dimensional scaffold sheet can be monitored while the tissue is growing.

Contributors and Collaborators

J.D. McColskey, C.N. McCowan (Materials Reliability Division, NIST), F.A. Landis, N.R. Washburn (Polymers Division, NIST)

Biomaterials Metrology: Cellular Level Measurements

Techniques and tools that facilitate the exposure of single cells (and arrays) to control and quantify mechanical forces, and at the same time allow for the characterization of other biological phenomena, are needed for the study of cardiovascular tissues and cells. The development and evaluation of one of these tools, an optical tweezer, is a focus of this year's effort.

Christopher N. McCowan and Andrew J. Slifka

Overall, the challenge is to develop mechanical test platforms and tools that can be integrated with currently used biological techniques for the evaluation and measurement of cellular response (*e.g.*, gene expression, cell morphology, area of adhesion, etc.). These types of studies are needed because the development of vascular smooth muscle cells in cardiovascular tissue, for example, depends on the variations in the stress–strain environment that result from the expansion and contraction of the vessel wall. The importance of the environment becomes apparent when one considers that engineered tissues have mechanical properties inferior to those of naturally grown tissues. This is possibly a bulk effect, but is clearly related to processes at the cellular level. Without a quantitative understanding of the mechanics and functionality of the building blocks (cells and fibers), the bulk properties of the tissues cannot be fully understood and modeled.

The focus for this year was to build an optical trapping system, including a force/displacement measurement loop. This system will be used to calibrate MEMS devices designed for testing single cells, in addition to measuring cellular components. To facilitate this effort, we are collaborating with David Marr at CSM, who has two optical tweezers. A fluid-flow channel for the calibration of the tweezers has been built. This flow channel will allow us to calibrate the tweezers using a drag force, or Stokes method, and will be compared with methods of calibration based on Brownian motion.

We have calibrated Bio-MEMS devices using the atomic force microscope (AFM). Figure 1 shows one of the devices and a 180 µm long AFM cantilever used for force calibration. The central platform of such a device would hold a cell that would then be subjected to a controlled, oscillating mechanical environment. This device operates in a suitable fluidic environment that keeps the cell viable and allows for biological assay measurements.

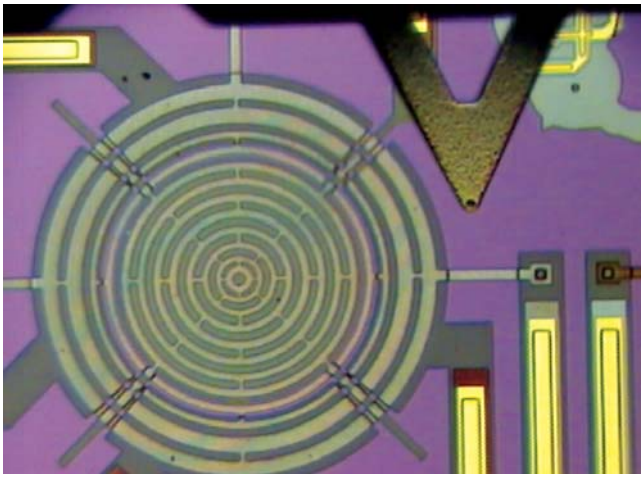


Figure 1: *A Bio-MEMS device used to apply controlled forces to cells.*

Our first application of the tools developed in this program will be to measure mechanical properties of leukocytes. This will be done in collaboration with Dr. Worthen of National Jewish Medical and Research Center, who has been studying the role of polymorphonuclear leukocytes, or neutrophils, in acute lung inflammation. The neutrophil is larger than the capillaries in the vascular bed of the lung. Therefore, details of the deformation of the neutrophil and capillaries are important in determining the mechanisms of pulmonary inflammatory response. We plan to develop tools such as optical tweezers and MEMS devices to apply and sense deforming forces on cells such as neutrophils.

