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# Biomimetic Morphologies in Synthetic Polymers via Melt Processing of Co-continuous Immiscible Polymer Blends

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## Abstract

PLLA/PMMA polymer composites were studied as a potential biomaterial system in which properties are adjusted by altering the blend ratio and/or the molecular weights of the end members. Blend compositions were prepared by extrusion near the co-continuous point as determined by empirical rheology based immiscibility rules and characterized by several relevant methods. SEM analysis of etched blends shows a columnar co-continuous morphology in which the domain size is approximately 20 – 30 μm. The architecture appears useful for tissue engineering applications either as scaffolding or implant material in which a transient phase is extracted by body fluids, enabling cell and tissue adhesion to the resultant porous surface. DMA and DSC analysis reveal that these blends have two $T_g$s and thus comprise an immiscible composite system, at least in the context of the extrusion environment. In addition to the $T_g$s of the end-members, the DSC spectra also revealed a broad, diffuse $T_g$ band centered around 80 $^oC$, which appears to be the marker of a new phase formed in-situ. Biocompatibility factors were studied in a preliminary sense by evaluating bone and muscle cell viability and adhesion and by testing the resilience of the blend elastic modulus in phosphate buffer solution at 37 $^oC$ over a 65 day period. Cell viability and adhesion performance was good and elastic modulus was retained over a two month test period.

## Introduction

Thermo-mechanical mixing of two or more polymers is a preferred method for new material development since it does not involve costly synthesis while process scale-up can be achieved quickly and inexpensively. Under optimal processing conditions, thermal polymer blends may exhibit synergistic and advantageous properties [1]. Miscible blends and/or compatibilized immiscible blends are the main objectives for thermal blending, but uncompatibilized immiscible blends have recently gained significant attention [2] due to their ability to provide unexpected and commercially useful properties. Studies have shown that for certain applications such as bone fillers and tissue scaffolds, immiscible blends may have specific advantages in morphological and mechanical properties. Presently, it is believed that co-continuous nature of immiscible compositions gives rise to the synergistic properties due to the intimate interaction between the components.

Multiphase biopolymer blends in which one phase is transient and another phase is persistent can provide the ideal balance of phases for in-vivo use with the phase ratio modified to meet specific application requirements [3]. Processing of the blend is important and numerous physical properties of the polymers, particularly the rheology, are critical in determining the properties and morphology of the resultant blend. An additional issue that requires consideration is the nature of the phase interface in immiscible blends. A weak interface can have an adverse effect on the mechanical properties of the blend [4], whereas a strong mechanically clamped interface, or even the development of reaction products at the interface, can impart important functionality to the blend.

Our research group is interested in the processing and properties of immiscible polymer blends. Previous works have shown that thermal processing of immiscible polymers is

morphologically bio-mimetic and possess biocompatibility. For previous work as well as work described in this paper, we have selected PLLA and PMMA to impart the blends with biodegradability and persistent mechanical support, respectively, over time. Unlike other immiscible systems such as PLLA/PCL blends [4], this system does not suffer from poor adhesion between phase, as evident in the synergism of the blends' mechanical properties shown later in this paper.

The objective of the present research was to exploit the special microstructures possible via immiscible polymer processing to produce a structure generally suitable for biomedical applications, with particular focus on hard tissue scaffolding and/or replacement materials based on in-vivo compatible immiscible polymers. In addition to the fabrication of polymer blends with useful architectures, preliminary experiments to investigate basic biological compatibility and cell adherence were part of this study. The goals of the preliminary experiments were three-fold: to understand if the cells could survive and grow when exposed to the polymer blends; to establish a procedure to measure adherence by the cells to the polymer blends; and thirdly, to determine if abnormal cell proliferation would result from cell contact with the polymer blends.

## Experimental Methods

*Materials and Blend Formulation*

Extrusion-grade PMMA was obtained in the form of clear pellets [GE Corporation, Pittsburgh, PA USA]. Medical-grade of poly(L-lactide) (PLLA), was obtained in the form of white granular powders from Boehringer Ingelheim Corporation [Ridgefield, CT 06877]. Two molecular weights of PLLA were obtained, L210 with a 200 $^{o}$C viscosity of 3739 Pa.s and L207S with a 200 $^{o}$C viscosity of 1563 Pa.s. Rheology measurements were performed on both polymers using a TA AR 2000 rheometer [TA Instruments, New Castle, DE, U.S.A] over a range

of shear rates for relevant temperatures. The data were used in predicting the composition range over which co-continuous blends are expected. The elected method is by Jordhamo [5], which states co-continuity exists when the following relationship between the volume fraction (Φ) ratio and the viscosity (η) ratio of the components at the processing temperature is satisfied:

$$\frac{\eta_A}{\eta_B} \cong \frac{\Phi_A}{\Phi_B} \tag{1}$$

The PLLA/PMMA composition that conforms to this relationship is 45% for Boehringer Ingelheim PLLA by volume as shown in Table I.

*Processing and Sample Preparation:*

The PLLA and PMMA polymers were dried for 47 hours in a vacuum oven (30 mmHg) at 45 °C and 70 °C, respectively, prior to processing. Batches of 200-g size were weighed out and melt processed in a 19-mm single screw laboratory extruder [C. W. Brabender, Inc., Hackensack, NJ] fitted with a mixing screw of 16.64 mm average root diameter. An average shear rate of 78.5 $s^{-1}$ was achieved at 200 $^{o}$C by operating the extruder at 100 revolutions per minute. No die was used, the extruded mass was collected as rods of 10 ± 2 mm diameter. Selected processed rods were cut into 1 x 2 x 20 mm segments for flexural 3-point bending test utilizing a dynamic mechanical analyzer (DMA) [Perkin-Elmer 7E, Wellesley, MA, USA]. Thin cross sections were sliced from extruded rods and trimmed with a sharp blade into smaller discs, approximately 10 mg in weight, for thermal analysis using Differential Scanning Calorimetry (DSC) [TA Instruments Q1000, New Castle, DE, USA].

*DSC, DMA, and image analysis*

Differential Scanning Calorimetry experiments were conducted in modulated mode (MDSC) to separate thermal (reversing, e.g. glass transition) from kinetics (non-reversing, e.g. crystallization) events. All runs included an initial heating, followed by a cooling and a reheating cycle. MDSC modulation parameters consisted of: amplitude = 2 °C, period = 40 s, ramp rate = 2 °C/minute and temperature range = 20 – 210 °C. The data shown here were taken from the reversing signal of the reheating cycle and shown as the derivative. Maxima of data represent inflection points on the original DSC thermographs, which indicated mid-point glass transition temperatures.

Dynamic mechanical analysis (DMA) using a Perkin-Elmer 7E helps determine the elastic modulus. Three-point bending flexural test was run on the prepared rectangular bars with loading applied dynamically at 1 Hz.

Scanning electron microscopy images were collected from fracture specimens with an Hitachi 2700 scope. Specimens were etched in dimethyl formamide for one minute to extract the more soluble phase and were then gold coated to reduce charging effects. Images were collected at 15 kV accelerating voltage both parallel and perpendicular to the extrusion axis.

*In-vitro mechanical degradation testing*

The DMA rectangular specimens were cleaned with Ethanol and dried under a sterile fume hood before submerging in individual 5-ml plastic vials containing sterile phosphate buffer solution (PBS) or deionized water with pH 7.0. Vials were kept in a water bath to maintain at 37 $^{o}$C and an agitation rate of 75 rpm to simulate physiological conditions. The samples were removed periodically for DMA measurements to determine the effect of these environments on the Young's modulus of the composites.

