Solvent effects on the microstructure and properties of 75/25 poly(D,L-lactide-*co*-glycolide) tissue scaffolds

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Abstract: Poly(lactide-*co*-glycolide) (PLGA) is used in many biomedical applications because it is biodegradable, biocompatible, and FDA approved. PLGA can also be processed into porous tissue scaffolds, often through the use of organic solvents. A static light scattering experiment showed that 75/25 PLGA is well solvated in acetone and methylene chloride, but forms aggregates in chloroform. This led to an investigation of whether the mechanical properties of the scaffolds were affected by solvent choice. Porous 75/25 PLGA scaffolds were created with the use of the solvent casting/particulate leaching technique with three different solvents: acetone, chloroform, and methylene chloride. Compression testing resulted in stiffness values of 21.7 ± 4.8 N/mm for acetone, 18.9 ± 4.2 N/mm for chloroform, and 30.2 ± 9.6 N/mm for methylene chloride. Permeability testing found values of $3.9 \pm 1.9 \times 10^{-12}$ m^2 for acetone, $3.6 \pm 1.3 \times 10^{-12}$ m^2 for chloroform, and $2.4 \pm 1.0 \times 10^{-12}$ m^2 for methylene chloride. Additional work was conducted to uncouple polymer/solvent interactions from evaporation dynamics, both of which may affect the scaffold properties. The results suggest that solvent choice creates small but significant differences in scaffold properties, and that the rate of evaporation is more important in affecting scaffold microstructure than polymer/solvent interactions.
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Key words: PLGA; microstructure; scaffold; permeability; compression

INTRODUCTION

Poly(α-hydroxy acids) are a commonly investigated class of biomaterials used in many applications, including scaffolds for tissue engineering. Poly(lactic acid), poly(glycolic acid), and a copolymer of the two, poly(lactic-*co*-glycolic acid) (PLGA), are the most encountered members of this polymer family. These polymers are attractive for tissue engineering because they are biocompatible, biodegradable, and can be tailored to possess a range of material properties. They have been used to create scaffolds for both *in vivo* and *in vitro* tissue-regeneration models in cartilage,[1] bone,[2,3] tendon,[4] and vascular smooth muscle tissue.[5]

A growing body of work suggests that scaffold microstructure (particularly pore size, interconnectivity, and permeability) is integral to the development of tissue constructs.[2,6,7] An ideal scaffold provides mechanical stability, directs tissue growth, and degrades as tissue develops.[8] The scaffold should also promote cellular attachment and infiltration, and possess sufficient porosity, interconnectivity, and permeability to satisfy transport requirements without compromising strength and durability.[9] Permeability may also influence cellular communication, and adaptation to mechanical stimulation,[10] and may improve tissue growth by reducing the localized accumulation of acidic by-products generated as the scaffold degrades.[11,12] These desired characteristics are directly related to the microstructure of the scaffold, which is, in turn, dependent on the scaffold fabrication process.

Several processing methods have been used to produce polymer tissue scaffolds, including solvent casting/particulate leaching,[13,14] phase separation,[15,16] gas foaming,[17,18] emulsion freeze-drying,[19] or some combination or modification of the above.[20,21] Of these, the most widely employed is the solvent casting/particulate leaching technique. This involves casting polymer dissolved in solvent over a leachable porogen, followed by solvent evaporation and porogen removal so that a porous polymer scaffold is left behind. Many of the studies that utilize this production method maintain the spirit of the technique but employ different

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porogens, polymers, and solvents, or protocol steps, yielding a variety of scaffold architectures. It is well documented that changing the nature or size of the porogen, or the polymer/porogen ratio, can affect pore size, porosity, and interconnectivity in a predictable manner.[22,23] One aspect of the solvent casting/particulate leaching technique that has not been fully evaluated for its effect on scaffold microstructure is the choice of solvent. The most commonly used solvents are acetone,[12,24] chloroform,[25,26] and methylene chloride.[3,14,27] It was hypothesized that polymer/solvent interactions may affect the microstructure and physical properties of the resulting scaffolds. Polymer chains that are extended in solvent may entangle to a greater degree than aggregated bulky chains. This may increase the stiffness of the resulting tissue scaffold and alter the permeability. However, the differing rates of solvent evaporation may also dictate or contribute to observed differences in scaffold morphology. The purpose of this study, therefore, was to investigate to what extent the interaction of solvent and polymer and the rate of evaporation influence the microstructure and properties of resulting tissue scaffolds.

