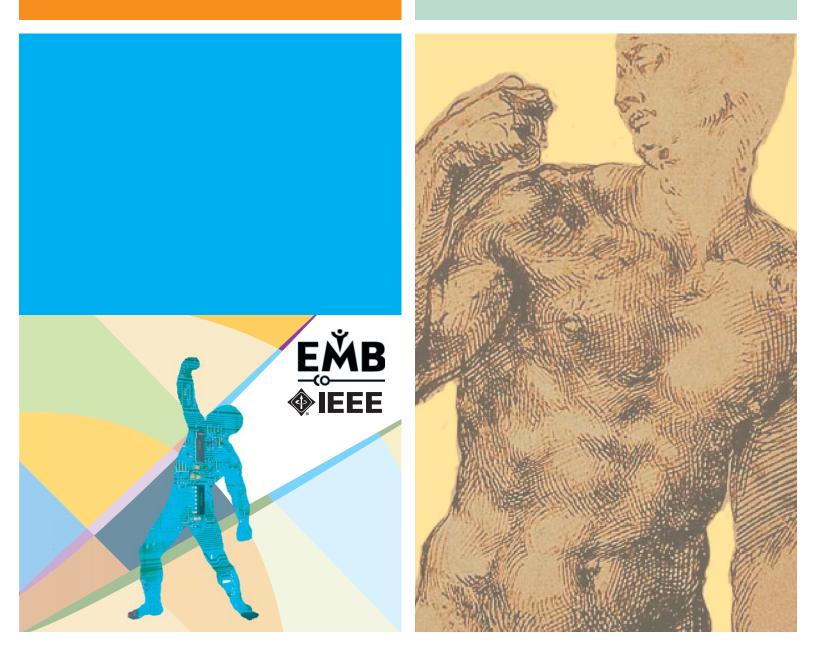# 2007 IEEE Dallas Engineering in Medicine and Biology Workshop

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Nano-featured highly interconnective macroporous elastic scaffolds for cardiovascular tissue engineering

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Abstract- The lack of suitable scaffolding materials and viable scaffold design is challenging the success of tissue engineering. In the present study, we developed a new scaffolding strategy using a secondary porogen, poly(ethylene glycol) dimethyl ether along with sieved salts based on traditional salt-leaching method. This new scaffolding technology could allow us to fabricate nano-featured highly interconnective macroporous biodegradable elastic scaffolds based on a newly developed crosslinked poly(1,8-octanediol citrate) (POC) for cardiovascular tissue engineering.

1. Introduction

Tissue engineering has emerged as an alternative technology to regenerate lost tissues or organs utilizing biodegradable scaffolds seeded with cells with the benefits of avoiding a second surgery. Significant progresses have been made for *in vitro* tissue engineering SDBV and cardiac tissues.[1-5] However, even with these successes, no graft has successfully achieved long-term patency when used for tissue-engineering small diameter blood vessels (<6 mm in diameter, such as coronary artery) and cardiac tissues to date. Why has tissue engineering not progressed as quickly as expected? The lack of suitable scaffolding materials and viable scaffold design challenges the success of tissue engineering along with the difficulty of finding suitable cell sources. Biodegradable scaffold is one of the key to the success of tissue engineering which requires the scaffold should be highly porous and interconnective to allow cells infusion and mass transport. The common used methods today are fiber bonding, solvent casting/particulate leaching, gas foaming, phase separation/emulsification etc.[6, 7] However the organic solvents used in these processes can be harmful to cells or growing tissue.[8] Uneven cell distribution and nutrient delivery in the deep portion of the scaffolds (> 200 μm deep), due to the random mobility of cell

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suspensions, often compromised the success of tissue engineering.[9] Cells tend to concentrate at the periphery of scaffold made of synthetic polymers.[10] After implantation, tissue ingrowth and vascularization are limited partially due to the hydrophobicity and unfavorable scaffold interconnectivity. Significant fibrous capsule formation, a normal inflammation reaction of the host to the implanted foreign materials, also significantly contributes to the lack of tissue ingrowth and neo-vascularization for the implants. Current materials such as Food & Drug Administration (FDA) approved polylactone materials are generally stiff and relatively inelastic, and the mechanical irritation to the surrounding tissues causes aggravation and contributes to inflammation reactions. This sort of response makes these materials unsuitable for regeneration of soft and elastic tissue, such as heart muscles and blood vessels.[11, 12]

In the present study, we focus on developing a method to fabricate nano-featured highly interconnective macroporous scaffolds for cardiovascular tissue engineering based on our recently developed novel biodegradable elastomers, poly (1,8-octanediol citrate) (POC).[12-15] This method attempts to greatly improve the interconnectivity of the salt-leaching scaffold and mimic the soft and elastic properties of the soft cardiovascular tissues.

