A portfolio of established methods from the New Jersey Center for Biomaterials

(June 2009)

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Black items are RESBIO technologies
Blue and italicized are NJCBM technologies
I. SYNTHESIS AND CHARACTERIZATION

Automated Parallel Synthesis Facility

AIM
RAPID EXPLORATION OF BIOMATERIALS DESIGN SPACE THROUGH AUTOMATED PARALLEL SYNTHESIS

The New Jersey Center for Biomaterials Parallel Synthesis Facility is equipped with a Chemspeed Accelerator SLT-100 Automated Synthesizer, which provides the capability for automated parallel synthesis of polymeric materials. This state-of-the-art robotic platform has the capability for carrying out 192 reactions in parallel, which can accelerate the creation of structurally-related polymer libraries and assist widely in reaction optimization. In the future, we plan to incorporate automated work-up, purification, and in-line characterization add-ons to enhance the automated and high-throughput capabilities of the automated synthesizer.

- In the near term, we can offer polymethacrylate libraries to the (bio)materials community. We have demonstrated this capability with the synthesis of a library of about 100 unique polymethacrylate compositions, synthesized by AIBN initiated RAFT polymerization.

- Optimization and validation of tyrosine-derived polycarbonate synthesis has recently been completed. Synthesis of 96 tyrosine-derived polycarbonates can be completed in 48 h using the automated parallel synthesizer compared to over a month with manual synthesis.

- Due to the capabilities of the Chemspeed Accelerator SLT-100, it is possible to investigate simultaneously several reaction conditions (e.g., type of monomer, initiator, solvent system, temperature). Thus, our chemists are able now to accelerate their throughput and explore efficiently polymer space in less time. This, together with the preservation of 'know-how', secures reproducibility and uniformity of materials as well as the ability to accelerate project timelines more efficiently.

![Summary of synthesis process and characterization results]

Exploration of several reaction conditions (e.g., reaction time, triphosgene addition, concentration) made possible the identification of a synthetic procedure that yields higher and more consistent molecular weights relative to manual synthesis for a model reaction (DTE polycondensation with phosgene).
Determination of Polymer Glass Transition Temperature

**AIM**
To determine the glass transition temperature (T<sub>g</sub>) of a polymer using a differential scanning calorimeter (DSC).

**Introduction**
The T<sub>g</sub> of a polymer marks the transition from the glass to the rubber-like state and is an important feature of polymer behavior. In this temperature region dramatic changes in the physical properties, such as hardness and elasticity, are observed. The changes are completely reversible and the transition from a glass to a rubber is a function of molecular motion, not polymer structure.

**Experimental**
Approximately 5-10 mg of the polymeric material is placed an aluminum DSC sample pan. To eliminate any thermal history, the sample first is heated above T<sub>g</sub> in the DSC (TA Instruments 2920) and then rapidly cooled to a temperature below T<sub>g</sub>. A second scan is then carried out and T<sub>g</sub> is determined from the midpoint intersection of the baseline shifts (see figure). Typical heating rates are 10 – 20 °C/min and measurements are carried out under nitrogen. For low temperature measurements, a refrigerated and a liquid nitrogen cooling system is available and modulated differential scanning calorimetry (MDSC) runs can also be measured.

A typical DSC thermogram of polymer sample, T<sub>g</sub> = 95.6 °C
Thermal Gravimetric Analysis

AIM
To characterize the volatile components in polymers and the decomposition of polymers.

Introduction
Thermal Gravimetric Analysis (TGA) is a simple analytical technique that measures the weight loss (or weight gain) of a material as a function of temperature. As materials are heated, they can lose weight from a simple process such as drying, or from chemical reactions that liberate gasses. Some materials can gain weight by reacting with the atmosphere in the testing environment.