*Cell Viability and Adhesion*

Available muscle (C2C12), and bone (MC3T3-E1) cells were selected for the experiments, to mimic the soft and hard tissue exposures that would be anticipated under bone replacement conditions. Untreated cell, positive (12-O-tetradecanoyl phorbal 13 acetate, tumor growth accelerant), and negative (cycloximide, growth inhibitor) controls were included as experiment treatments to provide baselines, along with assay reagent controls. The experiments were run one time to give an indication of whether further in-depth experimentation should be continued. Although experiments were run once, cell and control treatments were run in triplicate.

Seven polymer blend treatments were included in the cell culture experiments: pure PMMA, pure PLLA-210, and pure PLLA-207S represented polymer blend controls; PLLA-210/PMMA, and PLLA-207S base blends; PLLA-210/PMMA/ hydroxyapatite (HA), and PLLA-207S/PMMA/HA. The disks measured 7mm diameter by 1mm thickness.

Cell viability was measured by adenosine 5'-triphosphate (ATP) quantitation against controls. Supernatant from primary cell detachment was assayed once from each of the two cell types to ascertain positive growth, by reading through a luminometer.

Disks of polymer blends could not be analyzed for adherence by microscopy, due to the opacity of the blend depth, or thickness of 1 mm. After cells were detached from plates, disks of polymer blends were removed from wells, and isolated in 1.5ml centrifuge capsules. Adherence was determined by counting cells, which remained in disk inclusions after all cells were trypsinized from the plate wells. The isolated disks were further trypsinized, centrifuged, and the cells resuspended for hemocytometric quantitation.

## Results and Discussion

The PLLA/PMMA blend system processed uneventfully and samples were prepared over a narrow composition range near the co-continuous composition without difficulty. Immediately upon cooling, however, it was clear that this immiscible polymer blend system is different from most in that the extrudates were clear (figure 1) as compared to the milky, opaque specimens obtained from more traditional immiscible blend systems such as PS/HDPE or PMMA/PP. The clarity of the blends is also surprising considering the opacity of the neat PLLA. For such optical clarity to occur, light scattering centers must be absent or minimal, such as crystalline or immiscible phase interfaces. The possible reduction in crystallinity in these blends and the possibility of partial miscibility at the blend interface are interesting topics for study.

The SEM images (figure 2a and 2b) are remarkable in that they illustrate a dramatic architecture that was not unexpected from processing of immiscible polymer blends. The DMF etching as removed one phase and the remaining phase exhibits a continuous three-dimension morphology that surrounds the second phase (removed by etching in these images) which is also continuous in three dimensions. Furthermore, the structure and dimensions of the etched, or transient, phase is, to a first order approximation, similar to bone tissue and various forms of hard tissue scaffolding using in biomedical technology. The channels for a columnar structure traversing the specimen in the longitudinal extrusion axis dimension and the diameter of these channels is 20 – 30 μm, close to the 40 μm size of osteoblasts and other cells typically comprising hard tissue. Although the present work was exploratory and preliminary in nature, the results strongly suggest that immiscible polymer processing is a viable route to controlling pore size and volume fraction in biopolymer composites. Furthermore, the anisotropic structure

of these blends appears consistent with scaffolding and tissue compatibility architectures determine through cell viability studies.

From a polymer science perspective the results of this study shed interesting light on the physical nature of this immiscible polymer blend system. Given the transparent nature of these composites, the most logical question is: is this really an immiscible polymer system? Applying the criterion that immiscible binary blends possess two glass transitions, the answer is "yes," as demonstrated by DMA $T_g$ data in figure 3. Two transitions clearly exist, one at low temperature (~81 $^oC$) corresponding to PLLA and a second at high temperature (~116 $^oC$) corresponding to PMMA. These are DMA and the transition temperatures are dependent on the test procedure, but DMA is the most sensitive method for $T_g$ determination and the results clearly show two $T_g$s in this system.

More detailed information of the $T_g$s is obtained from modulated DSC analysis as shown in figures 4 and 5. Data from DSC runs on the neat polymers (figure 4a) shows the native $T_g$ behavior of PLLA (60 $^oC$) and PMMA (105 $^oC$). The profile at the top of the figure is the heat flow (left axis) and the derivative of the heat flow is shown at the bottom of the figure (right axis) to enable more accurate determination of the $T_g$. Similar measurements with similar results were obtained with PLLA 210 (figure 4b). The DSC data for the blends shows interesting and somewhat unusual behavior (figure 5). The glass transition of PLLA 207S (fig 5a) and PLLA 210 (fig 5b) are strongly evident at 63 $^oC$, as expected, but a broad, diffuse, unexpected $T_g$ signal is observed in the range of 65 – 100 $^oC$, and the PMMA $T_g$ at 105 $^oC$ is extremely faint. Both PLLA 207S and PLLA 210 show this behavior. The five compositions studied in this work and graphed in these figures are consist of the co-continuous composition bracketed by lower and higher compositions with ±10% PLLA levels as detailed in Table I. Within this narrow

compositional range, it is clear from the DSC traces that the phase generating the diffuse intermediate $T_g$ signal increases or decreases out of phase with the PLLA signal. Or, stated another way, the product of the two signals is approximately constant. This relationship offers the preliminary suggestion that these two phases constitute a product/reactant relationship. Further studies will be needed to clarify these new findings.

*Biocompatibility factors*

Cell viability and cell adhesion studies showed promising, if preliminary, results. Table II presents the hemocytometer count triplicate averages, and luminescence readings for each polymer blend treatment by cell type. Although mean differences between the data were not replicated sufficiently to ascribe statistical differences or significance, trends were observed among cell types between polymer blends. All cell treatments grew and survived exposure to all polymer blend treatments (Table II, luminescence). The glassy surface of pure PMMA would seem to restrict the attachment or adherence of both muscle and bone cells, when compared to pure PLLA polymers and blends. Also, bone cells tended to adhere in greater numbers than muscle cells (Table II, adherence). It is also of interest to note a distinction between PLLA-207 and PLLA-210 exposure to cells. The attachment of both muscle and bone cells seems to be higher when exposed to PLLA-210 over PLLA-207. Although preliminary data suggest that the polymer blends do not inhibit the growth and development of muscle and bone cells when exposed to polymer blends *in vitro*, further study looking at viability, adherence, proliferation, and other factors is warranted.

If PLLA/PMMA blends are to be used in-vivo, then the long term effect of body fluids on the structural properties is essential. In one manifestation of biomaterial application, body fluids attack the transient phase of the blend, presumably PLLA, and extract the phase to generate

morphologies similar to those shown in figure 2. Tissue will then grow into the continuous columnar pore to provide enhanced tissue bonding. As such, the elastic modulus of the blend will decline since it is becoming more porous, but the increasing incorporation of cellular matter and tissue will provide counterbalancing modulus contribution.

The effects of 65 days at 37 $^{o}$C in a phosphate buffer solution on the elastic modulus of the co-continuous composition compared to the neat polymers is shown in figure 7. Overall, there is very little change in modulus, which appears to be beneficial to the long term load carrying capacity of these blends in-vivo. However, these results may also mean that the degree of transient phase extraction is minimal and lower molecular weight PLLA polymers may yield better results in this regard. In any event, the 3.45 GPa initial modulus of PLLA and, coincidentally, of PMMA is reduced only slightly over the duration of the experiment. Perhaps the most interesting outcome of this segment of the study is that the neat PMMA material exhibited increasing modulus over time in this environment. In all cases, a measurable shift of PMMA modulus from 3.45 GPa to 3.55 GPa was observed over the 65 day period.