2. Methods and materials

2.1. Preparation of POC

The synthesis of POC was described elsewhere.[12-15] Briefly, POC was synthesized by reacting equimolar amounts of citric acid and 1,8-octanediol. Both the chemicals were added into a 250ml round bottomed flask and melted in an oil bath maintained at 160-165°C. The contents were allowed to react over a period of 1 hour. Once all the contents have melted the temperature is reduced to 140°C to create a POC pre-polymer (pre-POC). Pre-POC is mixed with PEGDM (poly(ethylene glycol) dimethyl ether, $M_n = 250$ and 500) which is used as a secondary porogen to create nano-features. For POC-PEGDM (nano-POC) films, a 1/2, 1/4, 1/6 (w/w) POC/PEGDM pre-polymer solution was blended and subsequently incubated at 120°C for 4 days under vacuum (2 Pa). The control POC film was polymerized for 4 days at 120°C. In case of scaffolds a 1/2/8, 1/4/8, 1/6/8 (w/w) POC/PEGDM/salt(150-250 μm) and polymerized for 1 day without vacuum at 120°C and another 3 days under high vacuum conditions. The PEGDM and salt was removed by leaching the films and scaffolds in deionized water.

2.2. Polymer characterization

The fourier transform infrared spectra (FTIR) of POC, PEGDM and POC-PEGDM mixture, POC-PEGDM (nano-POC) films were obtained at room temperature. Polymers were prepared on a KBr pellet directly for FTIR measurements.

Differential Scanning Calorimetry (DSC) was performed on a DSC 550 (Instrument Specialists Inc. Spring Grove) to determine the thermal behavior of the polymer samples. Samples are first scanned up to 150°C with a heating rate of 10°C/min under nitrogen purge (50ml/min). Thereafter cooled with a cooling rate of -40°C/min to -60°C and recorded a second time up to 230°C. The glass transition temperature (T_g) is determined as the middle of the recorded step change in heat capacity from the second heating run.

Tensile mechanical tests were conducted using an MTS Insight II mechanical tester. The samples were cut into dog-bone-shape samples according to ASTM D412. A 500N load cell was used at a crosshead speed of 500 mm/min.

2.3. Sample preparation for SEM

POC-PEGDM samples were then sputter coated with Cu and observed under the SEM. The images taken were nalyzed using Image J (NIH) to determine interconnectivity. The total area of the pore (A_t) and the area of each micropore above 20μm in diameter (A_1, A_2, A_3 etc) were calculated. The interconnectivity index was defined as follows.

$$Interconnectivity\ Index\ \% = \frac{Total\ Area\ of\ Micropores\left(A_1 + A_2 + A_3 + \ldots\right)}{Total\ Area\left(A_t\right)} \times 100 \ldots\ldots(2)$$

The interconnectivity index for all the different pores in the picture was calculated and an average of this value is taken as the final value. More than 10 SEM pictures for each sample were analyzed.

2.4. In vitro degradation study

The polymer scaffolds were cut out in circular discs of ~7mm diameter and 1.5-2 mm diameter, weighed and placed in tubes containing 10ml of PBS (phosphor buffered saline) at a pH of 7.4. The specimens were incubated at 37°C over a period of 6 weeks. Samples were taken out at predetermined time intervals to obtain the wet weight (W_t) and dimensions. The samples are then washed with deionized water and freeze dried for a week after which the dry weight (W_d) and dimensions are obtained. Mass loss % is calculated using the initial weight (W_0) with the mass at given time point (W_t) as shown in equation (1).

$$Mass\ Loss\ \% = \frac{W_0 - W_t}{W_0} \times 100 \ldots\ldots(1)$$

2.5. Porosity measurements

The porosity of scaffolds were measured according to a method described elsewhere based on the Archimedes principle.[16] The sample was cut out into a small strip and the initial weight of the sample was calculated. A density bottle containing ethanol (replacing liquid) is placed in a water bath maintained at 30°C. The density bottle is placed

inside the bath till there is no more ethanol spilling out. At this point the bottle is weighed (W_1). A piece of scaffold is suspended in the bottle and vacuum is applied for a few minutes to make sure there are no air bubbles. The bottle is filled with ethanol and balanced again and the weight W_2 is obtained. The sample is removed and weight W_3 is obtained. The porosity can be calculated from the formula.

$$\%Porosity = \frac{W_2 - W_3 - W_0}{W_1 - W_3} \times 100 \ldots\ldots(3)$$

2.6. Statistical analysis

Data were presented as means ± standard deviation. The statistical significance between two set of data was calculated by two-tailed student t test. Data were taken to be significant when a p value of 0.05 or less was obtained.

