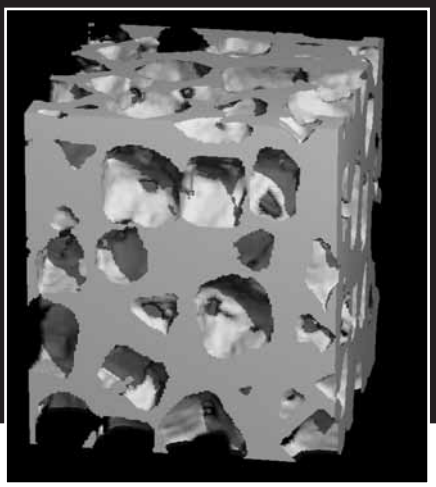
3D Tissue Scaffolds

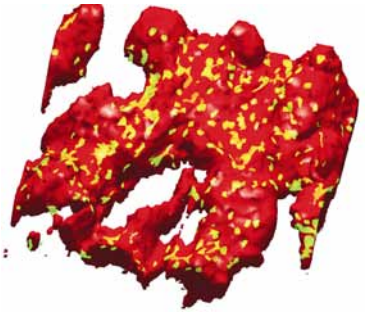
Objective

Our goal is to develop measurement tools and reference materials for assessing the impact of the physical and chemical properties of 3D tissue scaffolds on cellular response. These tools will be used to explore the relationship between cellular response on 2D surfaces to that in 3D scaffolds. In developing these tools we leverage non-invasive imaging techniques and combinatorial methods to enable a better understanding of cell-scaffold interactions for improved design of the scaffold-based medical products of the future.



Impact and Customers

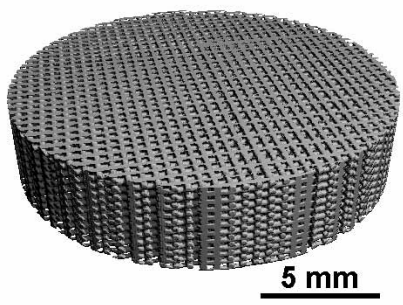
- The US spends $35 billion annually (3% of healthcare costs) to care for the 100,000 patients with end stage organ failure waiting for organ transplants. Our measurement solutions will accelerate development of engineered organ replacements to alleviate this burden.
- A reference tissue scaffold has been developed in collaboration with ASTM (F04.42.WK6507) that will enable companies to reliably characterize physical properties of their scaffold-based products.



- We have developed the world's first combinatorial platforms for screening cell response to 3D tissue scaffold properties. The platform encompasses several major classes of scaffolds including salt-leached scaffolds, hydrogels and nanofiber scaffolds.
- The National Institutes of Health support this work at NIST (R21 EB006497-01) in collaboration with the New Jersey Center for Biomaterials (RESBIO P41 EB 001046).
- We have compiled an invited special issue on "Combinatorial Screening of Cell-Material Interactions" for *Combinatorial Chemistry & High-Throughput Screening,* highlighting recent work from leaders in the field.

Approach

We are leveraging noninvasive imaging techniques and combinatorial methods to develop improved characterization techniques for 3D tissue scaffolds and cell-scaffold interactions. Due to the complexity of testing in 3D, cell-material interactions are typically tested in 2D formats, even though it is widely accepted that 3D formats are more likely to yield a clinically relevant response. The methods we develop will both simplify characterization of cell-material interactions in 3D, and allow us to connect results from 2D systems to expected results in 3D scaffolds.



We have pioneered the first combinatorial methods for screening cell response to properties of 3D scaffolds; demonstrating methods for fabricating polymer scaffold libraries in the forms of both gradients and discrete arrays. These combinatorial libraries contain scaffolds with variability over the full range of a particular variable, allowing complete characterization of cell response to that variable in relatively simple, compact experiments. The reference scaffolds we are developing will be used as calibration standards for the combinatorial libraries, as well as for industrial use in development of scaffold-based medical products.

BIOMATERIALS

Accomplishments

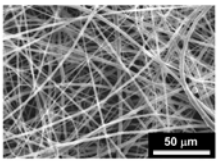
3D Scaffold Libraries
Previous combinatorial approaches for screening cell-material interactions have focused on planar (2D) surfaces or films. However, biomaterials are commonly used in 3D scaffolds and cells behave differently when cultured in a 3D environment.

