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Influence of Scaffold Forming Techniques on Stress Relaxation Behavior of Polycaprolactone Scaffolds

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ABSTRACT: This study evaluated the effect of scaffold processing methods on viscoelastic properties of polycaprolactone (PCL), a frequently explored biomaterial in tissue engineering. 80 kDa and 45 kDa PCL scaffolds were synthesized using salt leaching and electrospinning techniques. Also, films were formed by air drying. Scanning electron microscopy analysis confirmed that salt leached scaffolds had open pore architecture and electrospun scaffolds had randomly distributed uniform fibers. Using the tensile test results in phosphate buffered saline (pH=7.4) and 37° C, ramp-hold tests were performed for five stages by setting the strain rate to be 1%s^{-1} for 2 s followed by 58 s of hold. Also, tests were performed at various strain rates and total strain. Salt leached scaffolds of same MW showed less relaxation in each stage relative to electrospun scaffolds. 45 kDa salt leached scaffolds relaxed more than 80 kDa scaffolds. Stress accumulated in each stage was more in films than in scaffolds. However, relaxation function appeared similar between films and electrospun fibers. Strain rate and amount of applied strain had significant effect on relaxation characteristics; 0.6%s^{-1} strain rate had higher accumulated stress than 1%s^{-1} and 3%s^{-1}. Increased amount of loading had significant effect in the first stage with repetitive relaxation characteristics in subsequent stages. SEM analysis of tested samples showed no change in the microstructure with the exception of a few locations where pores oriented in the direction of the pull. In summary, viscoelastic characteristics vary based on the type of scaffold processing used, despite use of the same polymer. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2013

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INTRODUCTION

Three-dimensional (3-D) scaffolds of biodegradable polymers are used for cell culture to regenerate tissues in a required geometric configuration.[1] Varieties of polymers have been explored with the intent of tailoring the biological response, degradation rate, and mechanical properties. Synthetic polyester polycaprolactone (PCL) has gained much attention due to its low melting point (60°C) elastomeric properties, high elongation, and biodegradation.[2] The rate of biodegradation by hydrolysis increases with decrease in molecular weight (MW), involving the cleavage of ester linkages lowering the molecular weight.[3] Degradation rates and mechanical properties can be altered via polymerization techniques and processing conditions.[4]

Significant advances have been made in synthesizing porous scaffolds of precise size and shape.[5] Scaffolds have been generated using PCL by various additive techniques such as electrospinning,[6,7] rapid prototyping,[8] and subtractive techniques such as salt leaching,[9] freeze drying,[10] and modified melt processing with selective extraction.[11] Electrospinning produces non-woven fibers of diameters ranging from nanometers to micrometer. Since the technology allows the possibility of tailoring the biomechanical properties, there has been a significant effort to adapt the technology in tissue regeneration.[12] Various modifications to electrospinning have also been proposed such as incorporation of hybrid twin screw extrusion to form functionally graded PCL scaffolds.[13]

Salt leaching technique does not need special equipment to produce non-fibrous scaffolds where the walls are flat in configuration from the perspective of the cell size.[9] To tailor the biomechanical and degradation properties of PCL, many other materials have also been blended to either improve the biological activity for soft tissue applications,[14,15] or to increase mechanical properties for bone regeneration.[13,16] Nevertheless, the primary modes of mechanical property evaluation to understand the suitability of scaffold preparations are linear tensile and compression tests, despite the fact that many parts of the body show complex mechanical behavior and are exposed to smaller-magnitude cyclical stresses.

Biological tissues display a complex mechanical behavior to an applied strain or stress termed viscoelasticity which is time-dependent and load-history-dependent.[17,18] The viscoelastic properties of tissues create a conducive environment for the cells which is critical for their viability and function. When a tissue is being replaced by a synthetic scaffold for repair or utilized as *in vitro* tissue structures for research purposes, these tissues need to possess compatible viscoelastic properties with the native environment to accurately mimic the condition.[19] Thus understanding the viscoelastic behavior of the scaffold material is necessary to know how it performs under various applications. Though there have been many studies to understand the viscoelastic properties of the soft tissues,[20,21] those of the synthetic tissues are scarce.[22–24] Porous polymeric biodegradable structures utilized in tissue regeneration also show viscoelastic behavior.[25,26] Linear relaxation property in a single cycle of loading and unloading of PCL scaffolds with and without cell culturing has also been reported with comparison to bovine cartilage.[24] However, the effect of different scaffold preparation techniques on viscoelastic properties is not well understood. Thus to understand the utility and the quality of the regenerated tissues, one has to perform viscoelastic testing comparing it to the properties that native tissues in the body possess.

The objective of this study was to understand the effect of scaffold preparation techniques on the viscoelastic properties of low and high MW PCL. The scaffolds of 45 and 80 kDa MW were prepared using salt leaching technique[9] and electrospinning technique.[7] The effect of processing of the scaffolds in relaxation property was investigated. These results show significant effect of scaffold processing on stress relaxation characteristics.

MATERIALS AND METHODS

Materials

PCL of molecular weight 80,000 Da (referred as 80 kDa) was purchased from Sigma (St. Louis, MO), PCL of molecular weight 43,000–50,000 kDa (referred as 45 kDa) was purchased from Polysciences (Warrington, PA), Chloroform, 1 : 2 from Pharmco (Brookfield, CT) and phosphate buffer salts (sodium chloride, potassium chloride, potassium dihydrogen phosphate, and sodium monohydrate phosphate heptahydrate) was purchased from Sigma-Aldrich (St. Louis, MO). All chemicals were used as received without further purification.

