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Soluble poly(glycerol sebacate) and poly(ε-caprolactone) 3D scaffolds for blood vessel constructs

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ABSTRACT

Cardiovascular diseases, frequently associated to the formation of aneurisms, are the mayor cause of mortality and morbidity in the world. Due to the increased need for the regeneration of arteries and veins, several natural and synthetic biopolymers such as poly(glycerol sebacate), PGS, have been studied to make blood vessel constructs. PGS elastomeric properties develop after it is crosslinked; however, the poor solubility of the material limits the process to fabricate useful constructs for tissue engineering by electrospinning, casting, or other methods. The structure and properties of electrospun scaffolds made from soluble poly(glycerol sebacate) and poly(ε-caprolactone), are reported here. Soluble PGS oligomers (o-PGS) of different molecular weight, obtained by the polycondensation reaction of sebacic acid and glycerol, were analyzed, including molecular structure, physical properties and solubility. Temperature, reactor atmosphere, and time of reaction strongly influenced the solubility, the molecular weight and molecular structure. To improve o-PGS processing and properties it was mixed with PCL to make electrospun scaffolds. In order to process the mixture by electrospinning, homogeneous solutions o-PGS and PCL were prepared. Because PCL is hydrophobic and o-PGS is hydrophilic selected solvent mixtures were tested to form the homogeneous solutions; the materials dissolved in a mixture of THF:DMF:DCM. Typical electrospinning parameters for preparing the tubular scaffolds at room conditions were: voltage 17.5 kV, needle-collector distance 20 cm and, relative humidity 30-35%, flow injection 0.5 to 2.0 ml/h. The initial mechanical properties of the biodegradable scaffolds were better than those made of natural grafts; the Young's modulus ranged from 7.6 to 13.0 MPa, depending on electrospinning process parameters. The morphology and physical properties of electrospun PGS/PCL tubular scaffolds show useful features not found in similar constructs made by other methods. The 3D tubular scaffolds were built-up of layered porous walls to produce constructs of different pore size and fibers of different diameter. The porous area was one to two orders of magnitude higher than those produced at micrometer scale by conventional melting and dry/wet spinning methods. These scaffolds show useful characteristics for regenerative medicine such as physical properties; nanometric diameters; high surface/volume ratio; and potentiallity for adhesion and growth of living cells.

INTRODUCCION

Cardiovascular diseases are the mayor cause of mortality and morbidity in the world. An important disease related with the cardiovascular system is the formation of aneurisms that could be present in any part of the cardiovascular network. The use of natural grafts is limited because of their availability and the fast rate of aterosclerosis shown by some of these grafts. Thus, biodegradable synthetic polymer materials are often required for the treatment of vascular diseases.

Some of the associated risk factors are genetic, people with prolonged hypertension; age over 50 years, and smokers. The pathologies of arteries including the aorta have great influence over the mechanical properties of the arterial wall, as can be seen in affected vessels by arteriosclerosis, aneurism and genetic diseases like the Marfan syndrome [1]. However, the formation and rupture of aneurisms are recognized as complex problems associated to other medical severe risk factors. An aneurism is the abnormal dilatation of a portion of a blood vessel by the weakening of the vessel wall. Transplants (autotransplants, allotransplant and the xenotransplant) are useful alternatives, but frequently good results are limited. In Mexico 60-70% of patients who have had a transplant die from some type of infection (fungus). Another alternative to repair or regenerate the damaged tissues is the use of synthetic scaffolds made of different biopolymers.

Most tubular scaffolds are biodegradable and bioabsorbable, thus they must provide the functions of the tissue to be repaired while the patient extracellular matrix regenerates. Therefore they must be highly biocompatible with good physical, chemical and physiological properties. In addition, the materials must be processed easily in different inner diameters and wall thickness.

To comply with these requirements, we focus on two biodegradable and hemocompatible polymers: poly(glycerol sebacate), o-PGS, and poly(ε- caprolactone), PCL, based on the reports of Wang et al and Shilpa et al [2,3]. PGS elastomeric properties develop after crosslinking however the poor solubility of the cured PGS limits the process to fabricate useful constructs for tissue engineering by electrospinning, casting, or other methods. Therefore, soluble PGS oligomers (o-PGS) of different molecular weight, obtained by the condensation reaction of decanoic acid (sebacic acid) and 1,2,3-propanotriol (glycerol), were analyzed, including molecular structure, physical properties and solubility.

To improve o-PGS processing and properties, it was mixed with PCL. In order to process stable constructs by electrospinning, homogeneous solutions o-PGS and PCL were prepared. Because PCL is hydrophobic and o-PGS is hydrophilic, selected solvent mixtures of tetrahydrofurane (THF), dimethylformamide (DMF) and dichloromethane (DCM) at different ratios were used to obtain homogeneous solutions.