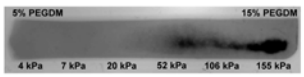
For these reasons, we have developed the world's first combinatorial platform for screening cell-material interactions in a 3D scaffold format. Our "combinatorial polymer scaffold library" approach has been adapted to many of the major classes of scaffolds including salt-leached scaffolds, hydrogel scaffolds and nanofiber scaffolds. Bone generation by osteoblasts has been screened while more recent work is with human mesenchymal stem cells (hMSCs).

hMSCs can differentiate into bone, fat, cartilage, muscle and nerve. Current work is focused on identifying optimal scaffold properties that direct stem cells to differentiate down these different lineages. These methods will enable us to develop a comprehensive understanding of how scaffold physical properties can be adjusted to direct stem cells to generate different tissues.

Combinatorial polymer scaffold libraries

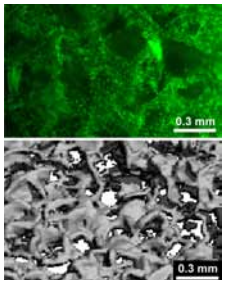
Examples of salt-leached scaffold libraries are shown in the figure above where red dye was used to visualize changes in scaffold composition. Scaffold libraries can be fabricated as gradients (left) or arrays (right). The image in this column shows the nanofibrous structure of an electrospun scaffold library. Below that is a transmitted white light image of a hydrogel library with a modulus gradient. The opacity in the right of the image is due to mineralization from osteoblasts seeded in the hydrogel libraries. The gel is stiffer on the right, which induces enhanced mineralization and bone deposition by osteoblasts.





Poly(ε-caprolactone) nanofiber scaffold (top)
Osteoblast mineral gradient in a hydrogel (bottom)

Non-Invasive Imaging
A significant difficulty in furthering the understanding of the cell/scaffold interaction is the lack of a high resolution, nondestructive imaging technique that is capable of penetrating deeply into the highly scattering scaffold medium. We are using a Small Business Innovative Research (SBIR) agreement to work with industry to build a high speed optical coherence/two photon fluorescence microscope (OCM/TPM) for imaging 3D tissue scaffolds. This microscope will perform simultaneous imaging of cell/scaffold structure (OCM channel) and function (TPM channel). The 10 KHz acquisition rate will allow collection of large, 3D image sets in a couple of hours. A 3D rendering from our OCM of osteoblasts distributed throughout a porous scaffold is shown on the previous page (image is 0.5 mm across).



Osteoblasts on polymer scaffold

We have used X-ray microcomputed tomography (μCT) for noninvasively measuring cell adhesion and proliferation in scaffolds. μCT has significant advantages over traditional microscopy in that it is an inherently 3D modality. In the images above, a confluent osteoblast cell layer adherent on the surface of a polymer scaffold has been imaged by fluorescence microscopy (top, green) and by μCT (bottom, grayscale). Staining was used to enhance cell contrast in the μCT image so that contributions from the polymer could be reliably removed, and only cells are visible in the image. This approach makes it possible to examine tissue formation within a scaffold without the tedium of serial sectioning, and enables 3D visualization and quantification of cell migration into scaffolds.

Reference Material Scaffolds
Reference Material scaffolds (RMs 8395, 8396 & 8397) have been developed with input from ASTM (F04.42.WK6507). The scaffolds were made by freeform fabrication since this approach offers tight control over scaffold structural morphology (image on previous page). These well characterized reference scaffolds will serve as standards during development of scaffold-based products.

Learn More

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Publications

Simon Jr CG, Yang Y, Dorsey SM, Ramalingam M and Chatterjee K *3D Polymer Scaffold Arrays* Methods in Molecular Biology: Biological Microarrays, in press (2009)

Simon Jr CG, Yang Y, Thomas V, Dorsey SM and Morgan AW *Cell Interactions with Biomaterials Gradients and Arrays* Combinatorial Chemistry & High-Throughput Screening, in press (2009)

Yang Y, Becker ML, Bolikal D, Kohn J, Zeiger DN and Simon Jr CG *Combinatorial Polymer Scaffold Libraries for Screening Cell-Biomaterial Interactions in 3D* Advanced Materials, 20: 2037-2043 (2008)

Yang Y, Dorsey SM, Becker ML, Lin-Gibson S, Schumacher GE, Flaim GM, Kohn J and Simon Jr CG *X-ray Imaging Optimization of 3D Tissue Engineering Scaffolds via Combinatorial Fabrication Methods* Biomaterials, 29: 1901-1911 (2008)