MATERIALS AND METHODS

PLGA scaffold fabrication

The poly(D,L-lactide-*co*-glycolide) (PLGA) (Birmingham Polymers, Inc., Birmingham, AL) used for this study possessed a monomer ratio of 75:25 lactide to glycolide, a weight-averaged molecular weight of 121,800 daltons, a number-averaged molecular weight of 83,200 daltons, and a polydispersity index of 1.46. Enzyme-grade sodium chloride was sieved to obtain diameters between 212 and 600 μm with an average salt-particle diameter of 323 ± 95 μm, with the use of USA standard testing sieves and a sieve shaker.

PLGA polymer scaffolds were fabricated by employing a derivative of the solvent casting/particulate leaching technique.[13] PLGA polymer weighing 0.95 g was transferred to a 10-mL beaker containing a stir bar. Acetone, chloroform, or methylene chloride (7 mL) was added, and a Parafilm sheet was stretched over the beaker top to minimize solvent evaporation. The polymer/solvent solution was mixed on a stir plate set at low speed for 1 h and then poured over 9.0 g of salt, evenly dispersed within a 50 × 15-mm (diameter × depth) perfluoroalkyoxy-polymer (PFA) Petri dish (VWR International, West Chester, PA), to form a 10.6% (w/w) polymer/salt solution. Each polymer/salt solution was covered with a matching glass lid, enclosed within a second glass Petri dish (100 × 20 mm, diameter × depth), and allowed to evaporate in a fume hood until no change in weight was observed (between 2 and 3 days). Polymer/salt composites were then heated for 4 h at 70.5°C and 15 mm Hg vacuum, cooled to room temperature, and subjected to continuous vacuum overnight to remove residual solvent. Polymer/salt composites were immersed in 500 mL of deionized water to remove salt from the polymer. Water was replaced daily for 3 days and the leaching progress was deemed complete when the addition of 0.1*N* silver nitrate in distilled water no longer produced precipitate in the rinse water. PLGA scaffolds were then dried overnight under continuous vacuum and stored under vacuum in a desiccator until testing. Three scaffold samples were made for each solvent treatment. Each sample was 50 mm in diameter and approximately 3 mm in thickness, and possessed a thin polymer film on the side of the scaffold that had been flush with the casting dish. From each sample, specimens 5 mm in diameter were obtained with a biopsy punch, the film removed with a razor blade, and the thickness measured with digital calipers.

Light scattering technique

A Wyatt Technology Dawn F scattering unit operating in batch mode was used for simultaneous measurements at 18 angles of the light scattered from the solutions studied.[28] The unit uses a 5-mW vertically polarized He-Ne laser at 633 nm.

The chief interest in these experiments was to ascertain the morphology of the polymers in each solvent. As such, an approximate value of the refractive index increment (dn/dc) of 0.10 was used for each solvent. PLGA solutions were prepared in three different solvents: acetone, chloroform, and methylene. A 0.45-μm PTFE filter was used to filter 8 mL of each of the solutions made at 0.5, 1, 2, and 4 mg/mL into 20-mL scintillation vials, used as scattering cells.

Solvent evaporation weight

During the course of solvent evaporation the weight of two samples from each solvent treatment was continuously monitored with a top-loading balance. Molar flux was calculated from a linear regression of the total number of moles of solvent lost per unit area (mass loss divided by the molecular weight of the solvent and the cross-sectional area of the casting dish) and the time.

Scanning electron microscopy

Scanning electron microscopy (SEM) was employed to observe microstructural differences between scaffolds. Circular specimens 5 mm in diameter were obtained from the scaffold samples and sectioned to reveal the interior. Specimens were sputter coated with gold/palladium (Poloron E6900) with the use of a 20-mA current, 1.5-kV voltage, and 9-min coating time and imaged with a JEOL SEM 820 scanning electron microscope at an accelerating voltage of 15 kV.