Generation of Scaffold by Salt-Leaching Technique

Solutions were prepared at room temperature and stirred for 24 h until the solutions became homogeneous. Scaffolds were prepared by salt-leaching technique using a previously published procedure.[9] In brief, sodium chloride salt crystals were pulverized using a mortar and pestle. These crystals were sieved using two trays (i) $>$274 μm sieve size and (ii) $<$246 μm sieve size to obtain crystals in the size of 246–274 μm. Then, 2.7 g of PCL was dissolved in 20 mL of chloroform (moisture content $<$ 0.001%) and 29 g of 246–274 μm salt crystals was added to the solution to form a homogeneous paste. The paste was spread in 5 $\times$ 5 cm rectangular wells prepared on Teflon sheets using silicone glue and air dried in a laminar hood. The formed structure was immersed in distilled water for 20 h to dissolve the salt and then analyzed by scanning electron microscopy and permeability to determine the pore architecture.

Generation of Scaffolds by Electrospun Fibers

Scaffolds were prepared by electrospinning using our previously published procedure[7] with minor modifications. In brief, the electrospinning setup consisted of one syringe pump (74900 series, Cole-Parmer Instrument Company, Vernon Hills, IL), 10 mL syringe (Luer-Lok Tip; Becton Dickinson and Company, Franklin Lakes, NJ), needle tips, high voltage power supply (ES30P-5W/DAM, Gamma high Voltage Research, Ormond Beach, FL), earth grounding, and a collection mandrel. Approximately 30 cm long PTFE tubing (Sigma Aldrich, St. Louis, MO) connected the syringe to the spinneret. 20%wt/v PCL solution in methanol/chloroform (1 : 2) was loaded into the syringe. A 12 kV voltage was applied between the needle and the conductive collector. PCL spinning solution was pumped to the spinneret (0.8 mm inner diameter) at 2 mL/h. Randomly distributed fibers were collected on a flat collector plate at a spinning distance of 10 cm. The collected fibers were then analyzed by SEM for fiber distribution and size and used for mechanical tests.

Generation of Films

PCL solution used for scaffold generation was air dried under laminar hood for 2 h to form the films. Similar to scaffolds, 5 mL of the solution was poured into the rectangular well which was prepared using silicone glue on Teflon sheets. Since films formed by evaporating 5 mL of the solution were less elastic, solution quantity was reduced to 2 mL to obtain thinner films.

Microstructure Characterization

Samples were analyzed using SEM similar to our previous publication.[6] In brief, samples were attached to an aluminum stub using a conductive graphite glue (Ted Pella, Redding, CA) and sputter-coated with gold for 1 min. Samples were characterized JOEL 6360 (Jeol USA, Peabody, MA) SEM at an accelerated voltage of 15 kV.

Determining Structure Thickness, Pore Size, and Fiber Size

Thickness of salt leached and electrospun scaffold was measured using a digital caliper (Fisher Scientific). Film thickness was determined by cutting the films into strips and orienting it orthogonally so that thickness was visible in the field of view of the inverted microscope equipped with a CCD camera.[27] Digital micrographs were obtained at various locations and quantified using Sigma Scan Pro image analysis software (SPSS Science, Chicago, IL) for the thickness. At least 3–4 images were analyzed per sample, and the calculated film thicknesses of three sample preparations (Table I) was used for determining the stress during tensile testing.

Porosity of salt leached scaffolds was determined using a thin strip of the samples adjacent to that used for testing. The cut strips were mounted to observe the cross section and digital micrographs were obtained at various locations using an inverted microscope. Then the net area of the pores was calculated using Sigma Scan Pro image analysis software. Assuming isotropic distribution of pores, porosity was calculated as the ratio of open pore area to the total image area. At least 3–4

Table I. Scaffold Characteristics

	Thickness (mm)	Porosity	Break strain (%)	Break stress (kPa)
Salt leached 45 kDa scaffold	2.10 ± 0.03	81.7 ± 1.50	20–25	76 ± 11.4
Salt leached 80 kDa scaffold	2.17 ± 0.03	82.6 ± 1.20	25–30	87 ± 21.5
Electrospun 80 kDa scaffolds	0.173 ± 0.045	–	140–150	132 ± 19.1
45 kDa films	0.124 ± 0.05		150–250	4420 ± 59
80 kDa films	0.129 ± 0.04		>700[22]	12,000[22]

images were analyzed per sample. The fiber diameters of electrospun scaffolds were calculated using SEM micrographs and Sigma Scan Pro image analysis software.

Mechanical Testing

All tests were conducted by immersing the samples in phosphate buffer solution at pH 7.4 at 37°C using INSTRON 5542 machine (INSTRON, Canton, MA) equipped with a 100 N load cell and a custom-built environmental chamber.[23] Data were collected using Merlin (INSTRON, Canton, MA) software and then exported to MS Excel for further analysis. The scaffolds and films were cut into 50 mm long and 10 mm wide strips. Each test was performed for three or more times using samples from different preparations.

Tensile Tests

Samples were pulled, where the cross head speed was set to 10 mm/min (0.17 mm/s) to break, similar to previous publications.[23] Break stress and strain were determined using the associated software, Merlin (INSTRON Canton, MA).