Contributors and Collaborators

D.S. Finch, T. Oreskovic, D. Lauria (Materials Reliability Division, NIST); R. Rorrer (Mechanical Engineering, CU Denver); D. Marr, J. Oakey (Chemical Engineering, Colorado School of Mines); D. Serrell, H. Panchawagh (Mechanical Engineering, CU Boulder)

Biomaterials Metrology: Quantitative Ultrasonic Characterization

Quantitative ultrasonic characterization of biological materials is the application of physical acoustics techniques to biological and medical problems. The mechanical vibrations of ultrasound involve frequencies from below 1 MHz to over 100 MHz with corresponding length scales from millimeters down to tens of micrometers. Quantitative measurement of propagation and scattering properties enables evaluation of the health and quality of biological materials.

Kendall R. Waters

Background

The biological and medical research communities are often interested in the health or quality of a biological material. Measurement of the mechanical properties of the material can provide information complementary to that determined from biochemical measurements. Furthermore, longitudinal studies monitor materials over an extended length of time, often requiring measurements to be performed nondestructively. Ultrasonic measurement techniques permit nondestructive evaluation of the mechanical properties of biological materials.

Materials can be characterized by their ultrasonic propagation and scattering properties such as phase velocity and attenuation coefficient. In quantitative ultrasonic characterization of biological materials, changes in ultrasonic properties can be correlated to changes in structure and morphology, which then can offer insight into a disease process, for example.

Technical Strategy

We are interested in several classes of biological materials, including soft and hard tissues and engineered biomaterial constructs. Ultrasonic measurements of pulmonary arteries (PAs) from rat models are performed to understand the mechanical effects of pulmonary hypertension. A collaboration with Dr. B. Hoffmeister investigates how ultrasonic properties of cancellous bone depend on bone mineral density, a key factor in the risk of fracture in osteoporotic patients. Another collaboration, with Dr. K. Anseth, investigates the propagation and scattering properties of a tissue-engineered construct of bovine cartilage and hydrogel seeded with chondrocytes (cartilage cells).

For the majority of measurements, we use an acoustic microscope in double-transmission and backscatter modes to determine propagation and scattering properties. Thickness and speed of sound are determined by measuring changes in times-of-flight of ultrasonic signals through a reference path and a path substituted with the biological material. Attenuation is determined by spectral analysis of the same ultrasonic signals.

Accomplishments

Measurements of PAs from three different rat models (controls, hypoxics, and knock-out hypoxics) have been performed. See the Technical Highlight on Pediatric Pulmonary Hypertension for a summary of results.

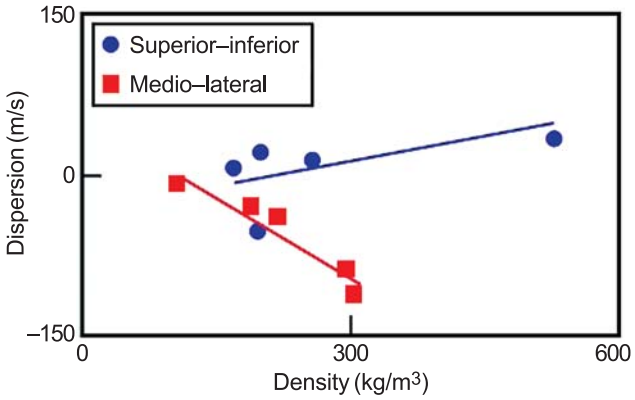


Figure 1: *Relation between dispersion and density for bovine cancellous bone in superior–inferior and medio–lateral directions.*

Phase-spectroscopic analysis of measurements in the medio–lateral (ML) and superior–inferior directions of ten cancellous bone specimens (bovine tibia) has been completed. Dispersion (frequency dependence of phase velocity) correlated well with density for ML specimens, as shown in Figure 1. Results indicate that dispersion may be a useful parameter for assessing fracture risk.

Preliminary investigations of the ultrasonic properties of a bovine cartilage-hydrogel construct have been performed. Results indicate that the reflection coefficient at the cartilage-hydrogel interface may provide a useful measure of integration with the native tissue.

