Designing Scaffolds with Optimal Chemical and Physical Properties for Tissue Engineering

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1. Abstract

The design of scaffolds that resemble natural organs is becoming increasingly important for tissue engineering. Cells ability to proliferate and function depends strongly on the mechanical and chemical properties of the scaffold. In this experiment we studied the effects of surface chemistry on cell morphology and proliferation. Polydimethylsiloxane (PDMS) was utilized as a scaffold due to its wide range of medical applications and the results were compared with those of cells cultured on tissue culture plates. The mechanical properties of the PDMS substrate were varied by adding different amount of a cross-linking agent. For each cross linking ratio, sets of samples containing 0, 0.5, 1.0, and 2.0 percent of Na clay were prepared. We observed the mechanical characteristics of the polymer samples using Instron tensile tester and Nicolet Spectra FTIR instrument. We measured the tensile modulus and extension at yield and break as a function of the degree of cross-linking. Cross-linked spherical plug molds were then made of each sample and placed in a 24 well plate where fibroblast-like cells (CF-29) were cultured. Cell counts were obtained after 24 hours and three days. The cells were also stained and imaged under a confocal microscope (Leica-SP). The results indicate that cell proliferation is hindered on PDMS substrates relative to tissue culture plastic (TCP) substrates. Addition of clays in the PDMS susbstrates increased the cell counts to values that were comparable to those obtained on TCP.

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2. Introduction

It has been shown that cells can sense the physical and chemical characteristics of their microenvironment. Cells measure the stiffness of the material by adhering to the surface and pulling on it. If the substrate is relatively soft, the cells are shown to express nerve-like protein, and if the substrate if relatively hard, they express bone protein[1]. Polymeric scaffolds can be used to mimic various tissues so the cells can proliferate and function normally.

Fibroblast cells play important role in production of extracellular matrix and are crucial for wound healing. To replicate the mechanical modulus of connective tissue, we cross-linked polydimethylsiloxane (PDMS) and then added clay nanoparticles. PDMS is widely used in the medical industry ranging from joint replacements to implants. PDMS hydrogel can be solidified by cross-linking using a curing agent. The hardness of the solid material depends on the cross-linking density[2]. The PDMS to cross-linker ratios were 20:1, 10:1 and 5:1.

Cross-linked PDMS presents a hydrophobic surface. To modify that surface chemically, we introduced various amounts of sodium Cloisite Na+ clay. Clays are layered compounds that can be exfoliated into particles of nanoscale dimensions. They are used extensively in producing many consumer materials ranging from car parts to beauty supplies. Cloisite® Na^+ is a natural montmorillonite often added to polymers in order to reinforce the polymeric matrix and make it impermeable to gases[3]. Each cross-linked PDMS was modified with 0, 0.5, 1 and 2% clay.

3. Methods

3.1 Materials

Sylgard® 184 Silicone Elastomer Base and Curing Agent were purchased from Dow Corning Corporation.
Cloisite NA^+ clay was purchased from Southern Clay Products, Inc.
Mechanical properties were analyzed using a Nicolet Spectra Fourier Transform Infrared Spectroscopy instrument and an Instron Tensile Tester.
Cell counting was performed under an Olympus inverted phase contrast microscope. Cell imaging was performed with a Leica-SP Confocal microscope.

3.2 Synthesis and Mechanical Analysis of PDMS

PDMS samples were made with varying degrees of cross-linking by mixing Sylgard 184 in 20:1, 10:1, and 5:1 ratios of base to curing agent.
Thin films of the samples were made for FTIR analysis by smearing gel onto Kaptom film and setting between two glass microscope slides. Spectra of Sylgard 184 base, curing agent and mixtures of 20:1, 10:1 and 5:1 were taken.
For each PDMS ratio, samples containing 0, 0.5, 1.0, and 2.0 percent Na clay were prepared. The samples were molded for Instron using appropriate steel plate molds. All

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samples were put under vacuum for one half hour followed by two hours in the oven at 65°C to complete curing as per manufacturer's specifications.

3.3 Cell Culture, Counting, Staining, and Imaging

Samples were incubated in 1% fibronectin solution for 24 hrs in a 5% CO_2, 37 °C, 100% humidity. Samples were washed once with PBS and human dermal fibroblast cells (CF-29) were plated with density of 2400 cells/cm^2 in DMEM (low glucose) 20% Fetal Bovine Serum (FBS) 1% Penicillin streptomycin (PS) media.