Stress–Relaxation Tests

Since salt leached scaffolds had lower break stress and break strain limits, the upper limit of total strain was set to 10% strain (Table I). Hence, the samples were stretched at a strain rate of 1% s^{-1} for 2 s in each stage which was repeated for five stages, accumulating to 10% strain for the entire experiment. Although the films had a different strain rate before their failure, in order to compare the stress relaxation behavior of films and scaffolds, the strain rate of 1% s^{-1} was maintained for both films and scaffolds. Five stages of ramp-hold experiments were performed on different structures with a constant strain rate of 1% s^{-1} for 2 s (ramp) followed by 58 s relaxation (hold). The cumulative strain limit was fixed to 10% based on the tensile behavior of the scaffolds. Films were tested under identical conditions. At least three samples from different preparations were analyzed by each method. Averages stress values and relaxation function values were determined along with the standard deviation. Obtained relaxation behaviors were analyzed using three types of graphical representations:

i. comparing the absolute values of stresses at different times for different samples.
ii. changes in stress in each stage were normalized to the origin and different stages for that sample were plotted on the same graph.
iii. relaxation function, $G(t)$, was plotted for the first stage by normalizing the relaxation data by the highest stress in that stage.

Cyclical Tests

Samples were stretched and relaxed toward the original length repeatedly between two preset stress limits of 10 and 35 kPa for five cycles at a cross head speed of 50 mm/min (0.864 mm/s).

RESULTS

Characteristics of Scaffolds

Obtained thicknesses for different structures (Table I) were compared between the scaffolds prepared by salt leaching and electrospinning techniques. As expected, high MW structures were thicker than low MW structures in both films and scaffolds. Microstructures of scaffolds in dry condition were characterized to better understand the observed mechanical properties. These results showed (Figure 1) that the electrospun scaffolds had uniform micro size fibers with random orientation. The fiber thickness of the electrospun 80 kDa PCL scaffolds was 2.08 ± 0.7 μm. There were no beads (small lumps due to salt leaching technique) in any part of the structure. When salt leached scaffolds were evaluated, both 45 and 80 kDa PCL scaffolds showed distribution of pores throughout the scaffold. The pores appeared interconnected.

Tensile Testing

Uniaxial tensile testing results in hydrated conditions at 37°C showed (Figure 2) that the scaffolds had non-linear stress–strain behavior even at small strain ranges. Break stress and break strain was lowest for the 45 kDa PCL scaffolds prepared by the salt leaching technique followed by 80 kDa PCL scaffolds prepared by the same technique (Table I). Electrospun scaffolds of 80 kDa PCL stretched more than salt leached scaffolds of same MW. Increased thickness of the electrospun scaffolds decreased the break strain significantly, lower than that of 80 kDa films we previously reported.[22] In general, high MW structures showed higher break strain than low MW PCL structures which was attributed to the increased chain length of the semi-crystalline PCL polymer.

Stress–Relaxation Behavior

Samples were subjected to five stages of ramp-hold tests to understand the stress relaxation behavior in PBS at 37°C. All the scaffolds and films showed (Figure 3) a progressive increase in stress value for each stage. The salt leached scaffolds accumulated up to 0.2 MPa stress at the end of five stages, whereas the scaffold by electrospun technique showed 0.7 MPa stress for five stages, for the same amount of net strain. These values were comparable to other reports for electrospun fibers,[19] although their tests were performed in dry conditions at 37°C and fiber sizes were significantly smaller. This suggested that the effect of

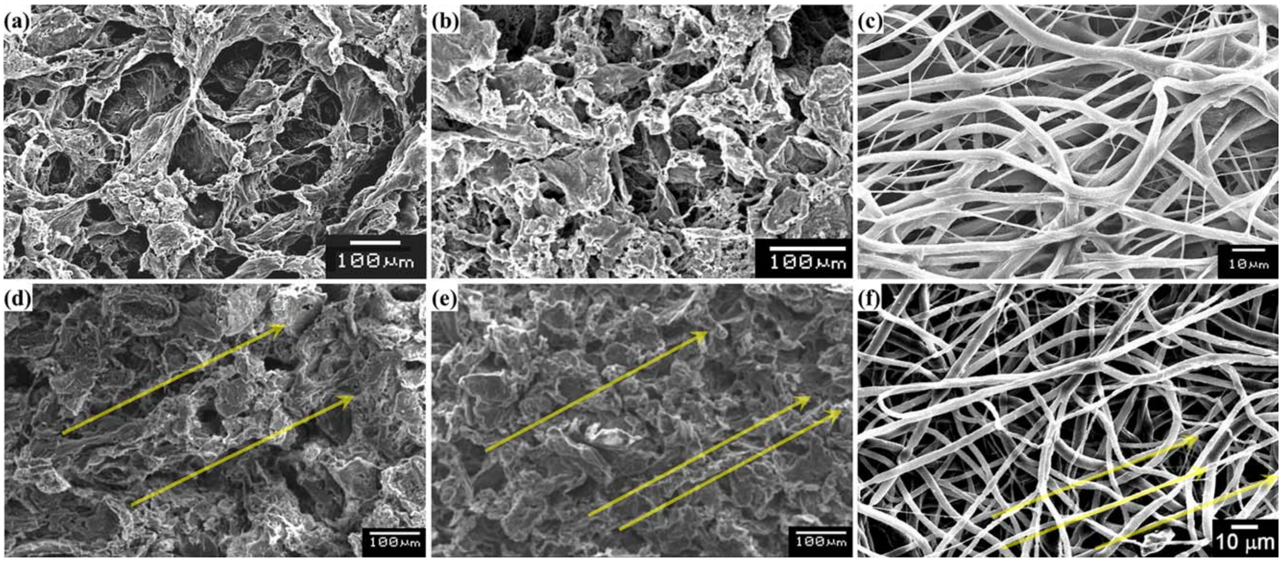

Figure 1. Microstructure of scaffolds before and after stress relaxation experiment. (a) 45 kDa salt leached scaffolds before stretching, (b) 45 kDa salt leached scaffolds after stretching, (c) 80 kDa salt leached scaffolds before stretching, (d) 80 kDa salt leached scaffolds after stretching,(e) 80 kDa electrospun scaffolds before stretching, and (f) 80 kDa electrospun scaffolds after stretching. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