Contributors and Collaborators

A. Slifka (Materials Reliability Division, NIST); K. Anseth (University of Colorado); B. Hoffmeister (Rhodes College of Memphis, TN)

USAXS and USAXS Imaging of Biomaterials

Modeling the behavior of complex materials requires detailed knowledge of the underlying three-dimensional microstructure. Ultra-small-angle X-ray scattering (USAXS) imaging is a new class of synchrotron X-ray imaging techniques that uses USAXS as the contrast mechanism. The technique provides imaging and statistical data that cannot be obtained using any other experimental methods. It has now been applied to characterize artificial tissue scaffolds and human cartilage in 3D.

Lyle E. Levine

USAXS imaging was developed by NIST researchers and was first demonstrated in May 2000. Advantages over existing X-ray imaging techniques are its inherently higher contrast and its USAXS-derived ability to provide quantitative data on object shapes and size distributions. USAXS imaging is applicable to a wide range of material systems including metals, ceramics, polymers, composites and biological materials. Over the past year, work has concentrated on two main areas. First, the final hardware components were installed, greatly improving the USAXS and USAXS imaging capabilities, and, second, a wide range of material systems, including biological ones, have been explored to determine where USAXS imaging can have the greatest impact.

The USAXS imaging experiments are conducted on the UNICAT sector 33 insertion-device beamline at the Argonne National Laboratory Advanced Photon Source (APS). A high-intensity, nearly-parallel, monochromatic X-ray beam passes through entrance slits that define the size, shape and position of the beam. The angular divergence in the X-ray beam is reduced by using multiple Bragg reflections on <111> Si crystals referred to as the collimator. Within the sample, local density variations from the microstructure produce X-ray scattering at small angles. The X-rays leaving the sample are then angle-filtered using a pair of <111> Si crystals referred to as the analyzer. The only X-rays from the sample that can pass through the analyzer are those scattered by the microstructure at a specific angle, where this angle can be selected by rotating the analyzer. Images are then formed by either exposing nuclear emulsion plates or using the NIST high-resolution X-ray camera system that was completed in FY 2003.

During FY 2004, the USAXS imaging instrument was declared fully operational after installation and testing of the last major hardware component, a new collimator system in which the surfaces of the two Si crystals were chemo-mechanically polished to minimize image aberrations. The new collimator was designed, built, and paid for by the APS and meets all of our specifications. Only minor problems were encountered during testing, and the required modifications are nearly completed.

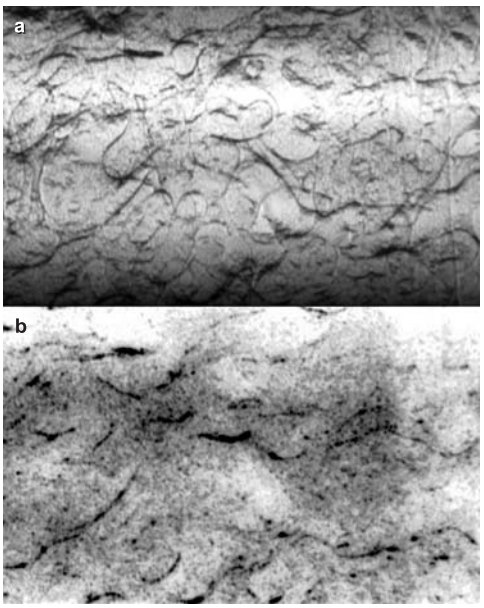


Figure 1: *Identical sample volumes of an artificial tissue scaffold imaged at scattering vectors of a) $q = 0$ Å^{-1} and b) $q = 0.0005$ Å^{-1}.*

Samples studied this year include artificial tissue scaffolds composed of porous poly-caprolactone (PCL), human cartilage, several metal alloys and a composite of carbon black in poly-methylmethacrylate. Figure 1 shows images of a PCL sample with 50 % porosity that was cultured with osteoblasts for 28 days. Figure 1a was taken with a 0º scattering angle (radiographic) and shows the overall structure of the scaffold. Figure 1b, from the same sample volume, was acquired at a scattering vector of q = 0.0005 Å^{-1} and shows the distribution of osteoblasts on the scaffold surfaces. Stereopairs were used to examine the cell positions in 3D. A full rotation sequence was acquired at q = 0 Å^{-1}, and a tomographic reconstruction produced a complete 3D description of the scaffold structure.