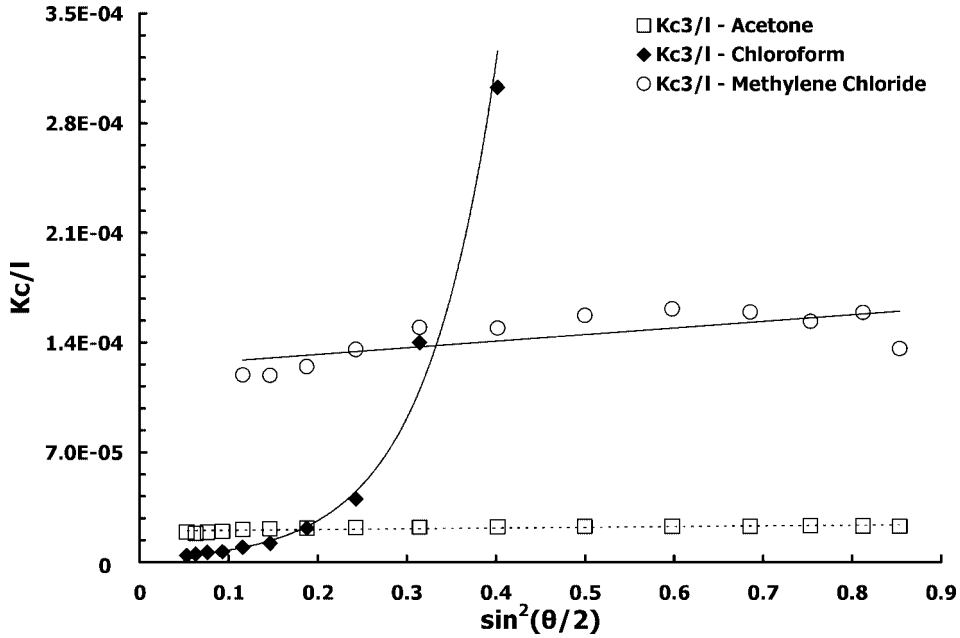

Figure 1. Reciprocal light scattering versus $\sin^2(\theta/2)$ of 75/25 PLGA in acetone, chloroform, and methylene chloride. PLGA in chloroform demonstrated exponential curvature that is characteristic of a large spheroidal aggregate. The curves for methylene chloride and acetone indicated a random coil conformation.

Mechanical testing

Between six and eight specimens from each scaffold sample were tested under simple unconfined compression between parallel platens with an Instron materials testing machine (Model 1122, Instron, Canton, MA) equipped with TestWorks® software (MTS, Eden Prairie, MN) and a 20-N compression load cell. The cross-head speed was set to 1.3 mm/min in accordance with ASTM Standard D695-02a. Specimens were loaded axially up to a load of 19 N. Sample stiffness was calculated from a regression of the linear region of the load-compression data (between 5 and 18 N). Stiffness, rather than a tangent modulus, was determined because the sample thickness was approximately 10 times the pore size, and too small to yield continuum-level results. The final specimens used in compression tests had the following thicknesses (mean ± standard deviation): acetone, 2.83 ± 0.27 mm (n = 21); chloroform, 2.69 ± 0.25 mm (n = 20); methylene chloride, 2.77 ± 0.26 mm (n = 18).

Permeability testing

Intrinsic permeability is used to determine the bulk flow properties of a material and is independent of the fluid used (provided the fluid is Newtonian). Although not a direct measurement of the microstructure, permeability can often provide information about interconnectedness and porosity. Permeability was measured with a custom-built, constant-flow-rate permeameter. Specimens were placed inline with a syringe pump that maintained constant flow rates of deionized water and a manometer that monitored pressure. Samples were tested at different flow rates to ensure that a linear relationship between pressure and flow rate existed.[29,30] The intrinsic permeability (k) was calculated according to Darcy's law,

$$k = \frac{\mu Q L}{A\ \Delta P}, \tag{1}$$

where μ is the viscosity of water, Q is the flow rate, A is the cross-sectional area of the sample, ΔP is the pressure differential across the samples, and L is the sample thickness. Permeability was measured from six specimens from each sample (and with the use of three samples from each solvent treatment).

Statistical analysis

Statistical significance, $p < 0.05$, was determined with the use of single-factor analysis of variance (ANOVA) and *post hoc* Tukey/Kramer tests. All statistical tests were conducted with StatView software (SAS Institute, Cary, NC).