Cells were counted from three separate dishes of each sample on day 1 and day 3 of incubation in standard conditions of the human body. Media was aspirated out of the dish and the substrate was washed once gently with phosphate buffer saline (PBS) solution. 300μL of trypsin EDTA was added to the substrate to lift the adhered cells and incubated for five minutes. Trypsin solution was then transferred to glass test tubes. Cells were counted under an Olympus inverted phase contrast microscope using a hemocytometer.

Cells were fixed for staining with 3.7% formaldehyde. The cells were then stained using Alexa-fluor 488 Phallodin for the F-Actin protein and Propidium Iodide for the nucleus. In order to determine cell morphology, the stained cells were imaged under the confocal microscope.

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4. Results and Discussion

4.1 FTIR

Infrared spectra of our samples were taken in order to quantify the cross-linking density. In order to obtain legible FTIR spectra, very thin films had to be prepared and the instrument dehumidified.

Table 4.1 summarizes the observed peaks from Figures 4.1a – c.

Peaks, cm^{-1}						
Literature value[4]	***Base***	***Curing Agent***	***20:1***	***10:1***	***5:1***	***Assignment***[4]
2850-3000	2962.72	2963.26 2904.05	2960 2900	2960	2962.87 2904.58	CH_3
2100-2360	-	2162.06	2160	2160	2159.79	Si-H
1900-2000	-	-	1950	1950	1943.86	C=C
1350-1470	-	1410.07	1410	1410	1411.85	CH_2
1250±10	1261.11	1261.04	1260	1260	1259.14	$Si-CH_3$