water on relaxation characteristics may be minimal as PCL is hydrophobic material. However, fiber size may be a significant factor. Nevertheless, comparison of the values by electrospun scaffolds to salt leached scaffolds showed reduced relaxation in electrospun fibers. The accumulated stress in films was greater than 4.5 MPa, which is significantly higher stress accumulation when compared to scaffolds for the same strain rate in five stages. Both 45 and 80 kDa scaffolds were more elastic than films, probably due to the presence of pores. In general, 80 kDa structures had higher elasticity than 45 kDa structures, similar to uniaxial tensile testing.

In order to understand the relaxation behavior in different stages, all stages of each sample were plotted by translating the stress pattern for each stage to its origin (Figure 4). For 45 kDa salt leached scaffolds and films, the relaxation progressively decreased in subsequent stages. In 80 kDa salt leached scaffolds, there was no significant effect of number of stages, unlike significant accumulation of stress observed in electrospun scaffolds. The stress accumulated in each stage and the number of stages had more effect on films than scaffolds.

We tested modeling the behavior using linear models. Since a linear Maxwell model for PCL scaffolds was used by others,[24] we first tested the fitness of that model for each stage within the sample. The results showed a poor fit, with r^2 ranging from 0.6 to 0.75. Others have used a standard linear solid model (spring in parallel with the linear Maxwell model) to describe

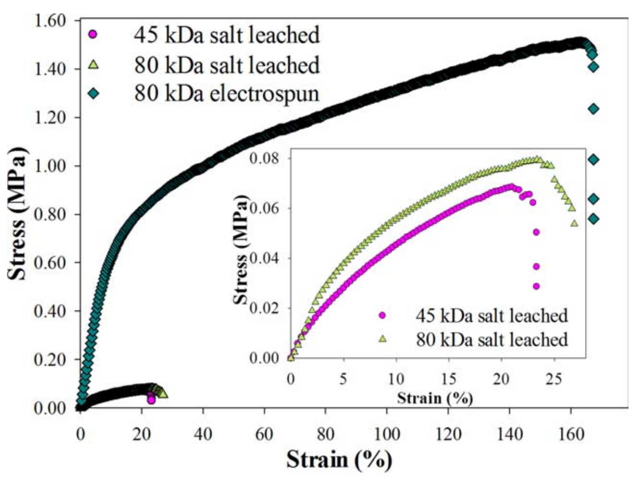

Figure 2. Uniaxial stress–strain behavior of scaffolds in phosphate buffered saline at 37°C. Inset is a graph showing the magnified view of the stress–strain behavior for salt leached scaffolds. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

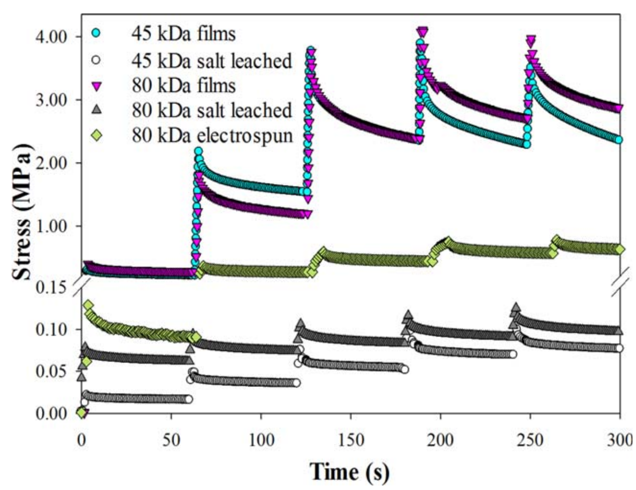

Figure 3. Stress relaxation behavior of PCL scaffolds and films with a strain rate of 1% s^{-1}. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