3. Results

The FTIR spectra of the PEGDM, POC and POC-PEGDM before and after polymerization are shown in Figure 1 A. The peaks at 1735 cm^{-1} were assigned to carbonyl (C=O) groups. The peak at ~ 2870cm^{-1} attributes to the methyl group of PEGDM. The peak at 1060-1030 cm^{-1} stands for the aliphatic C-O- in PEGDM and POC. The peaks at 2931 cm^{-1} were assigned to methyl groups. The spectra of POC-PEGDM mix includes all the peaks from PEGDM and POC but with a significant decrease of intensity of carbonyl group due to the relatively large amount of PEGDM mixed with POC. After leaching, a significant carbonyl peak appeared on POC-PEGDM (nano-POC) spectra. The DSC results indicated that the T_g of the nano-POC has been reduced to about -14.52°C compared to the -1.49 °C of POC.
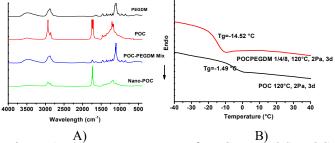

A)   B)

Figure 1: A) FTIR spectrum of PEGDM, POC, POC-PEGDM mix, and nano-POC after PEGDM leaching; B) DSC thermograms of POC and nano-POC.

SEM observation on POC-PEGDM films indicated that there are nano- or sub-micron-scale tortuous tunnels or islands presented on the surface and inside of POC-PEGDM films. The stress-strain curves of POC-PEGDM films in Fig 3A suggested that POC-PEGDM are characteristic of elastomers. The low Young's modulus and high elongation indicated that POC-PEGDM is soft and elastic which could well match those of the native soft tissues.[17] No permanent deformation was found after sample breaks during tensile tests. The in-vitro degradation results of POC/PEGDM porous scaffolds over a period of 6 weeks are shown in Fig. 3B. POC-PEGDM 1/2/8 scaffolds degraded as fast as POC

80°C 3 days (P>0.05) which are much faster than POC 120°C, 2Pa and 4 days (Fig. 3B). The samples of the POC-PEGDM 1/6/8 scaffolds decomposed into small pieces after
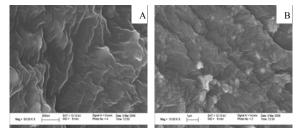

Figure 2. SEM pictures of POC-PEGDM (nano-POC) films. A) surface morphology, scale bar=300 nm; B) cross-section. scale bar=1 um.

one week degradation (Data not shown here).

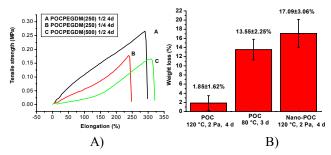A)   B)

Figure 3: A) Stress-strain curves of POC-PEGDM films; B) Degradation profiles of POC and POC/PEGDM 1/2/8 scaffolds over a period of 6 weeks in PBS at 37 °C.

The cross sectional morphology of the different scaffolds was analyzed by SEM imaging. POC control scaffolds (Fig. 4A) showed a lot of dead pores which is typical for salt-leaching scaffolds. It was observed that there were more open pores in the scaffolds using PEGDM as an additional porogen. With the amount of PEGDM increasing, the pore wall was evolved into fibrous structure (Fig. 4B-D) for POC-PEGDM scaffolds. For salt leaching method, scaffold porosity can be calculated as the weight ratio of salt/(polymer+salt). Our porosity measurements confirmed that the measured porosity for POC-PEGDM scaffolds could well match the calculated porosity when the weight of PEGDM is considered. (Table 1) Interconnectivity of POC-PEGDM scaffolds was increased significantly (P<0.05) with using PEGDM as secondary porogen when compared to POC control scaffolds. (Fig.5).

Table1 : porosity measurement for POC-PEGDM scaffolds.