Table 4.1. Infrared Spectrum of PDMS

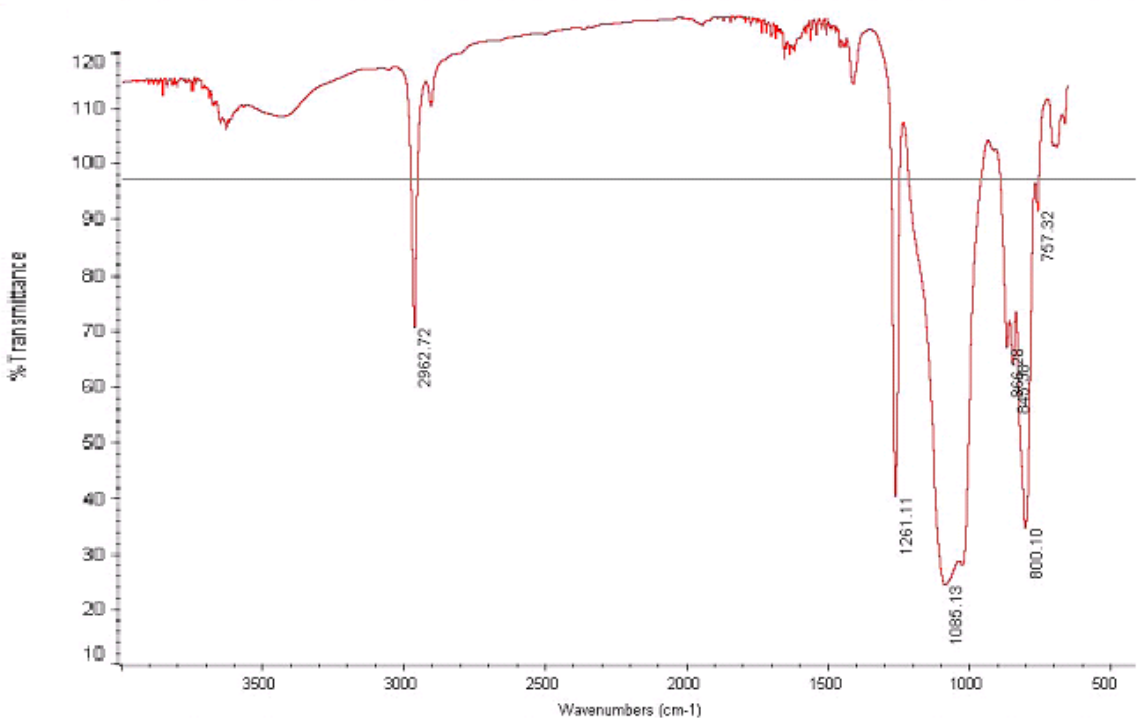

Figure 4.1a. Sylgard 184 PDMS Base

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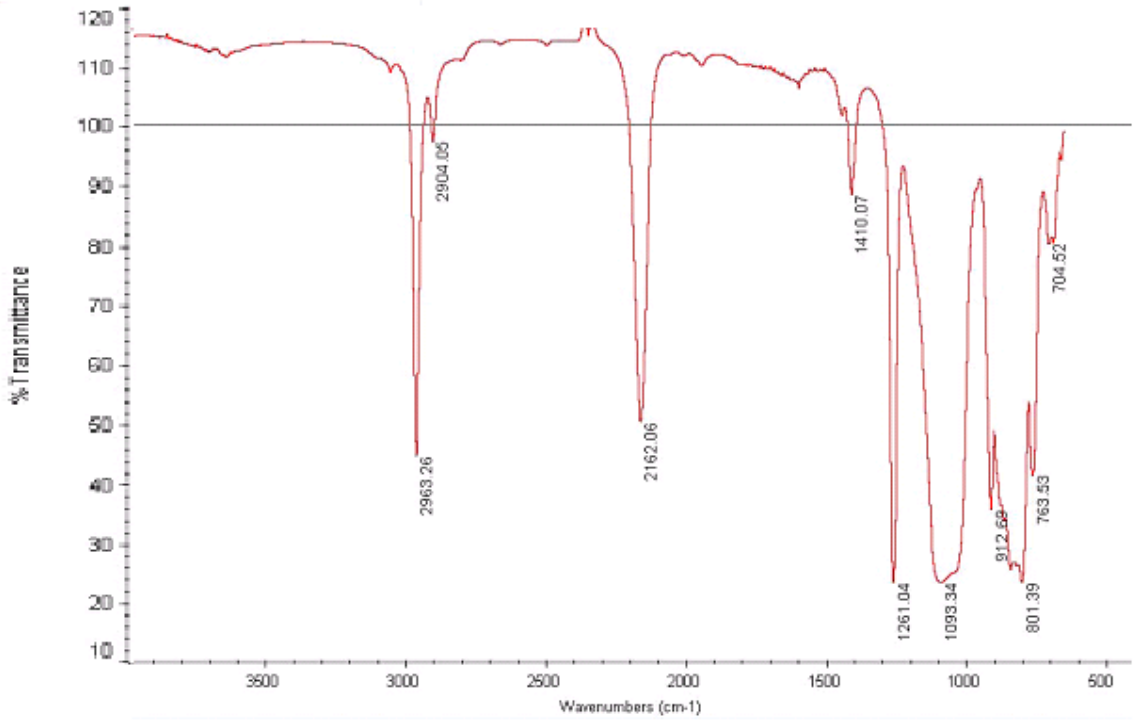