Experimental
Place 10-15 mg of the polymer sample in the platinum TGA pan. Use the TGA instrument (TA 2950) to select a suitable method depending on what measurement are being made
   a. For volatile component measurement, heat from room temperature to 250 °C at 10 °C/min
   b. To determine volatile component and decomposition temperature measurement, heat from room temperature to 400 °C (or a suitable temperature depending on the decomposition temperature) at 10 °C/min

Turn on the nitrogen and adjust the pressure using the pressure gauge attached and close the furnace using the furnace command on the instrument panel. Start the run and heat the sample as per the method. From the TGA thermogram (see figure) determine the volatile component (usually till 175 °C) and the decomposition temperature (either the onset temperature or the temperature corresponding to 10% mass loss

A typical thermogram of a polymer showing the release of volatiles. Decomposition starts at T > 200 °C
**Mechanical Property Evaluation**

**AIM**
To determine and understand the elongation and stiffness of biomaterials and biological specimens in ambient and simulated physiological conditions

**Introduction**
Success of materials in biomedical applications is crucially dependent on eliciting a precise and predetermined response from these materials to an external stress. Mechanical characteristics serve as important benchmarks in the development of new biomaterials. For this purpose biological specimens and “manmade” materials are usually tested under a variety of loading conditions. Using the MTS Sintech 5/D, which is available at the NJCBM, materials can be tested to failure under tensile, compressive and flexural stresses at loads from 10 to 20,000 N. Furthermore, these tests can be conducted under both ambient and simulated physiological conditions. Typical tests that have been conducted include tests for materials for use in stents, as neural implants and in the evaluation of scaffolds for various applications including skin replacement.

**Experimental**
Samples in variety of shapes can be accommodated although for interlaboratory comparison it is advisable to follow ASTM specifications for specimen preparations. A biological sample is conveniently attached to a tab, which is then held in a fixture. Tissue samples are typically tested with forces 1-10 N whereas samples used for load-bearing applications are tested with forces 10-100 N. The data are collected and analyzed using TestWorks® software.

**Results:**
Typical data obtained as a sample is relaxed after initial loading are shown in the accompanying figure. These data were obtained from a single filament typical of those that could be used in ligaments and stretched to about 1% of strain.

The apparatus for testing the mechanical properties of biomaterials under ambient and physiological conditions is shown on the left. The plot above shows the viscoelastic response of a ~70μm diameter filaments to the load of the type shown in the inset.

**Modeling the mechanical behavior:**
The properties of the material can be understood to a first approximation by simulating the stress-strain response using suitable analytical models. These models can be related to detailed structural characteristics at smaller length scales that can be experimentally determined or predicted using molecular mechanics and molecular dynamics simulations.
**Characterization of Polymer Molecular Weight by Gel Permeation Chromatography**

**AIM**
To determine absolute polymer molecular weight characteristics using GPC equipped with a triple detection system

**Introduction**
The molar mass and weight distribution of a polymer samples has significant influence on its properties and a knowledge of the shape of this distribution is fundamental to the full characterization of a polymer. A state-of-the-art Viscotek Gel Permeation Chromatography (GPC) equipped with triple detection system (TDA) boasting 4 detectors (refractive index (RI), viscosity, laser light scattering (right angle-RALS and low angle-LALS), and UV) is available. This GPC measurement provides absolute molecular weight of the polymer, the intrinsic viscosity, dN/dC, Mark-Houwink constants and many other characteristics of the polymers.

**Experimental**
For accurate and meaningful molecular weight determination, ensure:
A. The polymer sample should be completely dry
B. The polymer should be accurately weighed and dissolved in a known amount of solvent so that the exact concentration of the sample is known
C. The sample should be filtered

A typical chromatogram and values are listed below in Figures 1 and 2, respectively.

**Figure 1:** A typical TDA GPC-chromatogram. The red is from RI, Blue is from viscometer, and the green is RALS. LALS not shown

**Figure 2:** Typical results generated by the TDA-GPC
**Multiscale imaging of the distribution of water and the erosion in biodegradable polymers**

**AIM**
To map the distribution of water and the porosity that results as a result of hydrolysis in biodegradable polymers

**Introduction**
Biomaterials are invariably in contact with water, and hydration affects their mechanical properties. In the case of degradable biomaterials, the initial process of hydration affects the subsequent process of degradation, as well as cell-substrate interactions and drug release characteristics. Multiple technique are available at NJCBM and

The figures show the distribution of hydration at four different length scales. Top left: Schematic interpretation of SANS data that indicate the distribution of water in the form of 10 nm domains. Bottom left: TEM image showing 10 nm hydrated domains in a polymer immersed in water for 12 months. Top right: Raman confocal image showing the distribution of water into 10 μm domains. Bottom right: SEM image showing the 10 μm size voids that appear as a result of hydration-induced erosion.
associated facilities that are available for mapping the water distribution and the subsequent erosion of the degradable implant at different length and time scales using several measurement techniques. Mainly these are scattering techniques using x-rays and neutrons (~ 10 nm), transmission electron microscopy (~ 100 nm), confocal Raman imaging (5 μm) and scanning electron microscopy (10-100 μm).