**Summary and Conclusions**

Melt processing by extrusion of PLLA/PMMA blend compositions near the co-continuous composition as determined by empirical rheology based immiscibility rules yields clear blends with little visual evidence of crystallinity or immiscibility. SEM analysis of etched co-continuous blends shows an interesting columnar co-continuous morphology in which the domain size is approximately 20 – 30 μm. The architecture appears useful for tissue engineering applications either as scaffolding or implant material in which a transient phase is extracted by body fluids, enabling cell and tissue adhesion to the porous surface. DMA and DSC analysis reveal that these blends have two $T_g$s and are thus immiscible, at least in the context of the

extrusion environment. In addition to the $T_g$s of the end-members, the DSC spectra also revealed a broad, diffuse $T_g$ band, centered around 80 $^oC$, which appears to be the marker of a new phase formed in-situ.

Biocompatibility factors were studied in a preliminary sense by evaluating bone and muscle cell viability and adhesion, and by testing the resilience of the blend elastic modulus in neutral phosphate buffer solution at 37 $^oC$ over a 65 day period. Cell viability and adhesion performance was good for the blends, easily surpassing the performance of neat PMMA, and test bar elastic modulus was virtually unchanged over a two month test period. Overall, this blend system appears to be a promising system for the engineering of in-vivo compatible blends in which the pore structure and volume fraction can be readily and inexpensively adjusted to meet application requirements.

**Acknowledgements**

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**Table I:**
**Viscosity data measured from AR-2000 rheometer.**
**Data used to predict co-continuous compositions at 200 $^{o}$C**

| Raw Materials: | PLLA L210 | PLLA L207S | PMMA |
|---|---|---|---|
| **Density (g/cm$^3$)** | 1.25 | 1.25 | 1.18 |
| **Viscosity (Pa.s)** | 3739.2 | 1562.7 | 3989.1 |
| **$\eta$(PLLA) / $\eta$(PMMA)** | 0.937 | 0.392 | |
| **Co-continuous composition, volume percent PLLA** | 48.4 | 28.1 | |
| **Co-continuous composition, weight percent PLLA** | 49.8 | 29.3 | |

Note: Viscosity values in this table were obtained at T = 200 $^{o}$C and $\gamma$ = 78.5 s$^{-1}$

**Table II:**

**Preliminary experiments observing muscle and bone cell adherence measured by hemocytometer, and growth indicated by ATP in media measured by luminescence.**

| Blend Composition | Cells | Adherence | Luminescence |
|---|---|---|---|
| PMMA | C6C12 | 8.5 | 576.0 |
| PLLA L-210 | C6C12 | 123.5 | 511.0 |
| PLLA L-207S | C6C12 | 104 | 490.0 |
| L210/PMMA | C6C12 | 166.5 | 474.0 |
| L207/PMMA | C6C12 | 93.5 | 614.3 |
| L210/PMMA/HA | C6C12 | 115.5 | 646.4 |
| L207S/PMMA/HA | C6C12 | 27 | 756.0 |
| | | | |
| PMMA | MC3T3-E1 | 14 | 543.2 |
| PLLA L-210 | MC3T3-E1 | 55 | 558.3 |
| PLLA L-207S | MC3T3-E1 | 351 | 411.8 |
| L210/PMMA | MC3T3-E1 | 339 | 326.8 |
| L207/PMMA | MC3T3-E1 | 303 | 444.0 |
| L210/PMMA/HA | MC3T3-E1 | 650 | 562.6 |
| L207S/PMMA/HA | MC3T3-E1 | 92 | 539.6 |

## Figure Captions

**Figure 1:** PLLA/PMMA immiscible blend composites illustrating optical clarity.

**Figure 2a:** SEM image of PLLA/PMMA immiscible blend composites etched to show architecture of blend after transient phase is removed. Sample sectioned perpendicular to extrusion axis.

**Figure 2b:** SEM image of PLLA/PMMA immiscible blend composites etched to show architecture of blend after transient phase is removed. Sample sectioned parallel to extrusion axis.

**Figure 3:** Two glass transitions observed in PLLA/PMMA blends by DMA. The low temperature $T_g$ corresponds to PLLA and was measured by 3-point bending. The high temperature $T_g$ was measured by parallel plate methods and corresponds to PMMA.

**Figure 4a:** DSC data for neat end-member polymers PLLA 207S and PMMA showing glass transitions for each.

**Figure 4b:** DSC data for neat end-member polymers PLLA 210 and PMMA showing glass transitions for each.

**Figure 5a:** DSC derivative heat flow profile of PLLA/PMMA blends in the vicinity of the co-continuous composition for blends prepared with PLLA 207S.

**Figure 5b:** DSC derivative heat flow profile of PLLA/PMMA blends in the vicinity of the co-continuous composition for blends prepared with PLLA 210.

**Figure 6a:** Image of osteoblast cell line MC3T3-E1 used in cell compatibility testing. (200X).

**Figure 6b:** Image of myoblast cell line C2C12 used in cell compatibility testing. (200X).

**Figure 7a:** Elastic modulus retention under aging in PBS at 37 $^{o}$C for up to 65 days. Abscissa is weight fraction of PLLA 207S in PLLA/PMMA blend.

**Figure 7b:** Elastic modulus retention under aging in PBS at 37 $^{o}$C for up to 65 days. Abscissa is weight fraction of PLLA 210 in PLLA/PMMA blend.