Figure 4.1b. Sylgard 184 Curing Agent

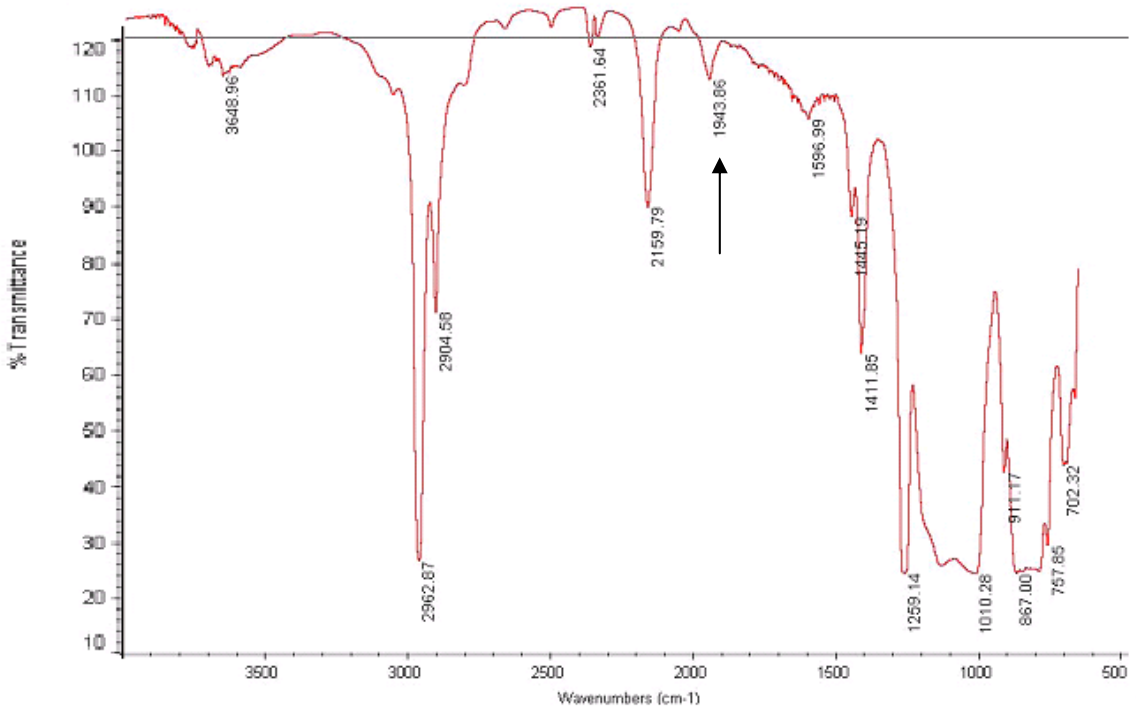

Figure 4.1c. PDMS, 5:1 Cross-linked

From Table 4.1 and Figures 4.1a - 4.1c, it can be recognized that a carbon-carbon double bond (C=C) forms after cross-linking. The peak at 1950 cm^{-1} does not appear on the base or the curing agent's spectrum. To quantify the cross-linking density, the peak area was measured by triangulation. The area of the C=C peak was divided by the area of the CH_3 peak, yielding the transmittance ratio. The results are summarized in Figure 4.3d.

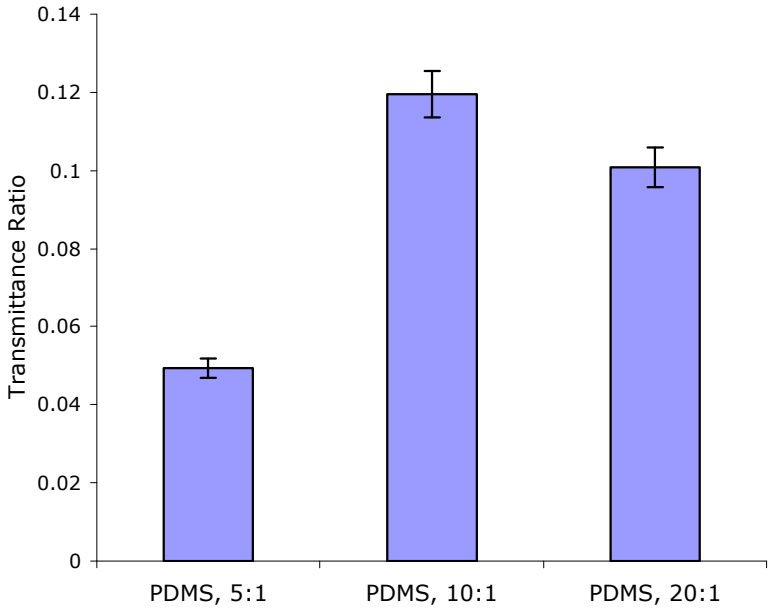

Figure 4.1d. Cross-linked PDMS versus Transmittance Ratio.

From Figure 4.1d, we can observe that 10:1 polymer-to-cross-linker ratio is the optimal one. The transmittance ratio doubles from 20:1 to 10:1, and it decreases for 5:1. The decrease for 5:1 symbolizes oversaturation of PDMS with the curing agent, which consequently inhibits cross-linking.

4.2 Tensile Test

Samples of varying cross-linker were analyzed with an Instron Tensile Tester. Young's Modulus, defined as the slope of the stress-strain curve at the yield point, is calculated and plotted as a function of cross-linking density in Figure 4.2a. The extension of the sample at yield is plotted in Figure 4.2b.