**Experimental**

The type of sample depends on the technique. Typically 1-2 mm thick samples are used for scattering, TEM requires a film < 100 nm thick deposited on a carbon or a copper grid. Confocal Raman imaging can be performed on 200 μm thick films. SEM images the surface exposed to the electron beam and the samples can be as large as a several mm.

Each of these techniques is highly specialized and require the attention of experienced personnel in collecting and interpreting the data.
Static Water Contact Angle Measurements

AIM
To characterize the wetting properties of a test surface by air-water contact angle measurement.

Introduction
Wetting of surfaces by liquids is a very important phenomenon in the technological application of materials. The determination of the contact angle of a liquid drop on a solid interface is a simple method for the evaluating surface wettability. The greater the spreading tendency of a droplet, the more hydrophilic a surface.

Experimental
Air-water contact angles are measured on a Rame-Hart goniometer model 100-00-115 (Mountain Lakes, NJ) using double distilled water as the probe. A water droplet is deposited onto the test substrate from above forming a sensible drop having a diameter of 2.5 mm. Results are the averaged over at least three measurements (typical error ± 3)

Radiolabeled Water Uptake in Polymer Films

AIM
To determine quantitatively the amount of water in a bulk polymer sample.

Introduction
Using radiolabeled water to measure water uptake in polymer films is a simple, accurate, and reproducible method, which also allows the parallel measure of many samples. However, it requires radioactive management, controls must be run each time, large amount of polymer is required, and it cannot be used for polymers that lose mass or fracture upon handling.

Experimental
Tritiated (3H) water (Sigma-Aldrich) with an activity of 1 mCi/ml is diluted with HPLC grade non-radiolabeled “cold” water to a concentration of 0.2 μCi/ml. Polymer samples of ~1 cm diameter (fabricated by compression molding) are incubated in separated vials with 7 ml of 3H water (0.2 μCi/ml) and stored at 37 °C in the incubator. After the incubation, the respective sample is removed from the vial, rinsed with distilled water, blotted dry and dissolved with 3 ml of Tetrahydrofuran (THF, VWR) and 12 ml of liquid scintillation cocktail (LSC, Ecolite). A control curve is constructed with 0, 6, 8, 10, 12, and 14 μl of 0.2 μCi/ml 3H water (n = 3), 3 ml of THF and 12 ml of LSC; and one background with 12 ml of LSC and 3 ml of THF. Radioactive counts are measured using a scintillation counter (Beckmann 6500). Water content (M, H₂O) is calculated using the calibration curve. Water uptake (WU) is calculated as the water content relative to the original dry weight (Msample):

\[ WU(\%) = 100 \cdot \frac{M, H₂O}{M_{sample}} \]
II. FABRICATION TECHNOLOGIES

Thin Spun-Cast Polymer Films

AIM
To fabricate thin polymer films on various substrates for use in applications such as cell culturing, quartz crystal microbalance with dissipation (QCM-D), atomic force microscopy (AFM), contact angle and X-ray Photoelectron Spectroscopy (XPS) measurements.

Introduction
Spin-coating is an extremely useful, versatile, and efficient technique for preparing thin polymer films. After a volatile solution is deposited on a substrate of choice, the centrifugal force generated by the spin coater both expels and evaporates excess solvent, leaving behind a thin (20 – 300 nm) uniform film under optimal conditions. Variable parameters range from solution concentration, spin speed, spin acceleration, atmospheric conditions and the choice of substrate.

Experimental
Pre-spin Coating Steps: Calcium-sulfate dried air is used to evacuate the spin-coater chamber to obtain a relative humidity <20%. A solution of the material to be coated is then prepared using a suitable volatile organic solvent and filtered. Solution concentrations typically range from 1 - 2% w/v. For solutions with extremely hygroscopic solvents such as tetrahydrofuran (THF), the solvent must be anhydrous (molecular sieves). Substrates such as glass, silicon, or quartz crystals must be cleaned and particulate-free prior to spin coating. Substrates used for cell studies may require additional cleaning, such as UV/ozone exposure or sonication in ethanol.