A detailed paper describing the USAXS imaging instrument along with the underlying theory was completed and accepted for publication.

Contributors and Collaborators

J. Dunkers (Polymers Division, NIST); J. Ilavsky, G. Long (Argonne National Laboratory); P. Jemian (UNICAT); C. Muehleman (Rush Medical College)

Structure-Property Relationships in Dental Nanocomposites

Polymeric dental materials are finding increasing applications in dentistry and allied biomedical fields. As part of a joint research effort supported by the National Institute of Dental and Craniofacial Research and also in collaboration with the American Dental Association Health Foundation Paffenbarger Research Center, NIST is providing the dental industry with a fundamental knowledge base that will aid in the prediction of clinical performance of dental materials.

Joseph M. Antonucci and Elizabeth Wilder

Inorganic fillers of various types, sizes and shapes are commonly used to modify polymer properties. In resin-based dental composites, fillers are added to enhance the modulus, hardness and strength of the polymer phase while also reducing its coefficient of thermal expansion and volumetric contraction during polymerization. In order to achieve composites with optimal properties, it is also necessary to provide an effective, high-quality interfacial phase that will make the filler both interactive and reinforcing with respect to the polymeric matrix phase.

Recent developments in nanotechnology have spurred interest in nano-size fillers and additives for dental materials. The interactions that occur between the disparate polymer matrix and inorganic filler phases are increasingly important to elucidate for nano-fillers due to their high surface area. Surface treatments of the nano-sized fillers, designed to generate desirable interfacial phases upon polymerization, assume even greater importance in nanocomposites compared to conventionally sized materials.

In this project, we investigate two types of nanofillers: (1) a pure silica particulate surface-treated with blends of two silane agents, and (2) a well-characterized amorphous calcium phosphate (ACP) particulate modified by exposure to the organogelator dibenzylidene sorbitol (DBS). Before treatment, both of these fillers exist as complex aggregates that are difficult to incorporate into dental resins.

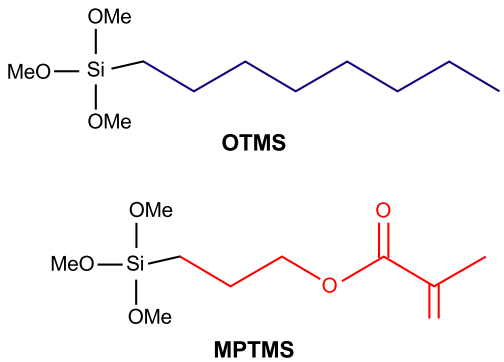


Figure 1: *Structure of (top) octyltrimethoxysilane(OTMS) and (bottom) methacryloxypropyltrimethoxysilane (MPTMS).*

The objective of our research with the nano-sized silica filler was to explore how the interfacial chemical structural variations arising from using a blend of two silane agents (Figure 1), one reactive (MPTMS) with the resin and the second non-reactive (OTMS), influence certain critical properties of the composites. It was found that processability, as measured by the facility of introducing the silica into the resins, was improved if the silane system contained OTMS. With equal parts by mass of MPTMS and OTMS, at least the same degree of reinforcement, measured by a biaxial flexure or 3-point flexural test, was achieved. The dual silane treatment also allows for the formulation of nanocomposites with higher filler contents.

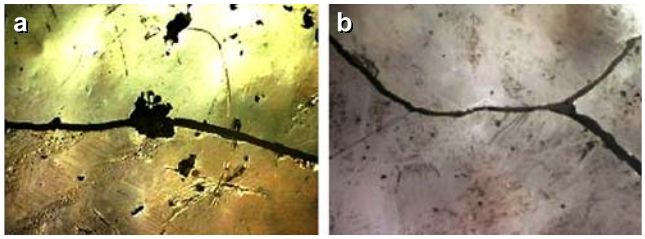


Figure 2: *Fractured ACP composite with: a) 0 % and b) 8 % mass fraction DBS.*

The second part of this project was aimed at determining the effect of DBS networks on shrinkage, stress development and mechanical properties of a bioactive composite utilizing ACP as the filler phase. DBS is capable of self-assembling into nano-scale fibrillar networks in a variety of dental monomers. The addition of DBS-reduced pore defects (Figure 2), increased biaxial flexure strength and reduced polymerization shrinkage and stress. These results suggest that dual silanization techniques and the use of organogelators may provide facile ways to enhance critical properties of polymeric dental composites and related materials.