RESULTS

Polymer morphology in solvent

The morphology of a polymer in solvent can be qualitatively determined from a plot of reciprocal light scattering versus $\sin^2(\theta/2)$, where θ is the scattering angle (Figure 1). The reciprocal scattering is represented as Kc/I, where K is an optical constant, c is the polymer concentration, and I the excess Rayleigh scat-

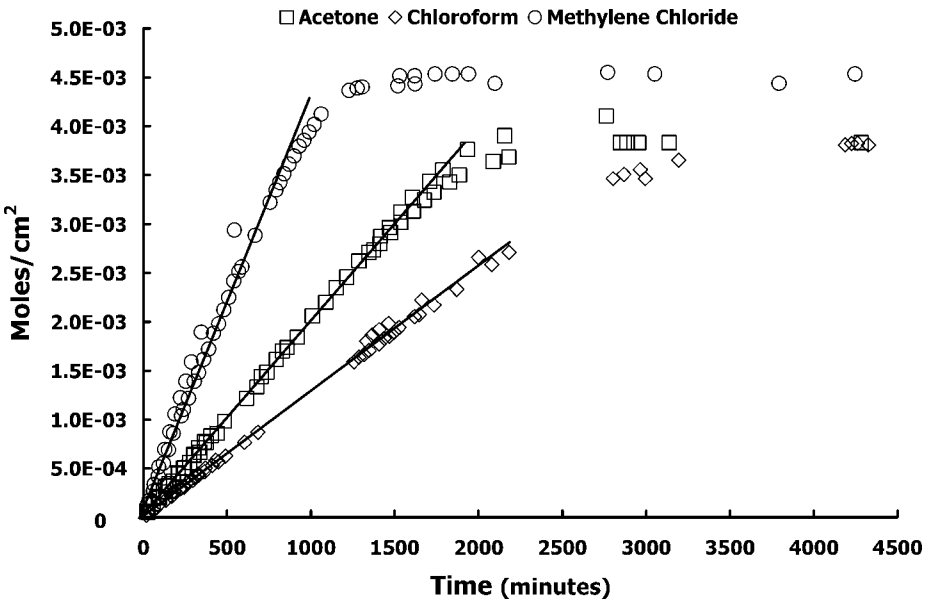

Figure 2. Evaporation rate of organic solvents during scaffold fabrication. The weight of the solvent/polymer/salt solution was monitored during the evaporation phase and is expressed here as molar flux. The molar flux for methylene chloride (4.26×10^{-6} moles/cm^2 · min) was more than double that of acetone (1.98×10^{-6} moles/cm^2 · min) and chloroform (1.28×10^{-6} moles/cm^2 · min).

tering ratio.[31] The shape of the curve indicates the morphology, which is dependent on the strength of polymer–solvent interactions.

PLGA behaved as well-dissolved polymer chains in acetone and methylene chloride, as can be seen in Figure 1. This conclusion is based on the fact that the plot of Kc/I versus $\sin^2(\theta/2)$ was a straight, virtually horizontal line in these two cases—a hallmark of objects whose dimensions are far smaller than the wavelength of light used (θ = 632.8 nm). The fact that the straight line for methylene chloride was significantly higher than that for acetone indicates a larger optical contrast between the polymer and methylene chloride than between the polymer and acetone. Although precise dn/dc (change in refractive index with change in polymer concentration) values are not available, the greatest optical contrast between polymer and solvent occurred in acetone (n = 1.3591), and yet, where contrast differs little (n = 1.447 for chloroform, n = 1.4244 for methylene chloride) the large qualitative difference in scattering was due to differences in polymer morphology in the two solvents. Consequently, acetone could be a better solvent than methylene chloride for further light scattering experiments. In contrast, the very low intercept and steep upward concave curvature of the Kc/I data for PLGA in chloroform is evidence of a massive, densely packed structure, with a size on the order of λ itself. Hence, the polymer existed in an aggregated form in chloroform, in contrast to the individual chain form in acetone and methylene chloride.