Sample Preparation and Spin-Casting: Set spin coating speed, acceleration, and duration. Common settings are 4000 RPM at 4000 RPM/s for 30 seconds. Place drops of the polymer solution onto the substrate such that the entire surface area is covered. Avoid air bubbles. The homogeneity of coatings may be characterized by AFM, contact angle, SEM and XPS.

Compression Molded Films

AIM
To fabricate polymer films of 200 - 500 μm by compression molding for application in water uptake, mechanical property, cell culture and degradation studies.

Introduction
Compression molding is a simple method for the fabrication of polymer films with typical thicknesses in the range of 200 – 500 μm. In conjunction with a temperature controlled compression molder, a stainless steel mold with smooth surfaces yields flat polymer films with homogenous sample thickness. For materials with low Tg’s, liquid nitrogen can be used to cool films for easy handling.

Experimental
The polymeric material is placed and distributed evenly inside the frame of a stainless steel mold. Place the mold in a Carver Press compression molder (Fred S. Carver Inc.) at 70°C above Tg for 5 min, and then slowly increase the press to 15,000 psi over 90 s, hold for 1 min, then slowly release
the pressure over 1 min. The film is then allowed to cool to room temperature before removing from the mold, and then annealed for 24 h at a temperature above $T_g$.

**Melt extruded filaments, fibers and films**

**AIM**
To fabricate filaments of up to 3 mm diameter, fibers down to 80 $\mu$m diameter and films by melt extrusion

**Introduction**
Melt extrusion is a standard technique for making thick polymer filaments, which can be later cut into shorter pins, thin fibers down to 80 $\mu$m diameter, and films. The extruded filaments, fibers and films can be further drawn and stretched so as to further modify the properties such as modulus and strength.

**Experimental**
The laboratory recently acquired a 3/8 in microtruder (Randcasatile, Cedar Grove, NJ). The extruder can process polymers as small as 20 g and produce 14 g of finished fiber. The extruder has a 3mm exit bore and is currently equipped with fiber dies, 0.5 and 2 mm dia. The extrudate is picked up on a fiber/film winder.

Microtruder with the control panel  
Microtruder with the fiber winder
Electrospun Polymer Scaffolds

AIM
To fabricate fibroporous, highly interconnected polymeric scaffolds with individual fiber diameters between 100 nm – 4 μm.

Introduction
Electrospinning is a facile technique for the fabrication of fibrous polymer scaffolds. The thickness of the scaffolds range from a sparse coating of fibers to 400 μm mats spun onto an aluminum foil. The experimental set up is shown in Figure 1 where a syringe pump is used to pass a polymer solution through a syringe needle under a positive charge onto a negatively charged substrate. Representative examples of cell permeable and cell-impermeable scaffolds are shown in Figure 2.

Figure 1: Typical electrospinning setup. Both flat plate and mandrel collectors are used in the NJCBM

Figure 2: Confocal microscopy images of fibroblast morphology at 5 days observed on electrospun scaffolds with fiber diameters of (a) 2.3 μm; (b) 1.0 μm; (c) 0.54 μm; and (d) 0.29 μm.

Experimental
- Prepare concentrated (typically 5-25%wt/vol) polymer solution
- Work in class 10,000 clean room to minimize particulate contamination of fabricated scaffolds
- Load polymer solution into glass syringe
- Wrap collector with an aluminum foil backing
- Use syringe pump to deliver polymer solution to stainless steel needle at a constant flow rate, typically 0.3-6 mL/hr
- Use a high voltage power supply to apply a positive charge (6-30 kV) to the needle and either ground or negatively charge the collector
- A very bright flashlight will allow one to observe whether fibers are being electrospun or not
- While in the clean room, place scaffolds on backing into sterilization pouches
- Sterilize by the standard ethylene oxides sterilization method
- Scaffolds are easily removed from the backing once the scaffolds have been wetted
**Porous Salt Leached Scaffolds**

**AIM**
**To fabricate polymeric scaffolds with macro/micro porous structure through solvent casting, salt leaching, and lyophilization process.**