Contributors and Collaborators

S. Lin–Gibson, N. Washburn, W. McDonough, K. Wilson (Polymers Division, NIST); D. Skrtic (American Dental Association Health Foundation, Paffenbarger Research Center)

Multi-Modal Imaging and Quantitative Data Reduction Methods for Regenerative Medicine

This project develops methods for determining the viability of tissue engineered medical products (TEMPs) through the use of in-vitro imaging coupled with data reduction techniques. These techniques are vital for distilling the voluminous amount of imaging data down to selected metrics of interest relating to TEMP viability. We illustrate this approach by using various imaging techniques, along with 3D image quantitation, to establish relationships between cell proliferation and scaffold microstructure.

Joy P. Dunkers and Forrest A. Landis

TEMP Imaging: Collinear Optical Coherence/ Confocal Fluorescence Microscopies

We use collinear optical coherence microscopy (OCM) in conjunction with one photon confocal fluorescence microscopy (CFM) as a multifunctional technique for characterization of TEMPs. OCM with its unparalleled combination of resolution (≈ 1 μm) and sensitivity (> 100 dB) is well-suited for imaging TEMPs. CFM has proven to be an extremely powerful technique for understanding cell viability, differentiation, and protein expression in tissue engineering and provides complementary information to the structural characterization provided by OCM.

Figure 1 shows OCM/CFM imaging data on a polymer scaffold. The OCM channel images changes in refractive index. In the image, the scaffold is shown in red and pores in black. The scaffold was seeded for 56 d with osteoblasts and subsequently the nuclei were stained. The presence of cells was detected using CFM (shown in yellow). From this image, it is clear that the cells are highly confluent along the surface of most of the scaffold including the pore walls but do not yet completely fill the pores. The image size is 500 μm × 500 μm × 100 μm.

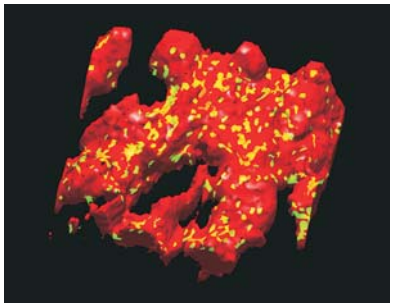


Figure 1: *OCM/CFM image of a polymer scaffold.*

Data Reduction Method: 3D Image Quantitation

We have developed methods to compute critical quantities from the imaging data, which can then be related to cell response. We have implemented algorithms that calculate pore volume, pore size distribution and structural anisotropy from 3D imaging data. Quantities such as pore connectivity and tortuosity are also expected to be influential, and algorithms are currently being developed.

Data Reduction Method: Immersive Visualization

Immersive visualization (IV) is a "virtual-reality" experience that literally puts you in the middle of your data. This is particularly advantageous when there are multiple types of volumetric data to be displayed simultaneously. IV allows one to see the entire data set at once and manipulate it in real time, which facilitates both qualitative and quantitative evaluation. Figure 2 shows a scientist exploring a TEMP imaging data set with a tool (arrow) in an immersive environment.

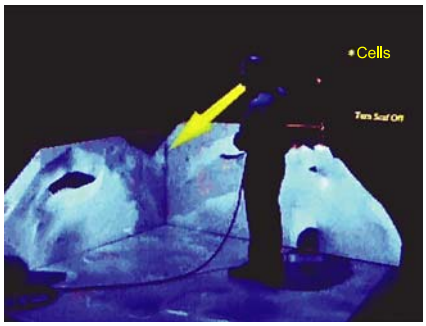


Figure 2: *A scientist exploring a TEMP imaging data set in an immersive environment.*

Impact

Our collinear OCM/CFM work has been highlighted by the biomedical community:

"Two Imaging Techniques Work Better Than One," *Biophotonics International*, January 2004.