Evaporation rate

The total number of moles lost for each solvent during the evaporation phase increased linearly up to a plateau that corresponded to the removal of bulk solvent from the casting dish (Figure 2). The molar flux, or rate of evaporation, for each solvent was determined from the slope of the line up to this plateau. The evaporation rates for methylene chloride, acetone, and chloroform were determined to be 4.26×10^{-6} moles/cm^2 · min, 1.98×10^{-6} moles/cm^2 · min, and 1.28×10^{-6} moles/cm^2 · min, respectively.

Scaffold microstructure

SEM images were obtained for several specimens from each solvent treatment and over a range of magnifications. Figure 3 illustrates representative SEM images taken originally at 50 and 150× for scaffolds fabricated from each solvent. In general, each solvent treatment produced open-cell foams. However, the acetone and chloroform specimens appeared to contain more irregularities. Specimens fabricated with methylene chloride appeared more ordered and had more intact unit cells.

Compression analysis

Compression tests were performed to determine whether differences in microstructure due to solvent

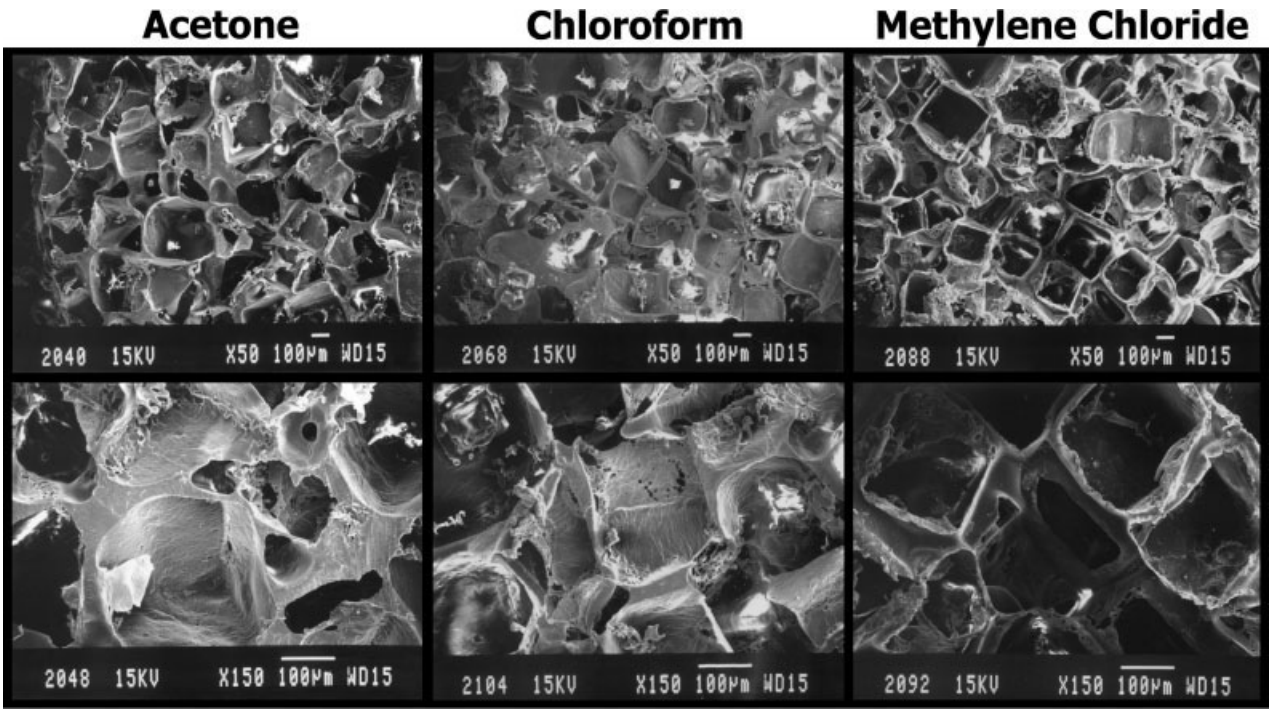

Figure 3. Representative scanning electron microscopy images of 75/25 PLGA scaffolds fabricated with acetone, chloroform, or methylene chloride. Scaffolds are shown at two magnifications (original) and are representative of the sample. Scaffolds formed with methylene chloride exhibited a more ordered structure than those formed with acetone or chloroform.