**Introduction**
A paradigm shift is taking place in medicine from using synthetic implants and tissue grafts to a tissue engineering approach that uses degradable porous material scaffolds integrated with biological cells or molecules to regenerate tissues. The fabrication of highly porous scaffolds, characterized by pore size and interconnectivity, is especially crucial to allow for cellular infiltration and subsequent tissue growth, diffusion of nutrients and clearance of wastes. While numerous techniques exist to fabricate macroporous scaffolds such as solvent casting, salt leaching, phase separation and fiber bonding, our standard protocol involves solvent casting the polymer solution over a bed of salt crystals to produce macropores (>100 \(\mu\)m), and a lyophilization process in order to generate micropores (<10 \(\mu\)m).

**Experimental**
1. **Solvent casting process:** The polymer solution is prepared by mixing 300 mg of polymer, 300 \(\mu\)L of nanopure water and 3 mL of 1,4 Dioxane (Sigma-Aldrich Inc., anhydrous, 99.8%) in a vortex mixer. The vial is sealed with parafilm during mixing, to avoid solvent evaporation.
2. **Salt-embedding process:** Once a homogeneous solution is obtained, 11 g of sodium chloride (size 212 - 425 \(\mu\)m) is placed in a Teflon petri dish and the polymer solution is slowly poured over the salt and kept undisturbed for 1 h to allow for the penetration of the viscous polymer solution throughout the bed of salt.
3. **Lyophilization process:** The mixture is then rapidly cooled in liquid nitrogen for 5 - 10 min and is dried overnight in a freeze drier.
4. **Salt leaching process:** The scaffolds are incubated and rinsed with nanopure water at least 7 times until the silver nitrate test is negative, indicating that absence of chloride ions in the washings. The scaffolds are maintained under vacuum for 24 - 48 h and then stored in an 8-well plate sealed with tin foil to protect them from dust.

![Pore architectures of poly(DTE carbonate) salt leach scaffolds. Scanning electron microscopy (SEM) images demonstrate a) macropores (scale bar = 100 \(\mu\)m) and b) micro-pores (scale bar = 10 \(\mu\)m). c) Optical coherence tomography image shows the interconnected pores. Red and green color represents the porous and the non-porous area of the scaffold respectively.](image-url)
III. IN VITRO METHODS

Hemocompatibility of Polymer Surfaces

AIM
To determine the hemocompatibility of polymer surfaces using an *in vitro* predictive method.

Introduction
The hemocompatibility of biomaterials is highly dependent on the adhesion and activation of platelets. Surface-adsorbed fibrinogen has a dominant role in promoting platelet adhesion to artificial surfaces by binding glycoprotein IIb-IIIa (GPIIb-IIIa), the major platelet membrane receptor. Using the Quartz Crystal Microbalance with Dissipation (QCM-D) technique, the binding kinetics of purified GPIIb-IIIa to surface-adsorbed fibrinogen is measured. This provides information on the dynamics and conformational properties associated with surface-adsorbed fibrinogen and the subsequent binding of GPIIb-IIIa. The use of GPIIb-IIIa to simulate platelet adhesion circumvents the need to work with potentially biohazardous blood components and the utility of this method is reported in *J. Biomed. Mater. Res.* (Weber et al. 2005).

Experimental

**Substrate preparation:** Gold-coated quartz crystals (Q-Sense, Inc. MD) are spin-coated at 4000 RPM using a spin coater with 1% (w/v) polymer solutions in suitable organic solvent (e.g., methylene chloride).

**QCM-D Measurement:** Human Fg adsorption to polymer-coated crystals is performed using the QCM-D model E4 (Q-Sense, Inc. MD) at 37 °C. After establishing a stable base line in PBS, the surface is exposed to human fibrinogen diluted in PBS (3 mg/mL), an albumin blocking step, followed by the platelet receptor GPIIb-IIIa.

**Modeling of the QCM-D response:** The viscoelastic properties (viscosity, elasticity, thickness) of the adsorbed layers are obtained using the Voigt model (Q-Tools, Q-Sense, Inc. MD). The layer density is fixed to 1200 kg/m$^3$. Parameters fitted are (i) layer viscosity between 0.0005 and 0.01 kg/ms (ii) layer shear between $10^5$ and $10^{12}$ Pa and (iii) layer thickness between $10^{-10}$ and $10^{-7}$ m.