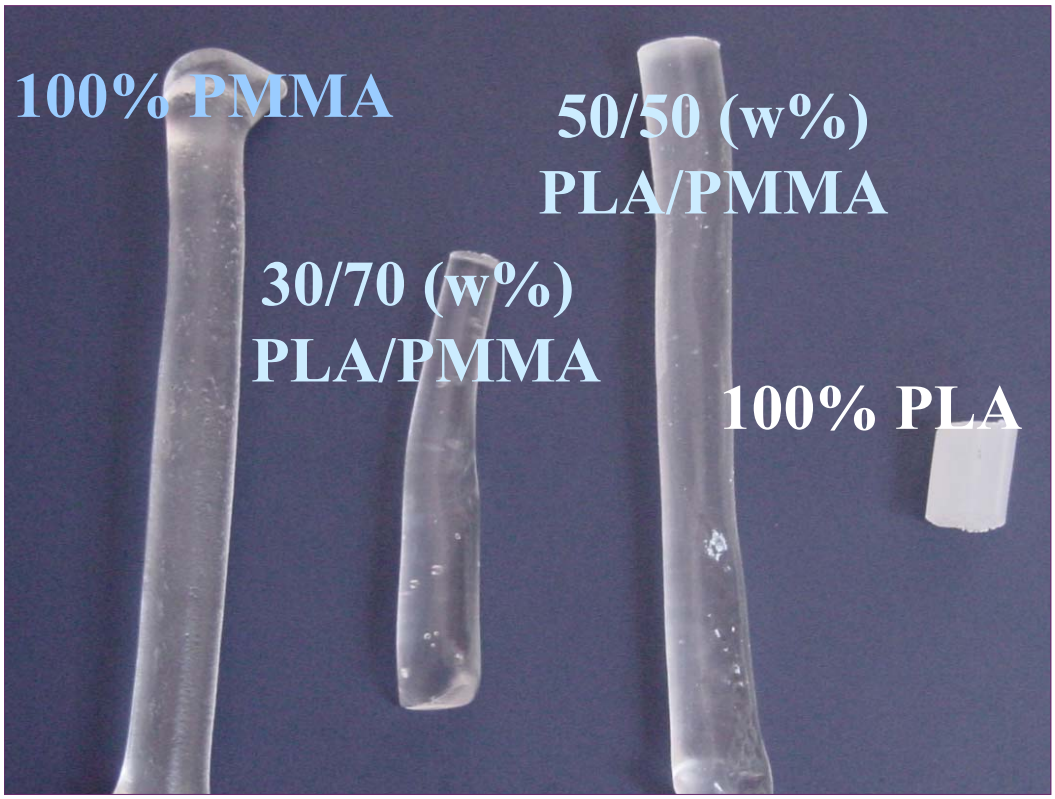



**Figure 1:** PLLA/PMMA immiscible blend composites illustrating optical clarity.

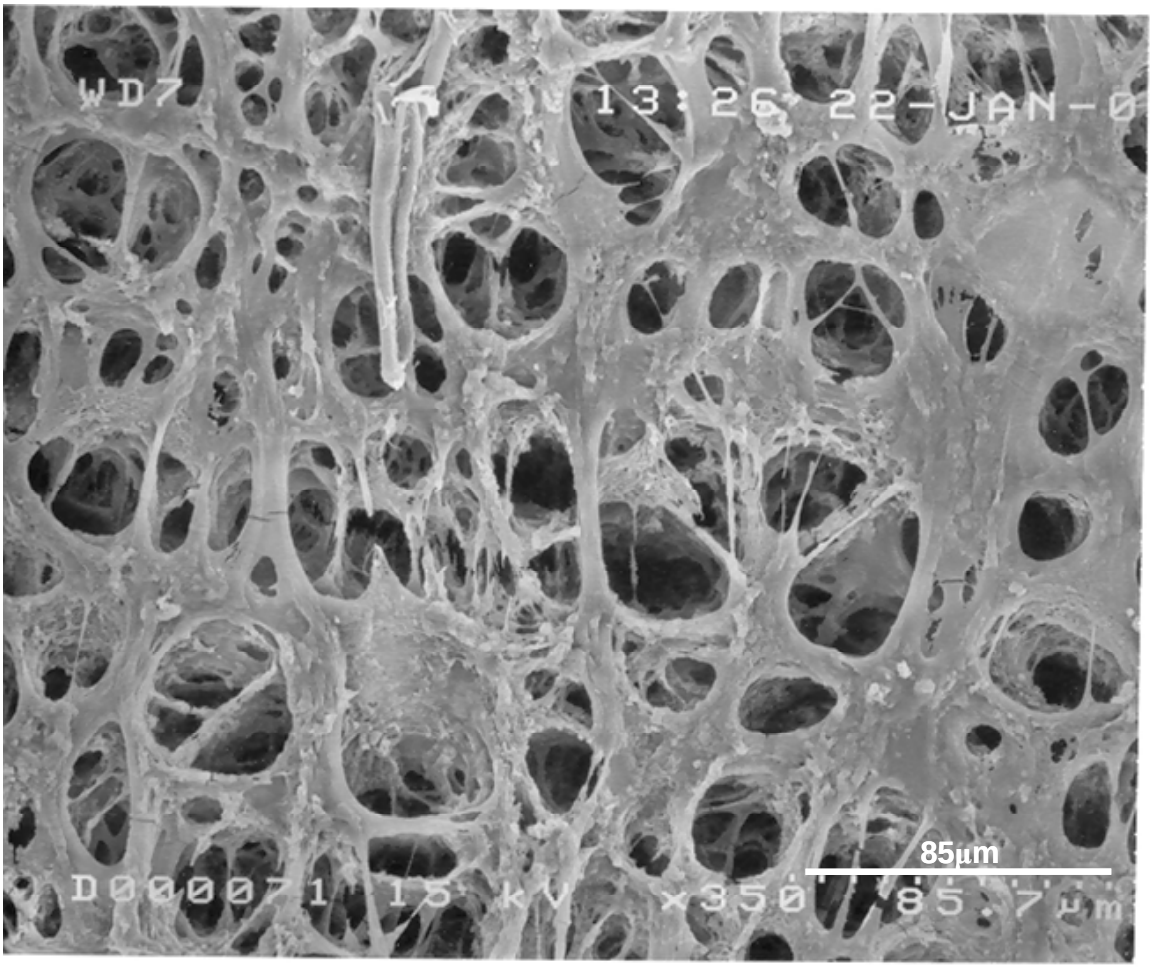



**Figure 2a:** SEM image of PLLA/PMMA immiscible blend composites etched to show architecture of blend after transient phase is removed. Sample sectioned perpendicular to extrusion axis.

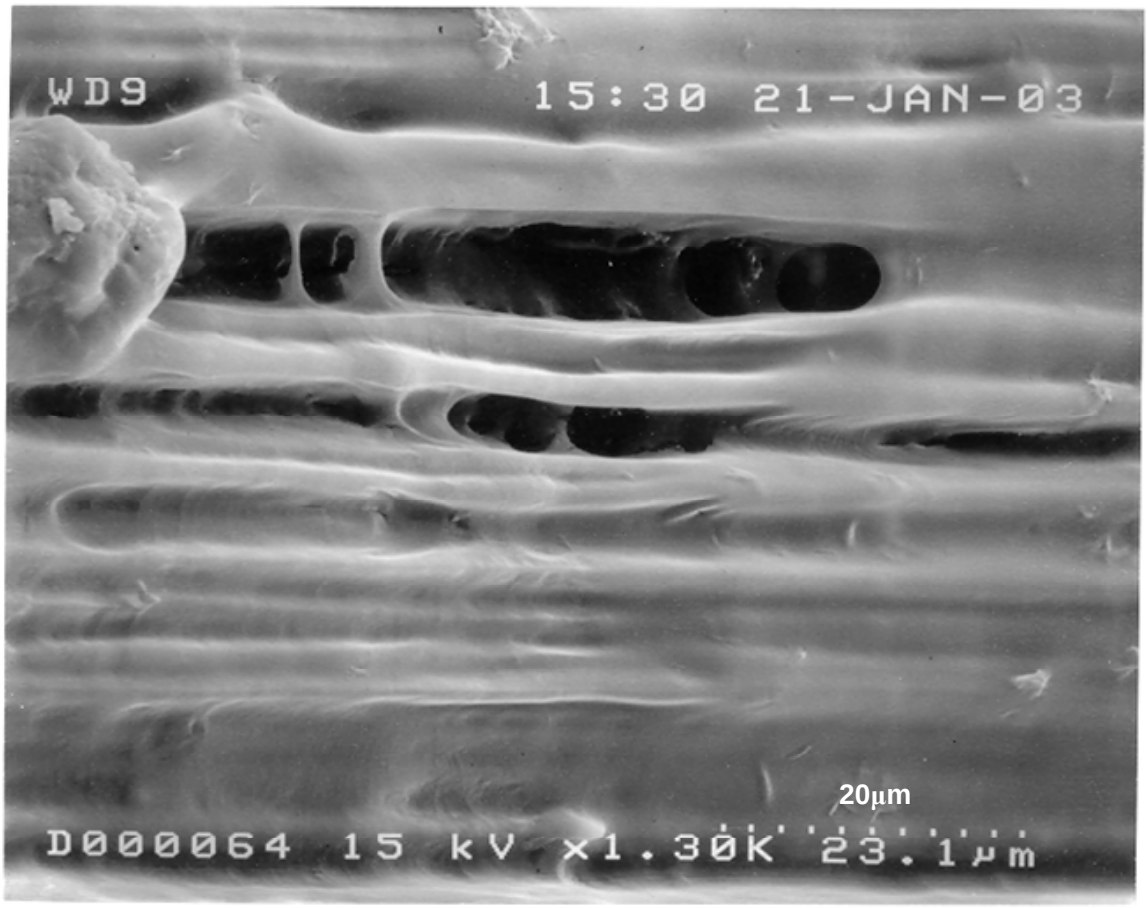



**Figure 2b:** SEM image of PLLA/PMMA immiscible blend composites etched to show architecture of blend after transient phase is removed. Sample sectioned parallel to extrusion axis.