"Microscopes Provide New View for Tissue Engineering," *Medical News Today*, December 10, 2003.

Contributors and Collaborators

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Tissue Engineering Scaffolds

Design issues relating to bioactive devices for regenerative medicine reflect the complexity of biological and materials issues, and their interactions. Regulatory issues also reflect this complexity. Quantifiable, reliable metrics that are relevant to success of tissue engineering (TE) constructs in properly supporting cell and tissue growth are needed to reduce the impact of this complexity. We are helping to define and develop these metrics.

Marcus T. Cicerone

The current efforts on this project are divided into two main areas: porosity measurements and useful definitions of porosity and chondrocyte response to dynamic environments. We are preparing to embark on another area — transferring results of cell-surface compatibility studies in 2D to 3D scaffolds. In each of these areas, we are emphasizing the use of unique volumetric imaging capabilities in the Polymers Division. We also apply real-time PCR (polymerase chain reaction) and other more common methods, such as histology.

Chondrocyte Response

Links have been established between a chondrocyte's dynamic environment and composition of the extra-cellular matrix (ECM) it produces. We are developing measurement tools to help determine how the dynamics of the environment is sensed by the cell, and stimulus levels required for engineering cartilage with specific ECM compositions. This work is in collaboration with top researchers at the National Institutes of Health's (NIH) National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).

Our working hypothesis is that physical deformation of chondrocytes influences ECM composition in the cartilage produced by both an intracellular transduction mechanism and increased perfusion of nutrients. This has been tested by applying variable pulsitile fluid stresses to chondrocytes encapsulated in poly(ethylene glycol) dimethacrylate gels. Cellular responses and matrix production are characterized by histological techniques, optical coherence tomography (OCM), and real time PCR. We are developing spectroscopic imaging methods that will aid in non-invasive determination of ECM composition.

Output of this work will be presented at the "Musculoskeletal Biology & Bioengineering Gordon Research Conference" and the "Polymer Network Conference" (both 2004). We also have a manuscript in preparation for *Journal of Orthopedic Research*.

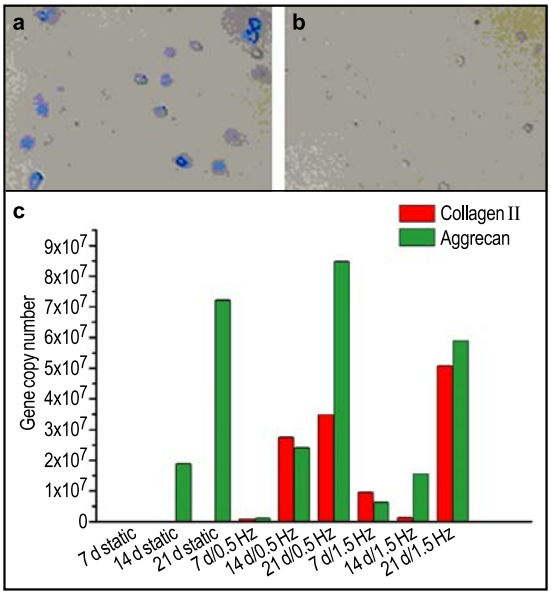

Figure 1: *Histology (panels a and b) showed an increase in ECM (sulfated proteoglycan) production for the dynamically cultured scaffolds as compared to the static culture scaffold. Real-Time PCR (panel c) showed the expression of genes for collagen type II and aggrecan under dynamic conditions and only aggrecan under static conditions.*

Porosity

It is generally agreed that "porosity" of tissue scaffolds is an important parameter; however, there is a general lack of agreement as to precisely what aspect of "porosity" is important, or how best to measure it.

We have initiated a collaborative project through ASTM (Task Force F04.42.06) to identify aspects of scaffold porosity that are most relevant and to come to consensus on how these aspects should be quantified. Seventeen laboratories throughout the world have joined us in this activity, which will be carried out in several stages, ultimately leading to the development of a reference scaffold for porosity measurements.

Output of this work thus far has included an ASTM draft guide for scaffold characterization (in preparation). Also, this work has been reported at the "Society of Biomaterials Meeting" and "World Biomaterials Meeting," both in 2004.