treatments affected mechanical properties. The data were expressed in terms of stiffness rather than a modulus because strain may not have been uniformly distributed through each specimen (the pore size was large in relation to the specimen size). Figure 4 presents representative load-compression curves for scaffolds produced from each solvent. Methylene chloride specimens ($n = 18$) exhibited a significantly higher mean stiffness (30.2 ± 9.6 N/mm) than acetone specimens (21.7 ± 4.8 N/mm, $n = 21$), and chloroform specimens (18.9 ± 4.2 N/mm, $n = 20$). To examine whether differences between specimens could be attributed to solvents and not sample-to-sample variation, comparisons were made between specimens obtained from different samples fabricated with the same solvent (Figure 5). Samples fabricated with chlo-

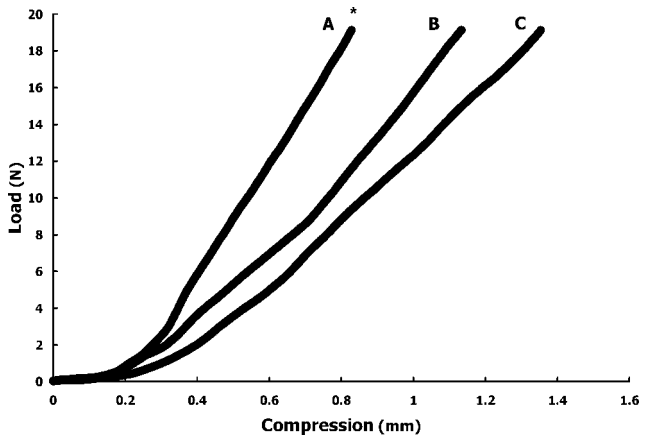

Figure 4. Load versus compression for scaffolds fabricated with different solvents. Specimen stiffness was determined from compression. The representative curves shown displayed compressive stiffnesses similar to the mean stiffness for each solvent treatment. Methylene chloride specimens (A) were stiffest ($n = 18$), followed by acetone (B) ($n = 21$), and chloroform (C) ($n = 20$). *Significant ($p < 0.05$) difference between methylene chloride and the other solvents.

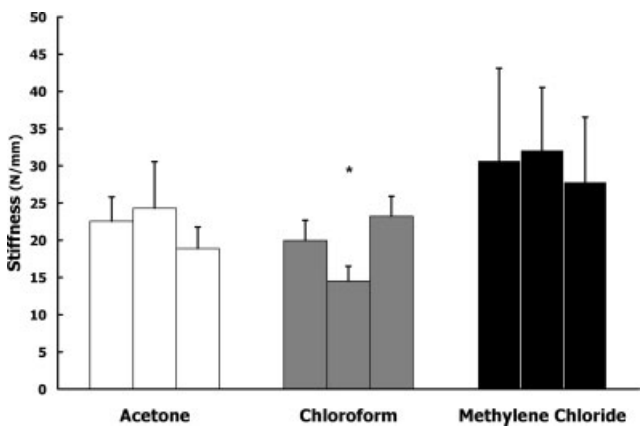

Figure 5. Average stiffness between samples of the same solvent treatment. Three distinct samples were made with each solvent. Specimens ($n = 6–8$) from each sample were compared to determine between sample variability. *Samples made with chloroform demonstrated significant ($p < 0.05$) differences in stiffness. Data are mean ± SD.

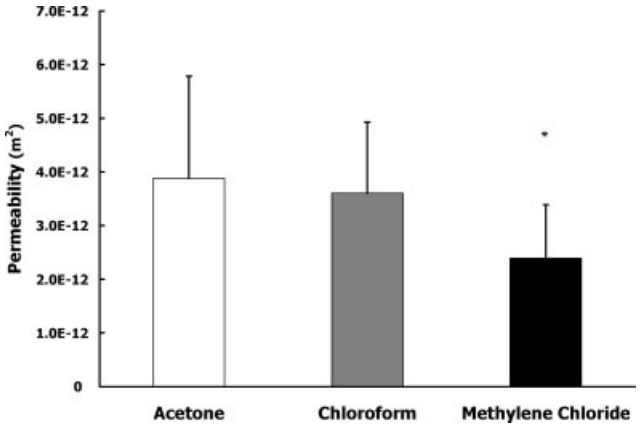

Figure 6. Intrinsic permeability versus solvent treatment. Methylene chloride specimens were the least permeable (n = 18), followed by chloroform (n = 18), and acetone (n = 18). *Significant ($p < 0.05$) difference between methylene chloride and the other solvents. Data are mean ± SD.

roform demonstrated significant differences in stiffness. Variations in specimen thickness, across all solvent types, did not significantly affect stiffness.