![Typical real-time QCM-D results of fibrinogen adsorption and BSA incubation followed by GPIIb-IIIa binding to a polymer coated quartz crystal sensor.](image)
Cell Attachment and Proliferation Assay Method

AIM
To quantify cell attachment and proliferation on spin coated polymer films,

Introduction
The evaluation of biomedical polymers’ biocompatibility represents a fundamental step in establishing and ensuring product applicability and safety. Analysis of cell proliferation or cytotoxicity can serve as a starting point to assess polymer biocompatibility. In this respect, in vitro cell culture represents a powerful tool for the preliminary screening of biomaterials cytotoxicity. One of the most popular quantitative techniques for the evaluation of cell viability is based on the measure of cell metabolic function dependent on the intact activity of the mitochondrial enzyme, succinate dehydrogenase, after exposure of cells to a test material. Tetrazolium salt such as MTT-([4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide) is converted by the succinate dehydrogenase into a blue/purple formazan product which is then quantified by spectrophotometry (Mosmann, 1983). In vitro cultured cells, such as mouse fibroblasts are seeded on polymer surfaces spin-coated on glass. Cell attachment and cell growth is quantified using the MTT/MTS assay described above.

Experimental
The setup used to study cell attachment and growth is shown in figure. Polymers are coated onto glass cover slips, sterilized and placed in wells of a 24 well plate. After placing a sterile silicone O-ring to secure the cover slip, ~1X10⁴ NIH3T3 fibroblasts are seeded on each polymer surface and incubated at 37 °C for 4 hrs, after which unattached cells are gently removed and cells attached to the surface quantified using the MTS assay (Attachment). In another identical set, attached cells are allowed to grow for 4 days and again measured using the MTS assay (Growth). Cell proliferation is calculated with respect to the initial cells attached to a polymer surface (Attachment) and expressed as relative to standard Tissue Culture surface (TCP).

References
Flouroreporter Cells

AIM
To engineer cell lines genetically to express Green Fluorescent Protein fusion genes.

Introduction
Cell-based, high throughput screening has revolutionized the development of small-molecule pharmaceuticals. Differential cell attachment, attachment strength, survival, and growth on diverse biopolymer substrates can confound the interpretation of standard live and fixed-cell assays and make screens to characterize the cellular responses to biomaterials labor intensive. We have attempted to develop non-invasive qualitative and quantitative tools for the rapid characterization of multiple cellular responses to biopolymer substrates. To this end, we have genetically engineered cell lines to express Green Fluorescent Protein (GFP) fusion genes that visually report on structural and functional properties in living cells, and have used these lines to demonstrate their ability to distinguish chemical and structural differences in biodegradable, polymer substrates (Figure 11).

Experimental
Plasmid source: GFP-based plasmids have been obtained from academic and commercial sources (Table 1) and are targeted to specific organelles through addition of a short localization signal (such as GFP-F) and to specific cytoskeletal proteins (such as GFP-paxillin).

Table 1: GFP-based Plasmid sources available at NJCBM

<table>
<thead>
<tr>
<th>Function</th>
<th>Plasmid</th>
<th>Location / Protein</th>
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<tbody>
<tr>
<td>Attachment, Spreading &amp; Migration</td>
<td>pEGFP-F</td>
<td>Plasma Membrane</td>
</tr>
<tr>
<td>Proliferation</td>
<td>pECFP-Nuc</td>
<td>Nucleus</td>
</tr>
<tr>
<td></td>
<td>pEGFP-RecA-NLS</td>
<td>Nucleus</td>
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<tr>
<td>Apoptosis</td>
<td>pCaspase3-Sensor</td>
<td>Cytoplasm → Nucleus</td>
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<tr>
<td></td>
<td>pEGFP-GAPDH</td>
<td>Cytoplasm→ Nucleus</td>
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<tr>
<td>Cytoskeletal Organization</td>
<td>pEGFP-Actin</td>
<td>Microfilaments (β-Actin)</td>
</tr>
<tr>
<td>Focal Adhesions (Structural)</td>
<td>pEGFP-Tub</td>
<td>Microtubules (α-Tubulin)</td>
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<tr>
<td></td>
<td>pEGFP-α-actinin</td>
<td>α-Actinin</td>
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<td>pEGFP-α5-integrin</td>
<td>α5-Integrin</td>
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<tr>
<td></td>
<td>pEGFP-paxillin</td>
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<tr>
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<td>pGFP-vinculin</td>
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<tr>
<td>Focal Adhesions (Regulatory)</td>
<td>pEGFP-rhoA</td>
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<tr>
<td>Signal Transduction</td>
<td>pEGFP-STAT1</td>
<td>Cytoplasm → Nucleus</td>
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Cell culture: CHO-K1 (ATCC; Manassas, VA) and Saos-2 (a gift from David Denhardt; Rutgers University) can be propagated in F12H (Invitrogen; Carlsbad, CA) supplemented with glutamine,
penicillin-streptomycin, and 10% fetal bovine serum (Sigma; St. Louis, MO). Human mesenchymal stem cells can be propagated in mesenchymal stem cell basal medium (Cambrex, East Rutherford, NJ) containing L-glutamine, Penn-Strep, and Mesenchymal Cell Growth Supplement (MCGS).