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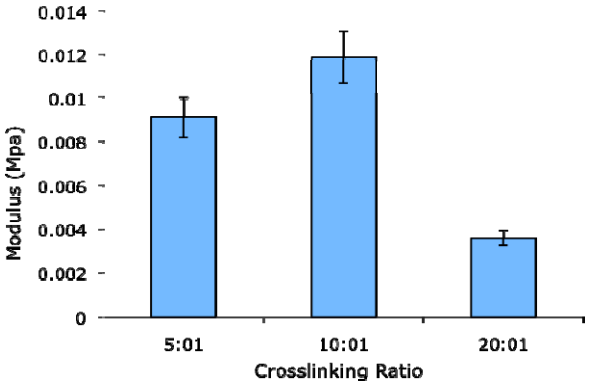

Figure 4.2a. Young's Modulus in PDMS Samples of 5:1, 10:1, and 20:1 Cross-linking Ratios

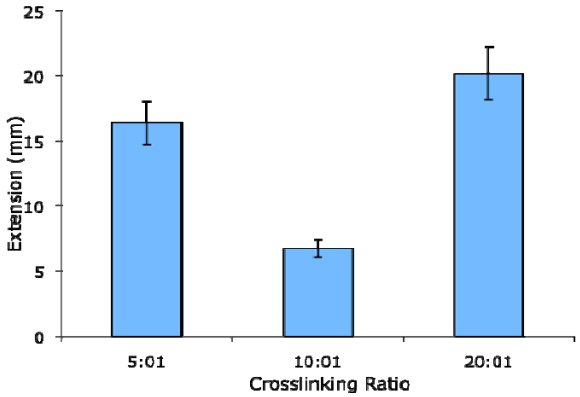

Figure 4.2b. Extension at Breaking Point in PDMS Samples of 5:1, 10:1, and 20:1 Cross-linking Ratios

This data suggests that PDMS with cross-linking density of 10:1 has the relatively highest modulus of 0.012 MPa compared to 5:1 and 20:1. The 10:1 sample had the shortest extension of 20.168 mm before yielding.

The 10:1 was chosen due to its highest modulus for mechanical analysis of varying clay concentrations. Figure 4.2c-d illustrates the Young's Modulus and extension at breaking point in PDMS samples of 0.5, 1, and 2 % clay concentrations. In the samples measured increased clay concentration resulted in a higher modulus material. All samples with nanocomposites had a higher modulus than those without.

In 10:1 samples, 1% clay exhibited the highest extension at breaking point, while 2% displayed the lowest. This suggests clay in amounts larger than 1% make PDMS more brittle than other samples, however still more elastic then samples without clay.

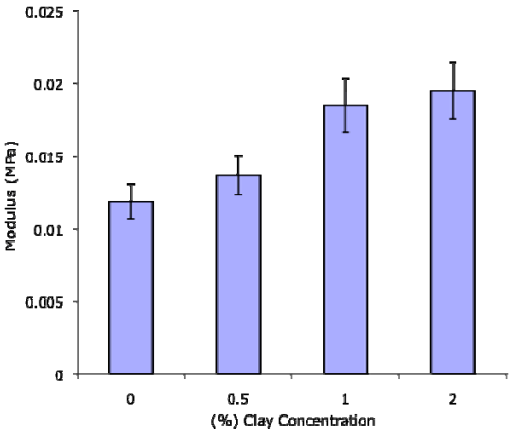

Figure 4.2c. Young's Modulus in 10:1 cross-linked PDMS Samples of 05, 1, 2% clay

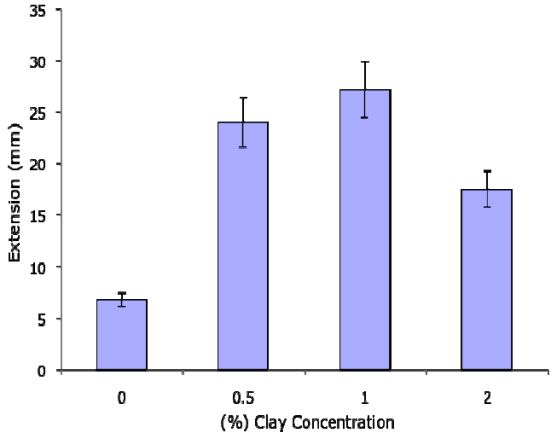

Figure 4.2d. Extension at Breaking Point of 10:1 cross-linked PDMS Samples of 0.5, 1, 2% clay