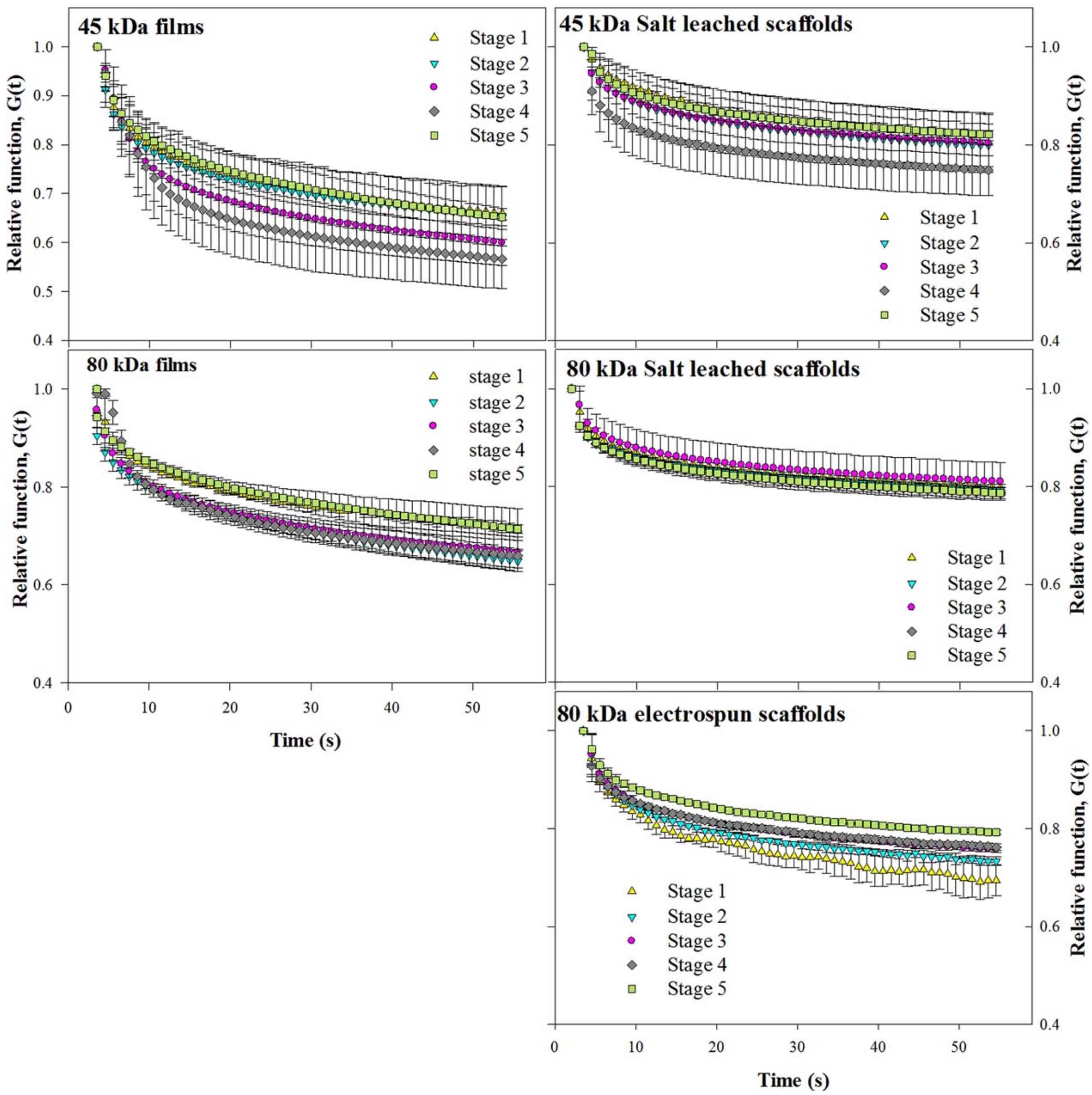

Figure 4. Changes in relaxation behavior between different stages. (a) 45 kDa Films, (b) 45 kDa scaffolds—Salt leaching, (c) 80 kDa Films, (d) 80 kDa scaffolds—Salt leaching, and (e) 80 kDa scaffolds—Electrospun. Shown values are average of three samples and error bars correspond to standard deviations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the behavior of polyethylene glycol-based tissue sealants.[28] Hence, we tested a standard linear solid constitutive equation derived for the condition of constant strain ε_0 in the form

$$\frac{\sigma(t)}{\varepsilon_0}=k_e+k_1 e^{-t/\tau}$$

where k_e is the stiffness of the equilibrium spring, k_1 is the stiffness of the Maxwell spring, and τ is the relaxation time. The equation fit to the relaxation portion of the experimental data for the first stage, showed goodness with $r^2 > 0.95$ for all conditions. The relaxation time showed a range of 10 s to 15 s for the first stage of all the samples. However, relaxation times varied for subsequent stages within the same range or lower. Shorter relaxation time is an indication of elastic behavior rather than the viscous behavior, and relaxation time is nearly zero for perfectly elastic materials. In any case, there were significant differences in the magnitude of k_e and k_1 values. k_e was 0.5 MPa for films, 0.1 MPa for electrospun scaffolds, and 0.02–0.05 MPa for salt leached scaffolds. Similarly, k_1 was 0.2 MPa for films, 0.05 MPa for electrospun scaffolds and 0.005–0.01 MPa for salt leached scaffolds.

We questioned how the stress relaxation tests affect the scaffolds for which changes in the scaffold morphology were analyzed via SEM (Figure 1). There was no change in pore orientation in both MW PCL salt leached scaffolds except in a few regions where the pores appeared to orient in the direction of pull. In electrospun scaffolds, largely no changes were observed in the

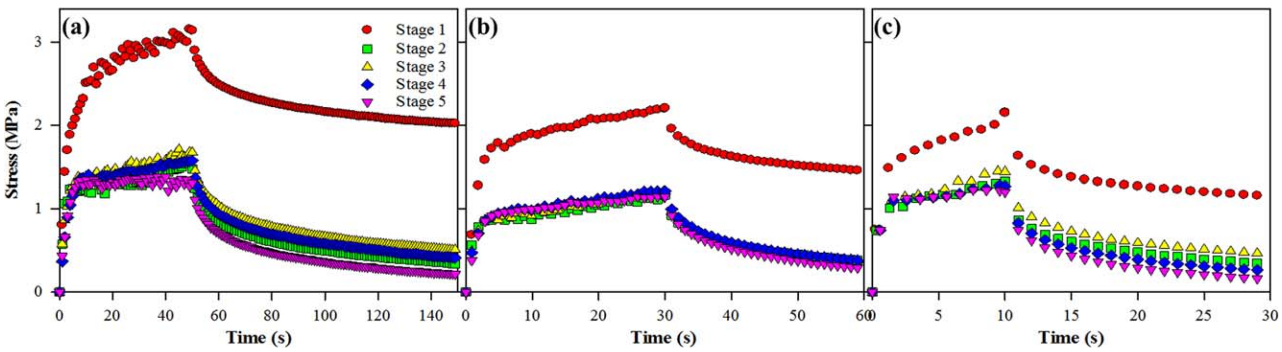

Figure 5. Effect of loading time on stress relaxation. (a) 0.6% s^{-1}loading for 50 s and 100 s relaxation time (b) 1.0% s^{-1}loading for 30 s and 30 s relaxation time and (c) 3.0% s^{-1}loading for 10 s and 20 s relaxation time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

distribution of fibers. This is unlike that reported for chitosan scaffolds, where majority of the pores oriented in the direction of pull.[23] Nevertheless, testing other models that incorporate the changes in the structure may provide a better fit.