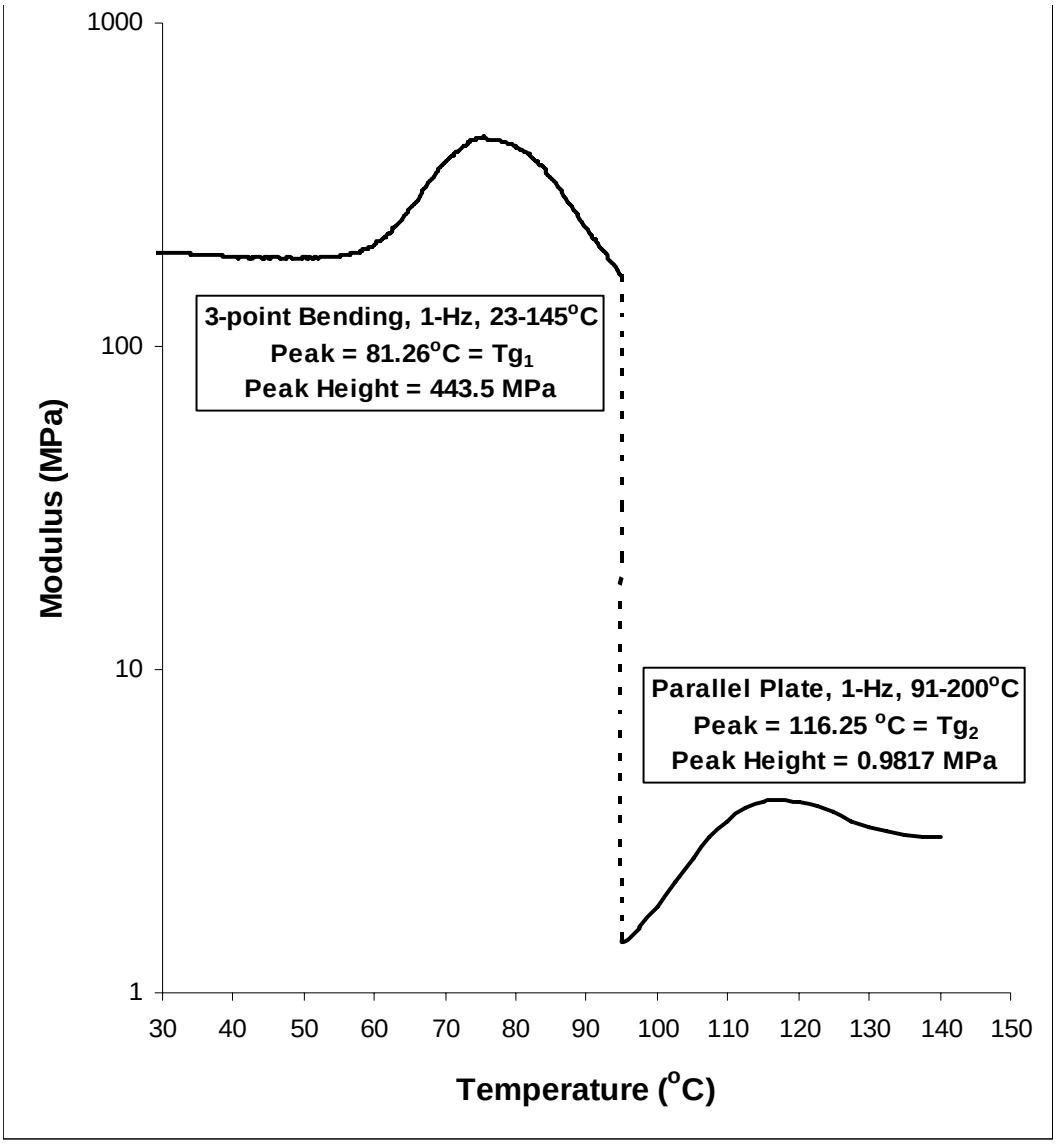
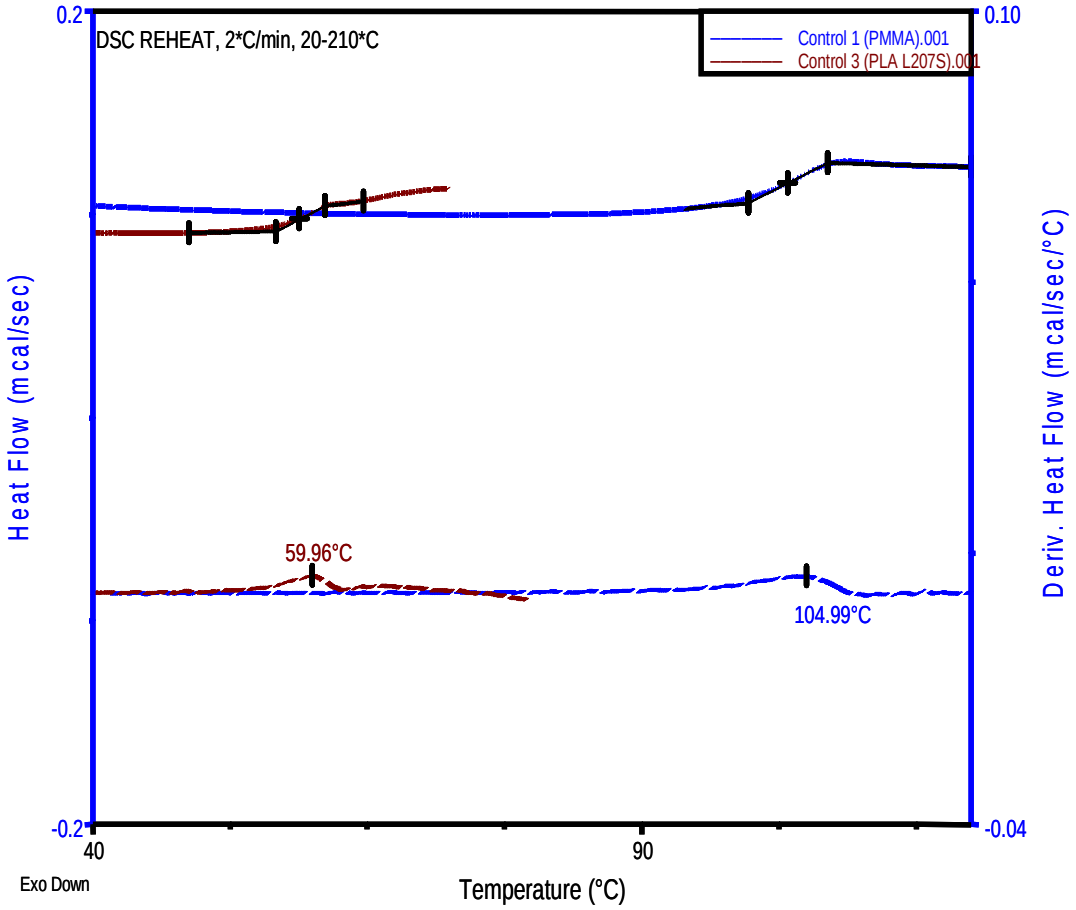



**Figure 3:** Two glass transitions observed in PLLA/PMMA blends by DMA. The low temperature $T_g$ corresponds to PLLA and was measured by 3-point bending. The high temperature $T_g$ was measured by parallel plate methods and corresponds to PMMA.