Permeability analysis

The intrinsic permeability (Figure 6) of specimens fabricated with acetone, chloroform, and methylene chloride was found to be $3.9 \pm 1.9 \times 10^{-12}$ m^2, $3.6 \pm 1.3 \times 10^{-12}$ m^2, $2.4 \pm 1.0 \times 10^{-12}$ m^2, respectively. Specimens produced with methylene chloride were significantly less permeable than those formed with acetone or chloroform. An examination of variability between samples fabricated from the same solvent revealed that specimens from one of the acetone samples were significantly less permeable than specimens from the other two samples fabricated with acetone (Fig. 7).

DISCUSSION

Solvent casting/particulate leaching is a widespread method for producing porous polymer scaffolds. The relationships between several processing parameters (such as the polymer/porogen ratio) and the resulting structural features have been determined, thereby enabling tissue scaffolds to be designed to possess features necessary for tissue generation. One parameter that has not been extensively investigated is how solvent choice impacts the resulting microstructure. Mikos et al. produced poly(lactic acid) foams cast with methylene chloride or chloroform and reported that the porosities were similar.[13] Chloroform was chosen by Mikos et al. as the preferred solvent because its lower vapor pressure and slower evaporation time likely improved the homogeneity of the scaffold. The present study, by contrast, observed small but significant differences in scaffold properties (as a result of microstructure) that arose from the solvent used. The results of this study are important for the many research groups that use the common solvent casting/particulate leaching technique. Nonetheless, care should be exercised in extending the differences noted here to every situation and/or to other fabrication techniques. The solubility of the polymer is dependent on many properties, including the molecular weight, copolymer ratio, and distribution of monomers within the chain.[32] Increased molecular weight and large blocks of glycolic acid can decrease solubility in some solvents, such as chloroform. Furthermore, other members of the poly(α-hydroxy acids) family will demonstrate different dissolution behavior than that of the polymer examined here. Despite these potential differences, the information presented in this study may be broadly useful because the solvents examined are commonly used, and the information that 75/25 D,L-PLGA exists in different conformations in different solvents may prove valuable to other fabrication techniques as well.

As a whole, the data from the present study indicate that scaffolds produced with methylene chloride were different from scaffolds produced with the other solvents. Methylene chloride samples were generally stiffer, less permeable, and possessed more regular morphology than acetone or chloroform samples. The irregularities observable in the acetone and chloroform scaffold microstructures likely increased the interconnectedness between pores and may have accounted for the higher permeability as compared to the methylene chloride scaffolds. Likewise, the scaffold stiffness may have been increased by the regular, unit cell microstructure of the methylene chloride foams, as compared to the acetone and chloroform scaffolds. Some variation was found between samples fashioned with the same solvent, which statistically obscured measurable effects of solvent treatments. This observation reinforces the fact that many variables are involved in scaffold fabrication, and care must be taken to produce consistent scaffolds time after time. Furthermore, the between-sample variability provides motivation for investigators to account for such variation by using block experimental designs and analyses when possible.[33] Block designs allow effects of batch-to-batch variance (for example, between scaffolds fabricated on two different days, but with the use of the same solvent type) to be isolated and considered separately from variance due to experimental treatments; a variety of block designs can be found in texts on experimental design.[33]