Contributors and Collaborators

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Combinatorial Methods for Rapid Screening of Biomaterials

Current methods for biomaterials development involve one-specimen-at-a-time characterization which is costly and time-consuming. In order to accelerate development, we have created a suite of high-throughput and combinatorial methods for rapidly screening and characterizing new biomaterials. Specifically, new methods for rapidly characterizing cell response to polymer crystallinity, composition, and surface chemistry have been developed.

Carl G. Simon, Jr. and Newell R. Washburn

Combinatorial and high-throughput methods hold the potential to accelerate research and development in any field of scientific study. In the Polymers Division, we are trying to lead the way in the application of these approaches to the characterization of biomaterials. Towards this end, we have utilized gradient technology to create libraries with varying material properties focusing specifically on *surface energy, polymer crystallinity,* and *polymer composition*.

Surface energy is a fundamental material property that affects cell interactions. This response may be through a direct cell-material interaction, but more often is an indirect effect where surface energy dictates protein adsorption, which subsequently dictates cell response. An automated stage is used to move a silanized glass slide beneath a UV lamp to create a gradient in oxidation from differential exposure to the UV light. Water contact angles for this gradient surface varies from 30 ° to 90 °. Cell behavior is assessed on the gradients, providing a unique tool to probe the fundamental correlations between cell response and surface energy.

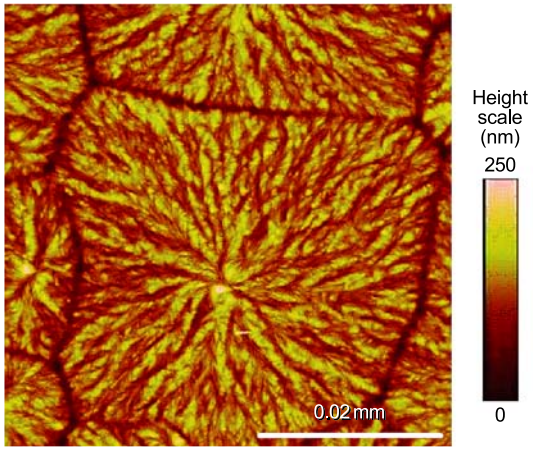

Figure 1: *Atomic Force Microscopy (AFM) height image of a poly(L-lactic acid) spherulite.*

Polymer crystallinity: Surface topology can strongly influence the performance of tissue-engineered medical products. Crystalline polymers used in biomedical applications, such as poly(ε-caprolactone) and poly(L-lactic acid) (PLLA), can have either a rough or a smooth surface depending upon processing. To create a gradient in surface topology, polymer solutions are first spread onto glass substrates to yield smooth, amorphous, thin films. The films are placed on a temperature gradient stage such that one end is held below the glass transition temperature (T_g) at ambient temperature and the other end is heated above the T_g to 100 °C. This produces gradients in crystallinity along the PLLA films where the room temperature-end remains smooth and amorphous while the 100 °C-end becomes crystalline and roughened. When combined with cell culture, we have a high-throughput method for studying cell response to the surface roughness that results from polymer crystallinity.

Polymer composition: Manufacturing industries have historically used polymer blending as an inexpensive method to create new materials with desirable properties. Blending can optimize modulus, strength, morphology, and crystallinity. For these reasons, blending is also receiving attention from the tissue engineering community. A gradient library of two polymers is created using a three-syringe pump system and a translation stage. The library is a strip-shaped film, and its composition is measured by Fourier Transform Infrared (FTIR) microspectroscopy. A gradient in composition spans the long axis of the film being PLLA-rich on one end and PDLLA-rich on the opposite end. Cells are cultured on the gradients to yield a high-throughput method for screening cell response to polymer blends.

The project accomplishments have been presented at six different symposia and in two journal articles, one of which was in the top-ranked tissue engineering journal, *Biomaterials*. Four other articles have been submitted or are in preparation. Finally, these activities were highlighted under "Government News" in *Biomaterials Forum* which is a quarterly news bulletin circulated by the Society for Biomaterials amongst its 1500 members.

Contributors and Collaborators

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