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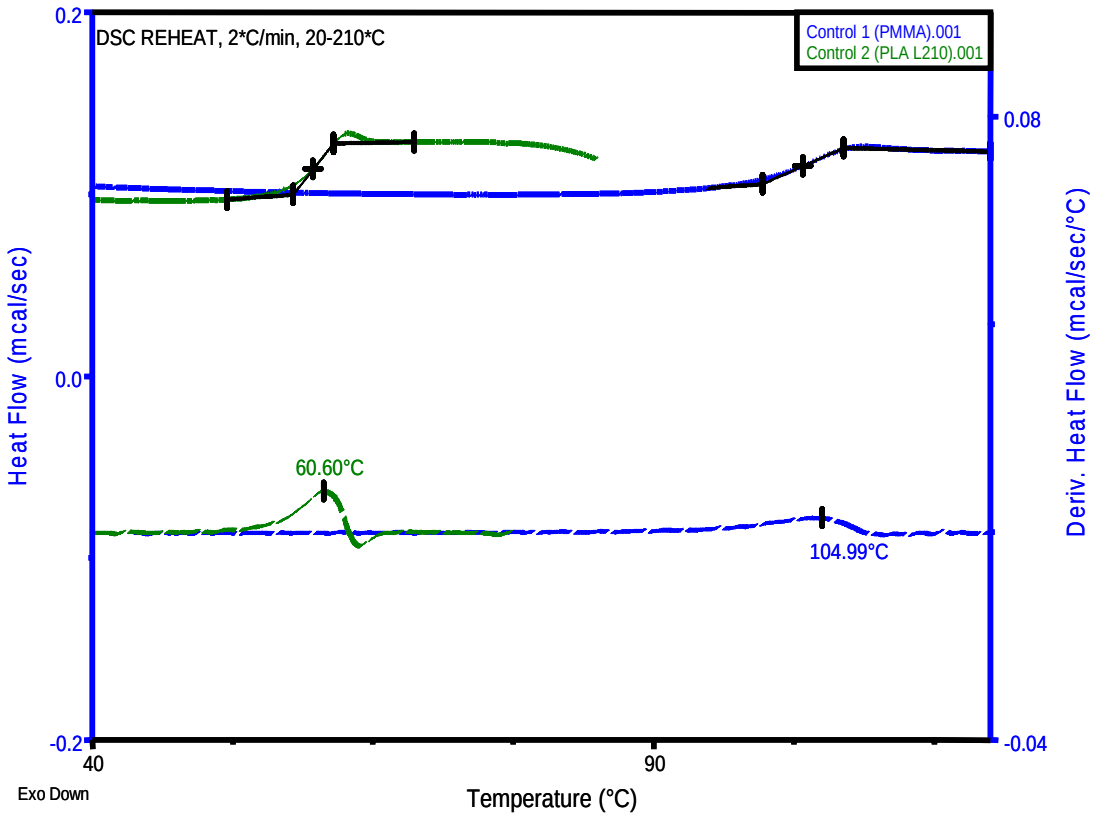



**Figure 4b:** DSC data for neat end-member polymers PLLA 210 and PMMA showing glass transitions for each.

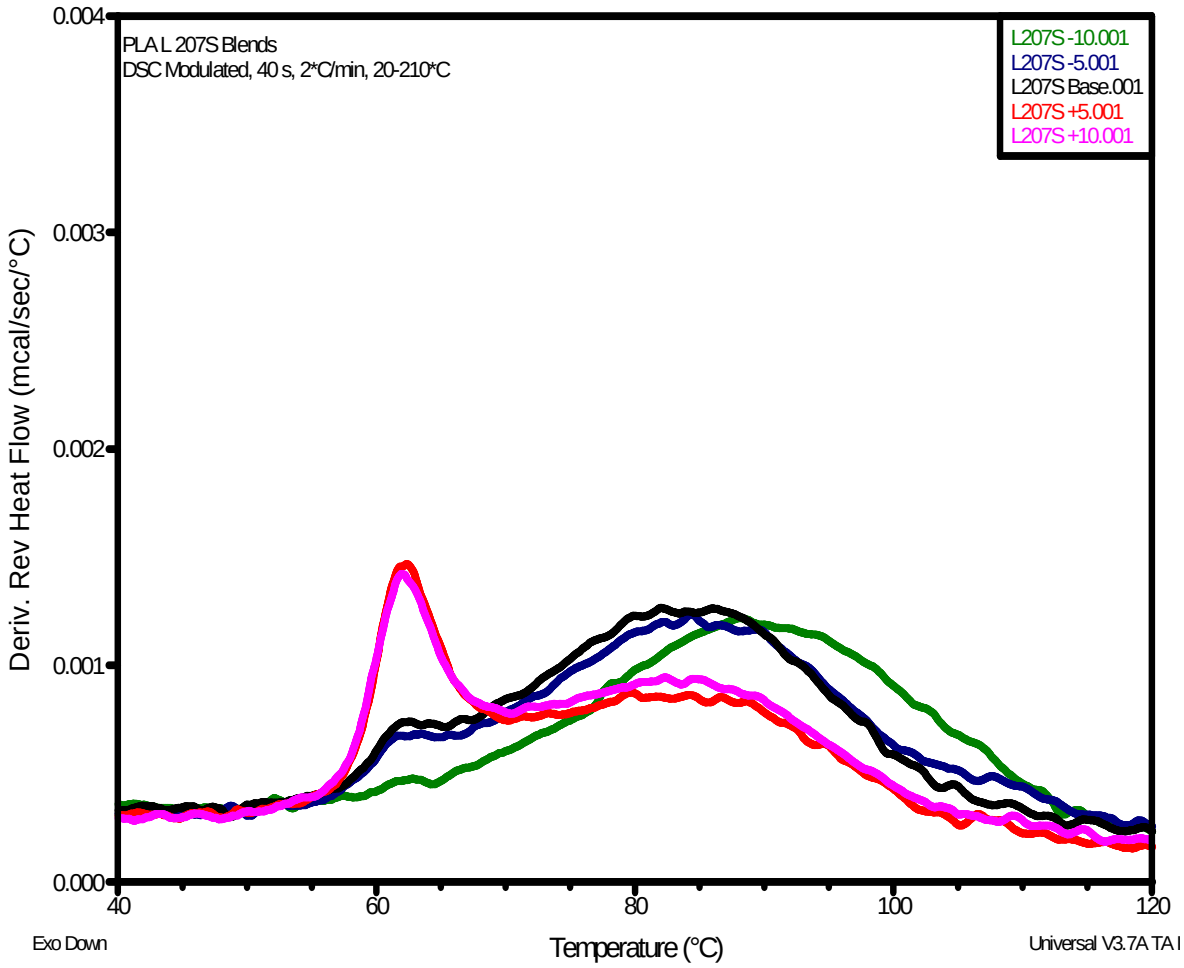



**Figure 5a:** DSC derivative heat flow profiles of PLLA/PMMA blends in the vicinity of the co-continuous composition for blends prepared with PLLA 207S