Effect of Applied Strain Rate to Stress Relaxation Behavior

Previously when films were stretched to 50% at the same strain rate of 1% s^{-1},[22] highest stress values were observed in the first stage with no difference in subsequent stages. These results agreed with the explanation of conditioning of films at a specific strain amount, i.e., repeat of relaxation behavior in successive stages after a specific strain could be viewed as the behavior after "preconditioning." To better understand the effect of loading rates and relaxation time on stress relaxation behavior of PCL, experiments were carried out by varying the loading and relaxation time with 45 kDa films. While performing these experiments, the total amount of strain applied in each stage was kept constant, by varying the loading time and relaxation time. Samples were subjected to five repetitive stage of ramp-hold tests in three conditions:

Condition 1. Constant strain applied at 3.0% s^{-1} for 10 s, and relaxed for 20 s.

Condition 2. Constant strain applied at 1.0% s^{-1} for 30 s, and relaxed for 30 s.

Condition 3. Constant strain applied at 0.6% s^{-1} for 50 s, and relaxed for 100 s.

Despite these changes, the behaviors were similar in all three conditions (Figure 5); the first stage showed the highest stress accumulation, and there was little difference in stress accumulation of successive stages. Others have shown similar frequency independent behavior in small amplitude ranges on PCL scaffolds formed by hybrid twin-screw extrusion coupled with electrospinning.[24] However, the stress accumulation at 0.6% s^{-1} strain rate was the highest of the three testing conditions, but similar to that from 1.0% s^{-1} and 3.0% s^{-1} strain rates. This suggests that when the amount of applied strain in each stage is less than that required for preconditioning, changes in accumulated stresses are expected in subsequent stages.

Alterations in Relaxation Behavior in the First Stage

As the maximum stress experienced by each sample was different, reduced relaxation functions $G(t)$ were plotted by normalizing the relaxation portion of the data to the highest stress experienced by each structure in the first stage. Further, to compare the results of 45 kDa films with different loading and relaxation times (which were pulled to 30% strain at the same 1% s^{-1} rate by loading for 30 s) the time axis was also normalized by dividing each time with 60 s, which is the total duration of each stage. From $G(t)$ plots (Figure 6), the salt leached scaffolds consistently showed less relaxation than films; only 15–20% of the stress is relaxed in each scaffold sample. 45 kDa scaffolds and films had a higher relaxation than 80 kDa scaffolds and films. Electrospun 80 kDa scaffolds showed relaxation behavior similar to 80 kDa films. The difference in percentage of relaxation between the 45 kDa and 80 kDa scaffolds and films were similar. 45 kDa films with a higher strain rate showed higher relaxation in the first stage but had a similar relaxation behavior at higher stages. This suggests that the polymer preconditioning occurs during the loading cycle. However, further experiments are necessary to better understand these behaviors.

Alterations in Cyclical Behavior

Apart from the viscoelastic experiments, cyclical tests are conducted separately to assess the fatigue characteristics of scaffolds as many biological loads are cyclical in nature. We

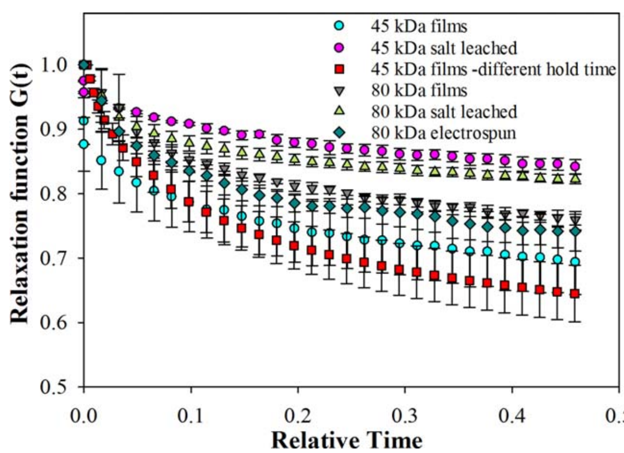

Figure 6. Normalized-relaxation function, $G(t)$, plot of first cycle of each strain rate. Shown values are average of three samples and error bars correspond to standard deviations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