D,L-PLGA is an amorphous hydrophobic polyester

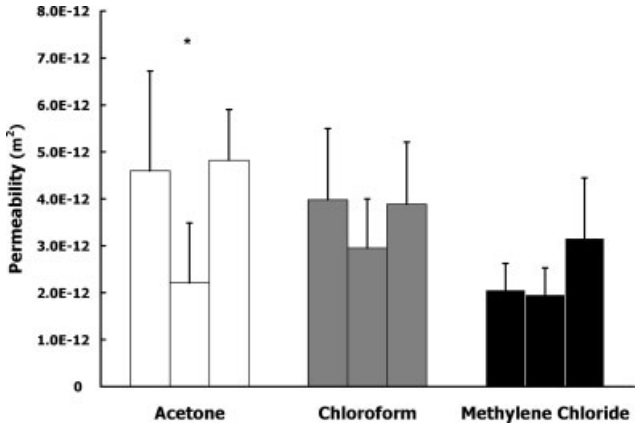

Figure 7. Average intrinsic permeability between samples of the same solvent treatment. Three distinct samples were made with each solvent. Specimens ($n = 6$) from each sample were compared to determine between sample variability. *Samples made with acetone demonstrated significant ($p < 0.05$) differences in permeability. Data are mean ± SD.

that is soluble in several organic solvents. In this study, 75/25 D,L-PLGA was dissolved in acetone, chloroform, or methylene chloride to form tissue scaffolds. Reciprocal light scattering data revealed that in chloroform, this polymer exists as large bulky aggregates, whereas in acetone and methylene chloride it maintains a random coil morphology. It was hypothesized that polymers in a random coil may entangle to a greater degree and subsequently influence the resulting scaffold architecture. However, the rate of solvent evaporation rather than the strength of polymer/solvent interactions appears to be the principle determinant of scaffold architecture. Each solvent possesses a vapor pressure indicative of its evaporation rate. The vapor pressures of methylene chloride, acetone, and chloroform at 20°C are 353, 184, and 160 mm Hg, respectively. As shown in Figure 2, methylene chloride evaporated more than twice as fast as either acetone or chloroform. The fact that the mechanical testing, permeability, and SEM data show that methylene chloride (rather than chloroform) produced specimens different from the other solvents suggests that the rate of solvent evaporation is more important in shaping the scaffold microstructure than the degree of polymer/solvent interactions alone. It is possible that the increased rate of evaporation in methylene chloride samples affected the polymer/solvent interactions through thermal mechanisms. For example, rapid evaporation of methylene chloride may have caused the formation of less porous polymer sheets around the salt particles; this could have reduced the permeability and increased the stiffness of the samples overall. Future work could focus on how the microstructure is affected by the rate of evaporation by, for example, changing the temperature at which scaffolds are fabricated for a given solvent. This would not completely isolate the effect of evaporation rate, but would provide additional insight.

High-porosity scaffolds are necessary to promote cellular infiltration and molecular transport, but small changes in microstructure can affect the extent to which these processes occur. In addition to the size of the pore space, tissue scaffolds must also possess sufficient interconnectivity to provide a path through the scaffold through which metabolites and cells can pass. The transmission of mechanical forces (either through the scaffold or as a result of fluid–solid interactions) will impact the functions of constituent cells and will be in part dependent on the scaffold microstructure. Ultimately, the choice of solvent for PLGA scaffold fabrication depends on the intended application. If, for example, mechanical and microstructural requirements can be met, acetone may be the preferred solvent because it is inexpensive and less hazardous than other solvents. Furthermore, for tissue-engineering applications, the greater permeability observed in acetone-formed scaffolds could improve nutrient transport to incorporated cells.

CONCLUSION

Many studies use some variant of the solvent casting/particulate leaching technique to generate porous polymer scaffolds. The choice of solvent varies between studies, but acetone, chloroform, and methylene chloride are the most commonly used. This study was motivated by results from static light scattering experiments that indicated that PLGA is well solvated in acetone and methylene chloride but forms bulky aggregates in chloroform. It was hypothesized that these polymer/solvent interactions may affect the resulting scaffold microstructure and properties.

The data indicated that small but significant differences exist between scaffolds fabricated with methylene chloride and scaffolds fabricated with acetone or chloroform. Scaffolds made with methylene chloride were the stiffest and least permeable, and possessed the most regular pore morphology. The evaporation rate of methylene chloride/polymer solution was more than double that of the other solvents. The observed differences in scaffold properties correlated to the solvent evaporation data but not the polymer/solvent morphology data. Consequently, although polymer/solvent interactions likely influence scaffold microstructure and mechanical properties, the rate of solvent evaporation appears more important in this regard.

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