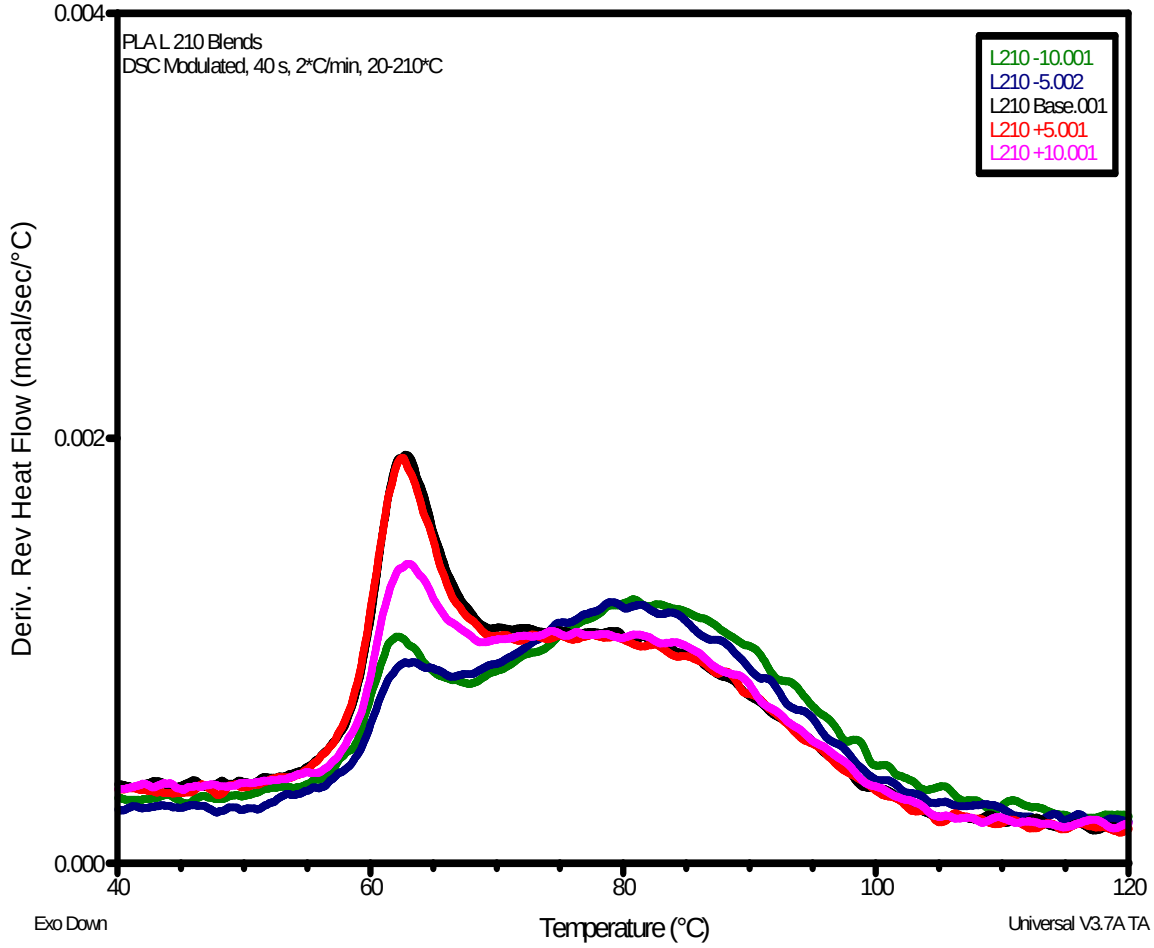



**Figure 5b:** DSC derivative heat flow profile of PLLA/PMMA blends in the vicinity of the co-continuous composition for blends prepared with PLLA 210