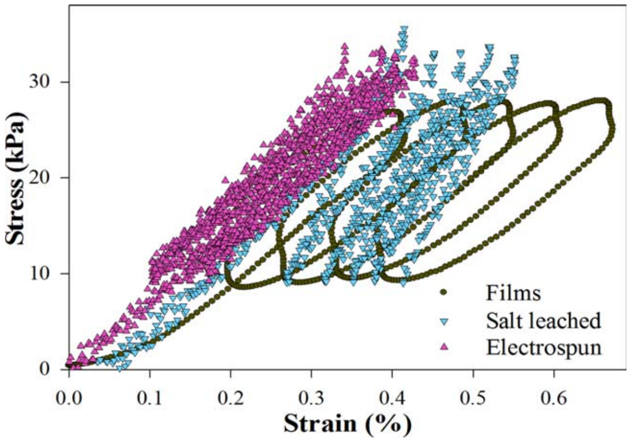

Figure 7. Effect of five cycles of fatigue on 80 kDa PCL structures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

questioned how the scaffolds would behave under cyclical loading. Cyclic tests were conducted using 80 kDa PCL structures in PBS at 37°C, similar to stress relaxation experiments. These results (Figure 7) showed that all samples had maximum deformation in the first cycle, and the subsequent cycles did not significantly deform any samples except the films. Also, films showed large hysteresis loops in each cycle. The electrospun scaffolds had the least deformation in the five cycles. However, salt leached scaffolds had maximum deformation and at each cycle there was an increase in the sample size. This could be attributed to less break stress and break strain in these samples in addition to the stiffness constants. Since films had larger hysteresis loops, we performed cyclical experiments using films in the stress range of 3 and 10.5 MPa. Under these conditions, no hysteresis loops, appeared and samples showed repetitive cyclical characteristics. Thus one has to determine the range in which these samples are used and their relationship to the break stress and break strain.

DISCUSSION

This study focused on understanding the effect of scaffold processing on stress relaxation characteristics of PCL scaffolds. The percentage of stress relaxed in PCL films in the first stage was similar to that of 50 : 50 poly-lactide-*co*-glycolide (PLGA) films.[26] However, the stress accumulation of PLGA films decreased in successive stages, leading to strain softening. This difference could be attributed to the fact that 50 : 50 PLGA is an amorphous polymer whereas PCL is a semi crystalline polymer. Nevertheless, further analysis is required to understand the preconditioning rationale in PLGA. Compared to our previous study in the same conditions, the stress relaxation trend and the value of stress accumulation were similar for both chloroform-casted and self-assembled PCL films despite their difference in MW. The relaxation behavior was different from that of chitosan and chitosan/gelatin[23] and also from small intestinal sub mucosa (SIS), a natural matrix with high amounts of type 1 collagen dispersed with other matrix elements.[26] The stress accumulation in SIS increased in successive stages but chitosan and chitosan–gelatin scaffolds showed no change in stress accumulation in successive stages. The percentage of relaxation in the first stage was similar to that of SIS. Also, chitosan scaffolds showed 90% relaxation property at the end of each stage but PCL scaffolds show only 25% relaxation at the end of each stage. Probably, blending of both the polymers may give a better relaxation property.

PCL polymers are viscoelastic in nature, i.e., the polymer begins to creep when a constant force is applied to the polymer above T_g, thereby affecting the degree of crystallinity of the polymer. PCL scaffolds by salt leaching shows a higher degree of crystallinity than scaffolds prepared from a melting compression technique.[29] PCL films cast from tetrahydrofuran (THF) showed that the degree of crystallinity decreases for the increase in concentration of the solutions. But for the same MW, PCL sponges show an increase in crystallinity for the increase in concentration. The characterization of the property varies for porous structure from that of films due to the effect of densification that produces the pore collapse.[30] Determining activation energy and changes in degree of crystallization are necessary to understand polymer mobility during scaffold processing. Others have analyzed activation energy for PCL mobility in various blends.[31,32] These results show that apart from horizontal shift factor (α_T) obtained using Williams Landau Ferry (or WLF) equation, vertical shift factor (β_T) has to be measured by combining dynamic mechanical analysis at various temperatures along with crystal structure changes. Further studies would help understand the observed changes in PCL properties due to the scaffold processing.

We used a simple linear model to understand the viscoelastic characteristics.[20] The variation in the pore size distribution of the scaffolds by electrospun and salt leaching causes the deviation in relaxation property. Others have reported on the effect of pore architecture on dynamic mechanical properties and increased stiffness with increased amount of polymer.[33] Our results also showed increased stiffness in the films and electrospun scaffolds but the relaxation time is not affected. Relaxation time is the ratio of Maxwell dashpot viscosity to the Maxwell stiffness k_1. At constant relaxation time, alterations in k_1 value lead to altered viscous contribution in the dashpot. Thus, the scaffold processing does affect the viscoelastic behavior. However, a linear model is accurate near the reference strain used in the model, and not for other strains. Hence, nonlinear models are preferred.[20]

From previous publications, the stress relaxation plots show concave downward trends for synthetic scaffolds, but are concave upwards for natural tissues such as ligaments.[19,34] One dominating model in biomechanics is the quasi-linear viscoelastic (QLV) modeling approach published by Fung in 1967.[18] QLV model needs a faster initial loading data from experimental analysis and suffers from many limitations, including inability to model non-stationary behavior. Another complementary modeling approach uses spring-and-dashpot based constitutive models, modified by including nonlinear hyper elastic "spring" elements.[23] Modeling of the relaxation property by different methods of processing needs to be further investigated to assess whether those models fit better than standard linear model.

Considering all stages of the "ramp-hold" experiment would be useful in the model development to account for changes occurring in different stages.[23] This could also be used in testing whether models can predict cyclical behavior, similar to our previous reports on chitosan and chitosan–gelatin scaffolds. We did not perform these tests in this study, but should be considered in the future. Nevertheless, tested stress ranges in cyclical tests were nearly ten times higher than chitosan–gelatin scaffolds previously reported,[23] where the stresses were between 0.5 and 1.5 kPa and the strain range was within 3% but 10 times lower than that reported for small intestinal sub mucosa (SIS).[27] Thus, while utilizing PCL scaffolds in different applications one has to consider these outcomes and then evaluate the influence on regeneration.