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4.3 Cell Count and Imaging

Cells were counted on Day 1 and Day 3 of incubation at 37°C. Three dishes were counted separately on each day to produce the growth curve in Figure 4.3a. The cell plating density was 2400 cells per cm^2. On Day 1, the 1% clay sample showed the most cell growth with 4350, followed by the 0.5% sample with 3950 cells. On Day 3, the 0.5% showed the most cell growth with 30950 cells, followed by the 1% sample with 29200.

Cells grew the least on pure PDMS with no clay, indicating the polymer itself is not beneficial for cell growth. The polystyrene dishes experienced higher cell growth than the PDMS alone.

All samples containing clays exhibited higher cell proliferation by day three than the PDMS alone and the cell culture dish, indicating scaffolds containing clays nanocomposites are not only cytofriendly but encourage cell proliferation.

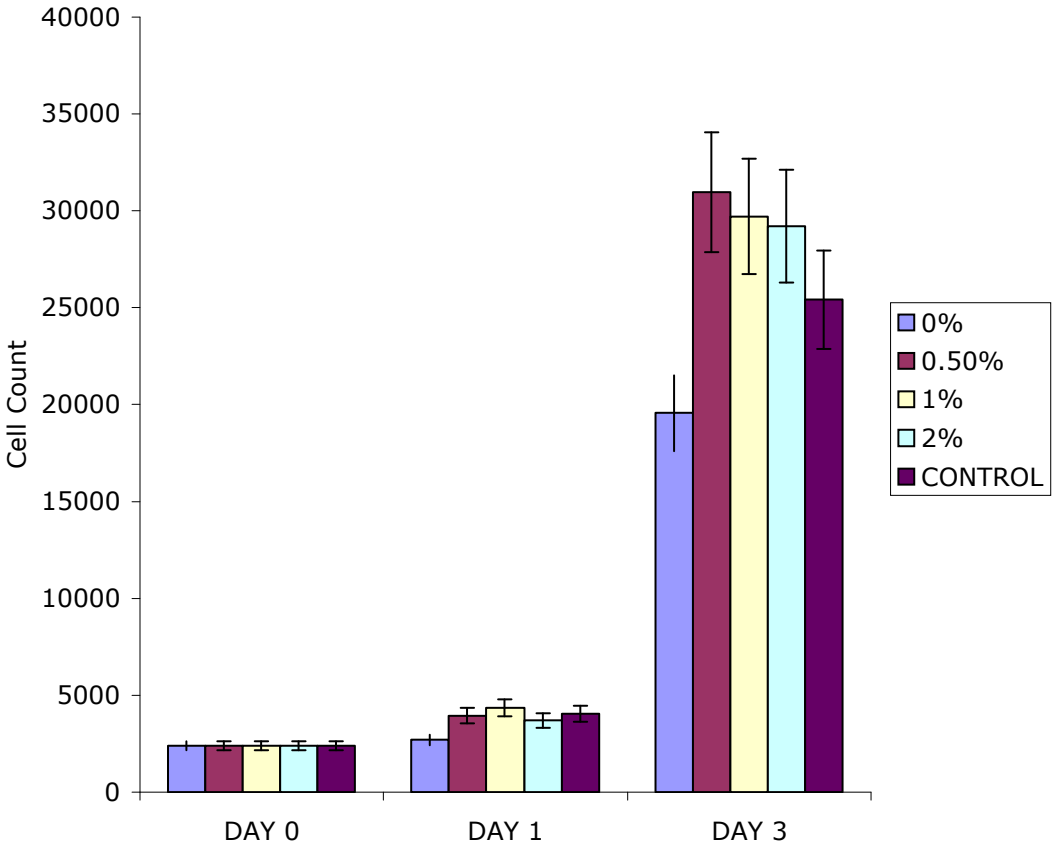

Figure 4.3a. Cell Cytotoxicity for Varying Concentrations of Sodium (Na) Clays in 10:1 Crosslinked PDMS

To determine the cell morphology the samples were examined under a Leica Confocal Microscope using a water lens at 20X magnification. These images may indicate the fibroblast have more preference for .5% clay based on the cell growth after three days.