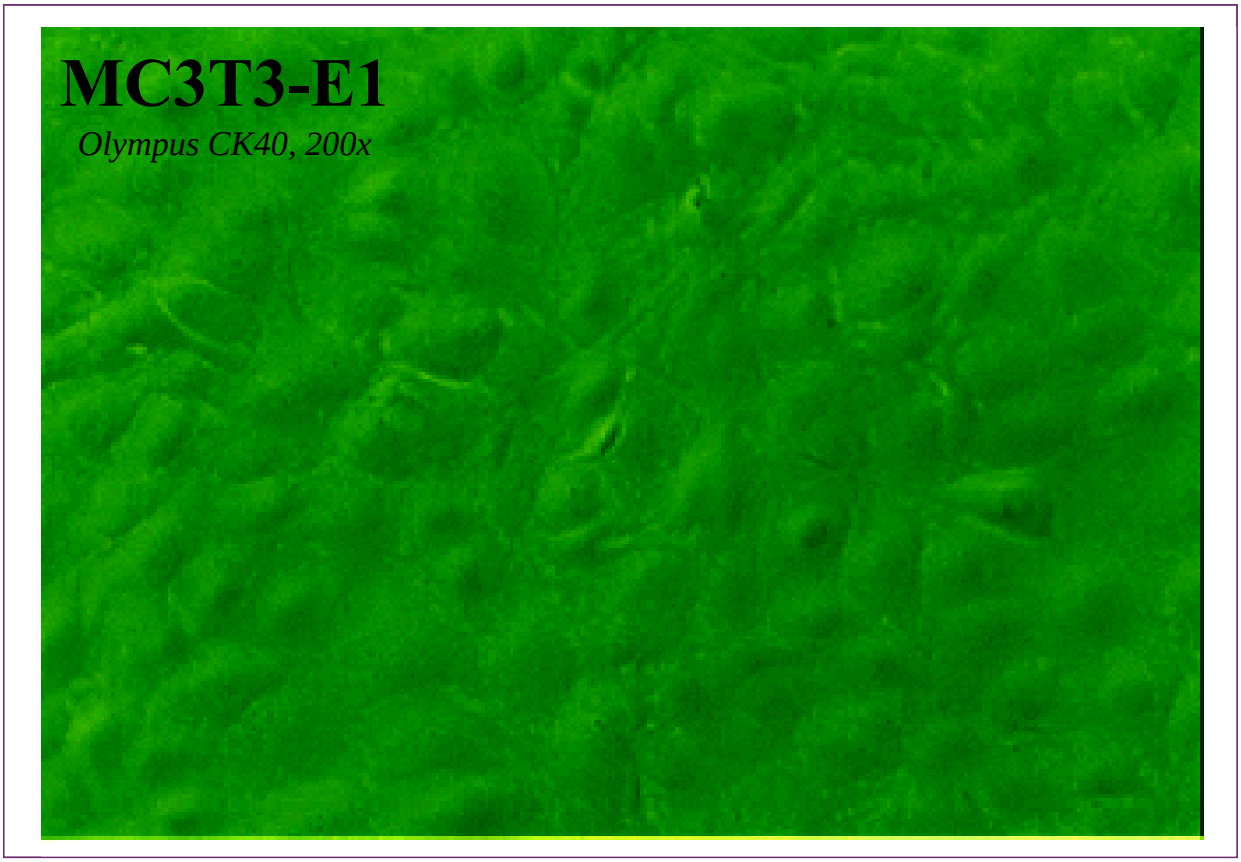



**Figure 6a:** Image of osteoblast cell line MC3T3-E1 used in cell compatibility testing. (200X)

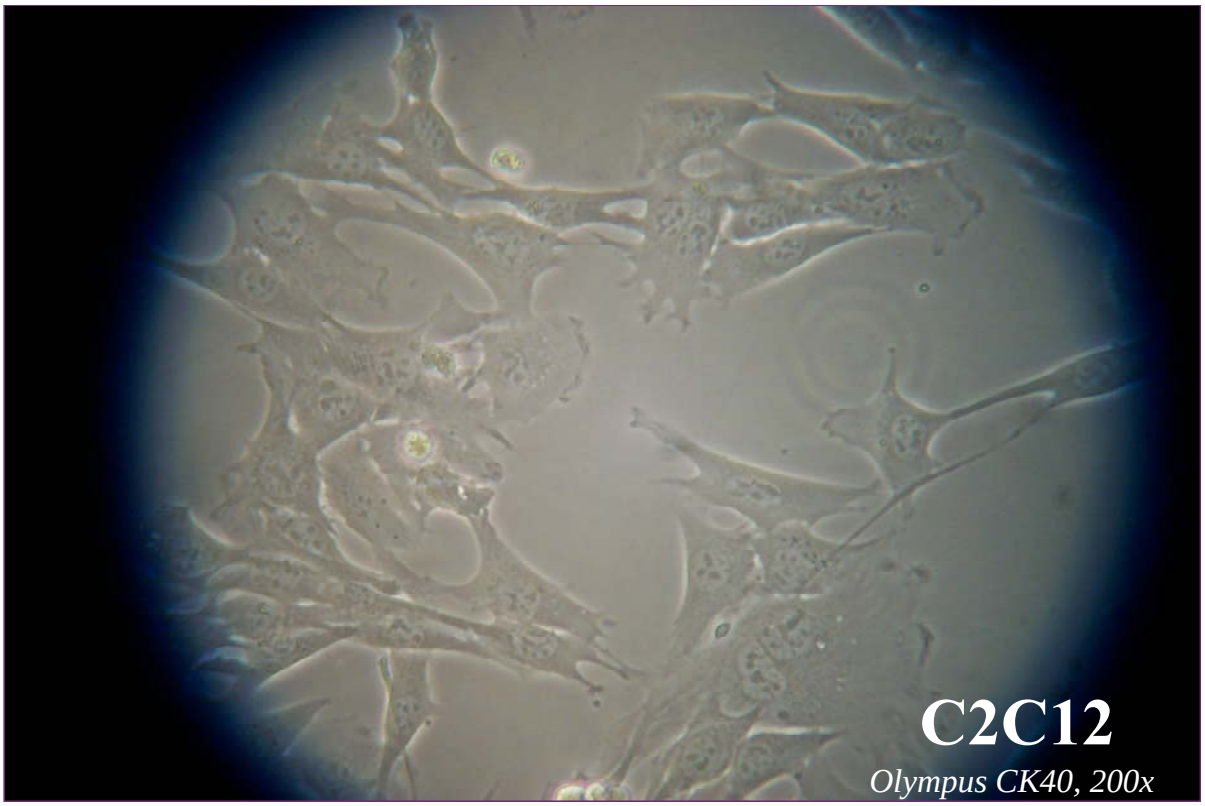
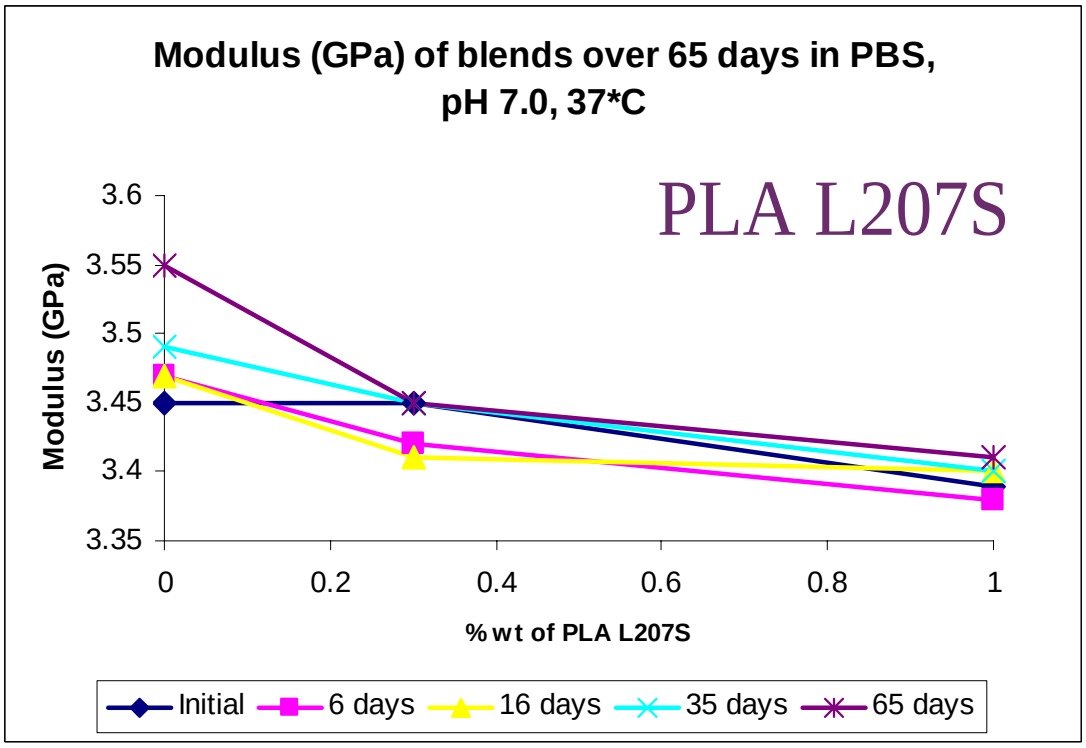



**Figure 6b:** Image of myoblast cell line C2C12 used in cell compatibility testing. (200X)

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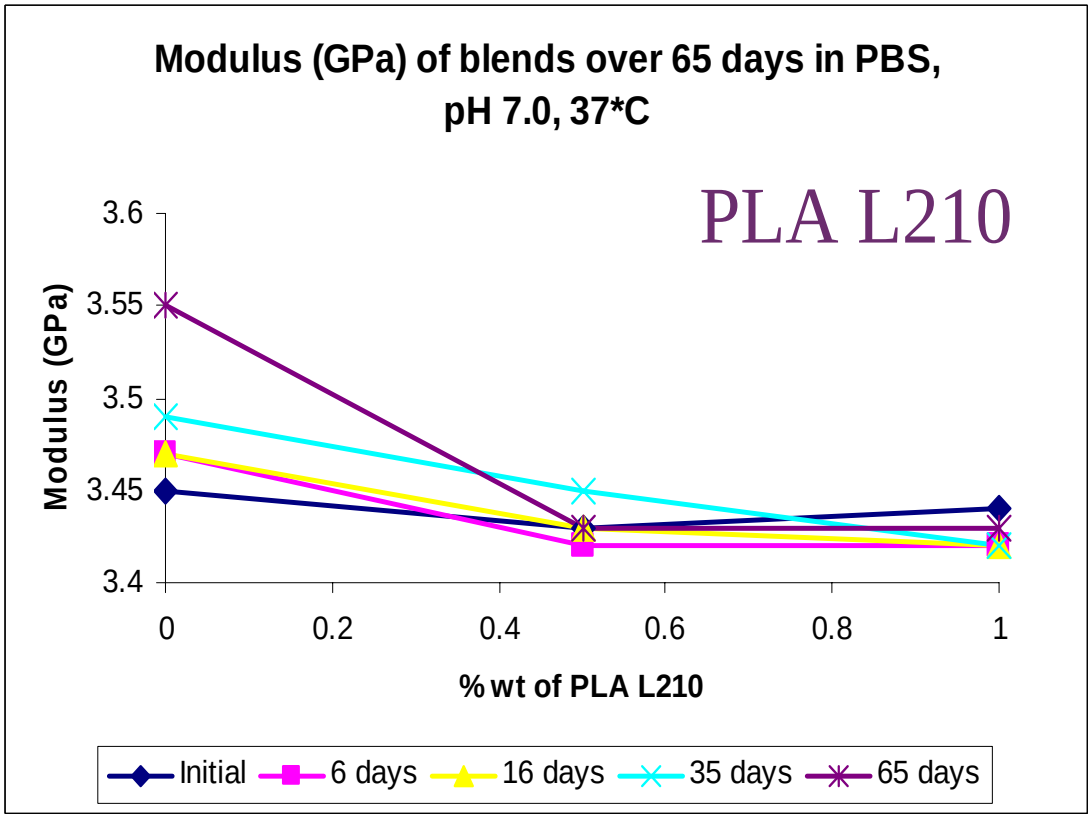


**Figure 7b:** Elastic modulus retention under aging in PBS at 37 $^{o}$C for up to 65 days. Abscissa is weight fraction of PLLA 210 in PLLA/PMMA blend.