In summary, the results show that PCL scaffolds prepared by electrospun technique relax more than the scaffolds prepared by salt leaching technique; however films relax more than scaffolds. High MW structures were more elastic than low MW structures. In both scaffolds and films there was a progressive increase in stress value for each stage in ramp-hold tests. Although the amount of loading was changed, 45 kDa films showed similar relaxation behavior, which implies that the polymer preconditioning occurs during the loading cycle.

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REFERENCES

1. Oh, S. H.; Park, I. K.; Kim, J.M.; Lee, J. H. *Biomaterials* **2007**, *28*, 1664.
2. Woodruff, M. A.; Hutmacher, D. W. *Prog. Polym. Sci.* **2010**, *35*, 1217.
3. Little, U.; Buchanan, F.; Harkin-Jones, E.; McCaigue, M.; Farrar, D.; Dickson, G. *Polym. Degrad. Stab.* **2009**, *94*, 213.
4. Ho, K. L.; Witte, M. N.; Bird, E. T. *J. Urol.* **2004**, *171*, 268.
5. Eberli, D.; Freitas Filho, L.; Atala, A.; Yoo, J. *J. Methods* **2009**, *47*, 109.
6. Hong, J. K.; Madihally, S. V. *Acta Biomater.* **2010**, *6*, 4734.
7. Hong, J. K.; Xu, G.; Piao, D. Q.; Madihally, S. V. *J. Appl. Polym. Sci.* **2013**, *128*, 1583.
8. Heo, S. J.; Kim, S. E.; Wei, J.; Hyun, Y. T.; Yun, H. S.; Kim, D. H.; Shin, J. W. *J. Biomed. Mater. Res. A* **2009**, *89*, 108.
9. Hou, Q.; Grijpma, D. W.; Feijen, J. *Biomaterials* **2003**, *24*, 1937.
10. Gercek, I.; Tigli, R. S.; Gumusderelioglu, M. *J. Biomed. Mater. Res. A* **2008**, *86*, 1012.
11. Ozkan, S.; Kalyon, D. M.; Yu, X.; McKelvey, C. A.; Lowinger, M. *Biomaterials* **2009**, *30*, 4336.
12. Hong, J. K.; Madihally, S. V. *Tissue Eng. Part B: Rev.* **2011**, *17*, 125.
13. Erisken, C.; Kalyon, D. M.; Wang, H.; Ornek-Ballanco, C.; Xu, J. *Tissue Eng. Part A* **2011**, *17*, 1239.
14. Sarasam, A. R.; Samli, A. I.; Hess, L.; Ihnat, M. A.; Madihally, S. V. *Macromol. Biosci.* **2007**, *7*, 1160.
15. Sarasam, A. R.; Brown, P.; Khajotia, S. S.; Dmytryk, J. J.; Madihally, S. V. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 1083.
16. Ergun A.; Yu X.; Valdevit, A.; Ritter, A.; Kalyon, D. M. *Tissue Eng. Part A* **2012**, *18–24*, 2426.
17. Craiem, D. *Phy.s Med. Biol.* **2008**, 53.
18. Fung, Y. C. *Am. J. Physiol.* **1967**, *213*, 1532..
19. Duling, R.; Dupaix, R.; Katsube, N.; Lannutti, J. *J. Biomech. Eng.* **2008**, 130.
20. Funk, J. R.; Hall, G. W.; Crandall, J. R.; Pilkey, W. D. *J. Biomech. Eng.* **2000**, *122*, 15.
21. Haslach, H. W. *Jr. Biomech. Model. Mechanobiol.* **2005**, *3*, 172.
22. Pok, S. W.; Wallace, K. N.; Madihally, S. V. *Acta Biomater.* **2010**, *6*, 1061.
23. Ratakonda, S.; Sridhar, U. M.; Rhinehart, R. R.; Madihally, S. V. *Acta Biomater.* **2012**, *8*, 1566.
24. Erisken, C.; Kalyon, D. M.; Wang, H. *J. Biomech. Eng.* **2010**, *132*, 091013.
25. Abramowitch, S. D.; Woo, S. L.; Clineff, T. D.; Debski, R. E. *Ann. Biomed. Eng.* **2004**, *32*, 329.
26. Mirani, R. D.; Pratt, J.; Iyer, P.; Madihally, S. V. *Biomaterials* **2009**, *30*, 703.
27. Raghavan, D.; Kropp, B. P.; Lin, H. K.; Zhang, Y.; Cowan, R.; Madihally, S. V. *J. Biomed. Mater. Res. A* **2005**, *73*, 90.
28. Shazly, T. M.; Artzi, N.; Boehning, F.; Edelman, E. R. *Biomaterials* **2008**, *29*, 4584.
29. Barbanti, S. H.; Zavaglia, C. A. C.; Duek, E. A. D. R. *Mater. Res.* **2008**, *11*, 75.
30. Más Estellés, J.; Vidaurre, A.; Meseguer Dueñas, J.; Castilla Cortázar, I. *J. Mater. Sci.: Mater. Med.* **2008**, *19*, 189.
31. Aoki, Y.; Arendt, O. *J. Appl. Polym. Sci.* **2001**, *82*, 2037.
32. Kwak, S. Y.; Oh, K. S. *Macromol. Mater. Eng.* **2003**, *288*, 503.
33. Moroni, L.; de Wijn, J. R.; van Blitterswijk, C. A. *Biomaterials* **2006**, *27*, 974.
34. Wang, C. C. B.; Hung, C. T.; Mow, V. C. *J. Biomech.* **2001**, *34*, 75.