Sample	MEASURED POROSITY (%)	Calculated Porosity
POC/PEGDM/ salt 1/2/8	92.82 ± 2.68	90.91
POC/PEGDM/ salt 1/4/8	91.2 ±7.26	92.31
POC/PEGDM/ salt 1/6/8	89.56 ± 6.36	93.33

A)   B)
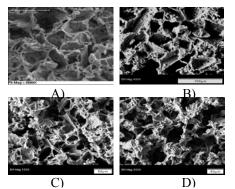
C)   D)

Figure 4: SEM images of a.) POC control, b.) POC/PEGDM scaffold 1/2/8, c.) POC/PEGDM scaffold 1/4/8, d.) POC/PEGDM scaffold 1/6/8.
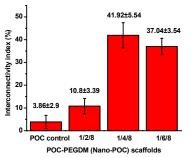

Figure 5: Interconnectivity index of nano-POC scaffolds with POC/PEGDM250/salt ratio of 1/2/8, 1/4/8, and 1/6/8 and POC control scaffolds.

4. Discussion

Finding suitable biomaterials and scaffold fabrication based on the biomaterials is an intense focus in tissue engineering field. For soft tissue engineering soft elastic scaffolds should be able to sustain and recover from cyclic deformation without irritation to the surrounding tissues.[11] Scaffold compliance mismatch has been long recognized to be associated with grafts failure in cardiovascular tissue engineering. Many polymers like poly(L-lactic acid), poly(glycolic acid) and collagen I are not elastic and subject to permanent deformation under cyclic mechanical stimulation thus increasing the chances of graft failure[18]. The ECM is naturally a mixture of pores, ridges and fibers at nanometer scales[19]. Porous scaffolds facilitates cell infiltration and repopulation[20]. Thus scaffolds for soft tissue engineering should possess high interconnectivity and mechanical properties similar to the native soft tissues.

We have developed a novel biodegradable elastomer POC which has shown its potential for soft tissue engineering with excellent biocompatibility and hemocompatibility *in vitro* and *in vivo*. [12-15] However, previously made POC scaffolds by salt-leaching consists of a large amount of dead pores which are not suitable for cell infiltration and mass transport. In the present study, we developed an improved scaffold fabrication technology by using a secondary porogen, PEGDM along with sieved salts. In FTIR results, no carbonyl group was shown in PEGDM spectra. The high intensity of carbonyl groups re-appeared after PEGDM

leaching as shown in POC-PEGDM spectra confirming that the most of PEGDM can be leached out. The Tg of POC-PEGDM decreased compared to that of POC suggested PEGDM might have partially participated in the formation of crosslinking network of POC due to trans-esterification under high temperature during reactions. The leaching of the remaining PEGDM in the POC could create nano-tortuous tunnel or island features which are expected to promote cell adhesion and proliferation. Preliminary bovine serum albumin (BSA) release experiments showed that BSA loaded permeable elastic POC membrane could maintain significant BSA release even at the 4th day while regular POC membrane stopped release in 1 day (data not shown here). This was explained that BSA could penetrate into tortuous POC film while only adsorbed on the regular POC film surface. Therefore the nano-featured POC scaffold can potentially be used as drug vehicles in tissue engineering in addition to as cell carriers. In addition to selecting proper monomers and synthesis conditions for POC,[12] introducing PEGDM as a secondary porogen could be an additional way to modulate the mechanical properties and degradation properties of POC scaffolds as shown in Fig. 3 due to the partial participation of PEGDM in crosslinking network formation which may change the molecular weight between crosslinks and hydrophilicity/hydrophobicity of the polymers.[12] The majority of PEGDM will be removed in the leaching process. Using PEGDM could also significantly enhance the interconnectivity of the porous scaffolds. The highly interconnective open pore structure of POCPEGDM scaffolds should facilitate the cell seeding and tissue formation within it. Biocompatibility evaluation of the fabricated POC-PEGDM scaffolds is under active investigation in our lab through cell culture and animal implantation.

In conclusion, we have identified a new porogen, PEGDM for scaffold fabrication in tissue engineering. PEGDM could effectively modulate the pore interconnectivity, scaffold morphology (nano-features, fibrous pore structure), mechanical properties and degradation rates of POC scaffolds. Salt-leaching is a convenient and widely used scaffold fabrication technology. Our study should further improve the utility of the salt-leaching method in tissue engineering application and expand the repertoire of the available scaffold fabrication technologies in tissue engineering.

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