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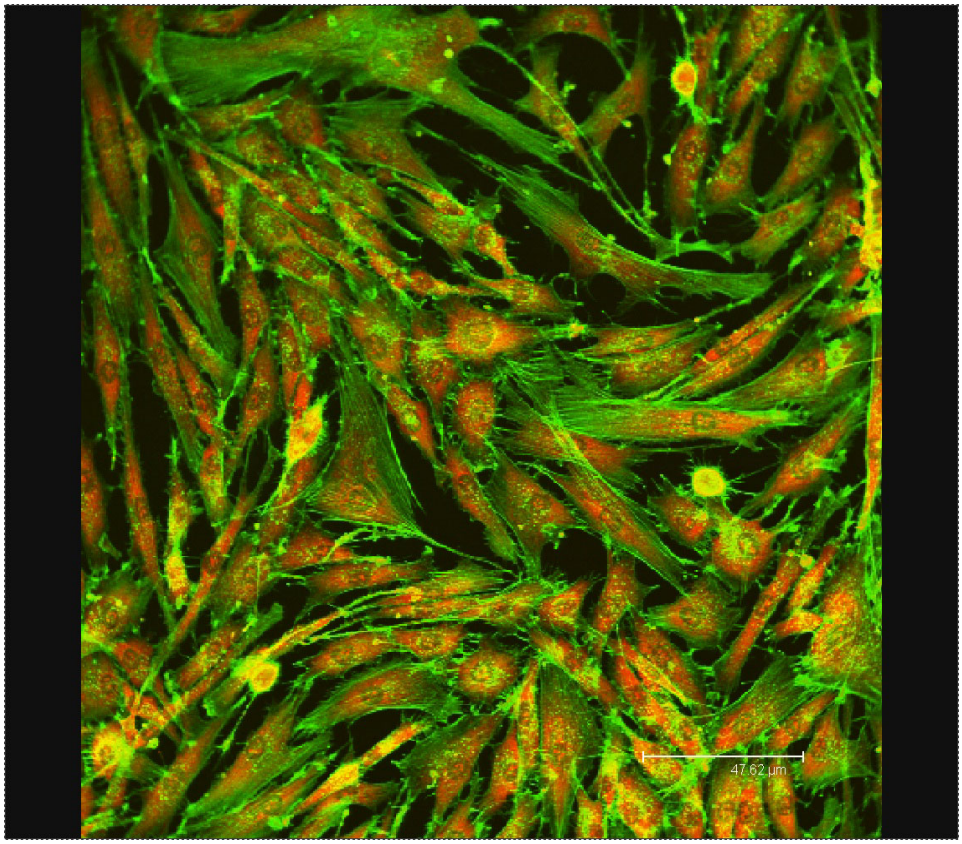

Figure 4.3b. Confocal Image, 5% clay on 10:1 PDMS

5. Conclusions

The FTIR spectra suggest that 10:1 cross-linking ratio yields the optimal cross-linking density for PDMS. The cross-link density increases up to 10:1 PDMS ratio, and then decreases for ratios larger than 10:1. A carbon-carbon double bond appears after the cross-linking reaction. This indicates that the base-curing agent bond contains a C=C bond.

Samples prepared with 10:1 cross-linking ratio had highest Young's Modulus of al10:1 cross-linked PDMS increased the Young's Modulus samples analyzed, whereas 20:1 had the lowest. Adding sodium Cloisite clay nanoparticles to PDMS revealed an increase in modulus in all samples.

Experiment with cell culture reveals sodium clay nanocomposites are not only cytofriendly but encourage cell growth. Cell imagining on confocal microscope discloses normal cell morphology in the presence of clay.

This data may be valuable in tissue engineering when designing scaffolds with more favorable mechanical properties for cell proliferation. Future research will reveal more information on the cell morphology in reaction to varying crosslinking ratios and types of clay nanocomposites, in addition to more detailed mechanical analysis of the materials.

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6. References

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3. "Polydimethylsiloxane." Wiki in the Department of Chemistry. 7 Dec. 2006. Imperial College London. 28 Mar. 2008 <http://www.ch.ic.ac.uk/wiki/index.php/It:Polydimethylsiloxane>

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