Transfection methods of plasmids to cells:
A. Liposomal method: Cells can be transfected using Lipofectamine supplemented with PLUS reagent (Invitrogen).
B. Electroporation method: Cells can be transfected using electroporation (KITE-R, World Precision Instruments, Inc., Sarasota, FL).
C. Nucleofection method: Cells can be transfected using AMAXA II system with an optimized nucleofection kit (Amxa Inc., Gaithersburg, MD).

Differential response to polymer substrate by Saos-2 cells expressing GFP fusion genes: Confocal images of live Saos-2 cells expressing (A) GFP-F, (B) GFP-actin, (C) GFP-actinin, and (D) GFP-paxillin. (630x; zoom 2).
IV. MICROSCOPY AND IMAGING

Multiphoton/Confocal Microscope

AIM
To obtain real-time 3D fluorescent images of cells, scaffolds and cytoskeletal elements

Introduction
The Leica TCS SP2 AOBS with the Spectra Physics Mai tai Titanium Sapphire femtosecond laser is a standard confocal laser scanning (CLSM) microscope that can be run as a multiphoton microscope (MPM). Our microscope is also equipped with a Ludin box that allows for atmospheric and temperature controls for time lapse imaging of live cells. CLSM and MPM are relatively non-destructive methods that provide images of both scaffold meso/microstructures and individual cell responses.

Specifications
Z drive:
• Precision focusing nosepiece (DM RXE): 2 mm travel, 10 nm resolution
• High-resolution z stage: 170 micrometer travel, xyz: 40 nm resolution, xz: < 1 nm resolution

Objectives
• 10x, 20x, 40x, 63x dry objectives
• 63x glycerin immersion objective
• 100x oil immersion objective

Lasers & Attenuation
• ArKr 458 nm, 476 nm, 488nm, 496 nm, 514 nm
• HeNe 543 nm, 594 nm, 633 nm
• Photomultiplier Tube (PMT) detectors for imaging three channels (Two fluorescent modes & reflected or transmitted mode)

Confocal Unit: Optics
• Differential Interference Contrast (DIC) optics
• Spectral range of detector optics: 400-850 nm
• Adjustable pupil illumination
• One pinhole, variable diameter size

Scanner
• K scanner with two independent galvanometers
• Line frequency: up to 2000 lines/s
• Frame rates: 3 fps (512 x 512 pixels), 20 fps (512 x 32 pixels)
• Scan resolution: up to 4096 x 4096 pixels
• Scan zoom 1 - 32 x
• Scan rotation -5 to +95 degrees
• Scan field: 22 mm diagonal in intermediate image plane

Other Features
• Motorized specimen stage in x, y, and z-directions with a minimum stepsize of 50nm
• Incubator-stage apparatus providing atmospheric and temperature control
• Vibration-free compressed air table
• Stage adaptability to diverse specimen carriers (ie - slides, LabTek & MatTek chambers
Osteoblasts seeded on poly (I2DTE carbonate) 72 hrs. and stained with calcein AM (green) and ethidium homodimer-1 (red). The scaffold (blue) was imaged in reflection mode. The image is a maximum projection image from fifty individual slices taken every ten microns (100x).