The 3D scaffolds were prepared by electrospinning because it is the most useful, simple and versatile method for controlling the structural parameters of 3D tubular scaffolds such as polymeric composition, texture, fiber diameter and orientation, inner tube diameter and wall thickness, mesh porosity, and mechanical properties.

The structure and properties of the materials were determined with the support of GPC, MALDI-TOF, FTIR, 1H and ^{13}C NMR, XDR, DSC, TGA, SEM, water contact angles and micromechanical tests. The morphology and physical properties of the o-PGS/PCL scaffolds were evaluated to determine their potential as blood vessels constructs for the treatment of aneurisms and regeneration of blood vessels.

EXPERIMENTAL DETAILS

Analytical grade PCL (70-90 kDa), decanoic acid (sebacic acid) and 1,2,3-propanotriol (glycerol), tetrahydrofuran, dichloromethane, and dimethylformamide, (Sigma-Aldrich) were used as received.

The o-PGS's were synthesized by polycondensation at an equimolecular ratio of sebacic

acid and glycerol. After mixing under 0.18 torr during 5h, the monomer mix was put in an oil bath at 120ºC under dry N_2 atmosphere for 24 h. Molecular weights were determined by GPC and MALDI-TOF. The o-PGS synthesis was monitor by the infrared carbonyl (C=O at 1732cm^{-1}) and ester (C-O at 1163 cm^{-1}) bands. Properties were correlated to the reaction parameters.

To improve processing and properties of the scaffolds the o-PGS was mixed with the PCL. A mixture of o-PGS and PCL, with a weight ratio of 2:1, was dissolved in a mixture of dichloromethane (DCM), tetrahydrofuran (THF) and dimethylformamide (DMF), in a volume ratio of 7:2:1 to form homogeneous solutions. Polymers concentration was adjusted at 33% w/v. According to CAS registries, the toxicity of the solvents used to dissolve o-PGS/PCL is 10 fold below those reported elsewhere.

An electrospinning set-up [4] was used to make the o-PGS/PCL scaffolds**,** according to reported procedures and previous tuning of the equipment, Wang et al and Sant et al [2, 5]. Typical electrospinning parameters were: voltage 17.5 kV, needle-collector distance 20 cm, relative humidity 30 to 36, and injection flow rate 0.2 to 0.5 ml/h.

A rotatory collector with a mandrel to fix exchangeable shafts made of cooper of different diameters (between 0.6 and 2.0 cm) and a motor that makes the shaft to spin at controlled speeds were designed. A rectangular cooper frame collector of obtain 2D scaffolds was also used for the mechanical tests. The collectors prevent deformations of the scaffolds when they are retired from the collectors; in order to preserve the morphology of the constructs.

The samples were analysed by infrared spectroscopy (FTIR Nicolet 6700) with the aid of a reflectance attenuated total reflectance accessory (ATR, Smart Orbit). The structures were confirmed by proton nuclear magnetic resonance (H^1 –NMR, Bruker Advance, mod. 400).

The morphology of the scaffolds was studied by scanning electron microscopy under field emission (SEM Jeol JSM-7600F). Hydrophilicity was determined by measuring the contact angle of a sessile drop of water on the sample (goniometer Ramé-hart Inc, mod. 100-07-00). In order to compare the mechanical properties with those of blood vessels the mechanical properties of scaffolds under tension were measured (Ultim Universal equipment of Exel test of special design, with a certificate system of calibration FUTEK, (Advanced Sensor Technology Inc. and a uniaxial traction MTS mini Bionix 858 with a charge cell LCM300)).

Crystallinity was determined by X-ray diffraction, (WAXS SIEMENS D-500). The thermal transitions of the materials were evaluated by differential scanning calorimetry (TA Instruments DSC Modulated Q 2000 V24.10 Build 122). The sample, ~ 10 mg, was placed in a standard aluminium sealed pan. Runs were programmed at heating ramp of 10ºC/min under dry N_2. The heating-cooling cycle was repeated itself twice from -70 to 150ºC to register the thermal history. The thermal weight loss was determined by thermogravimetry (TA Instruments TGA Q 5000 V3.15 Build 263).

DISCUSSION

The molecular weight, Mn, of the obtained o-PGS, ranged between 12,800 and 13,900 kDa. The observed infrared signals of the scaffolds in Fig. 1(a); they agree with those reported by Wang et al [2]; the OH groups appear at 3442 cm^{-1}, the CH_2 at 2925 and 2850 cm^{-1} and the carbonyl group and ester at 1729 and 1163 cm^{-1} respectively. The ^{1}HNMR spectrum and the structure of the o-PGS

are shown in Fig. 1(b); the signal of glycerol is in the range 3.5 to 5.5 ppm and sebacic acid peaks were identified in 1.30, 1.62 and 2.35 ppm.

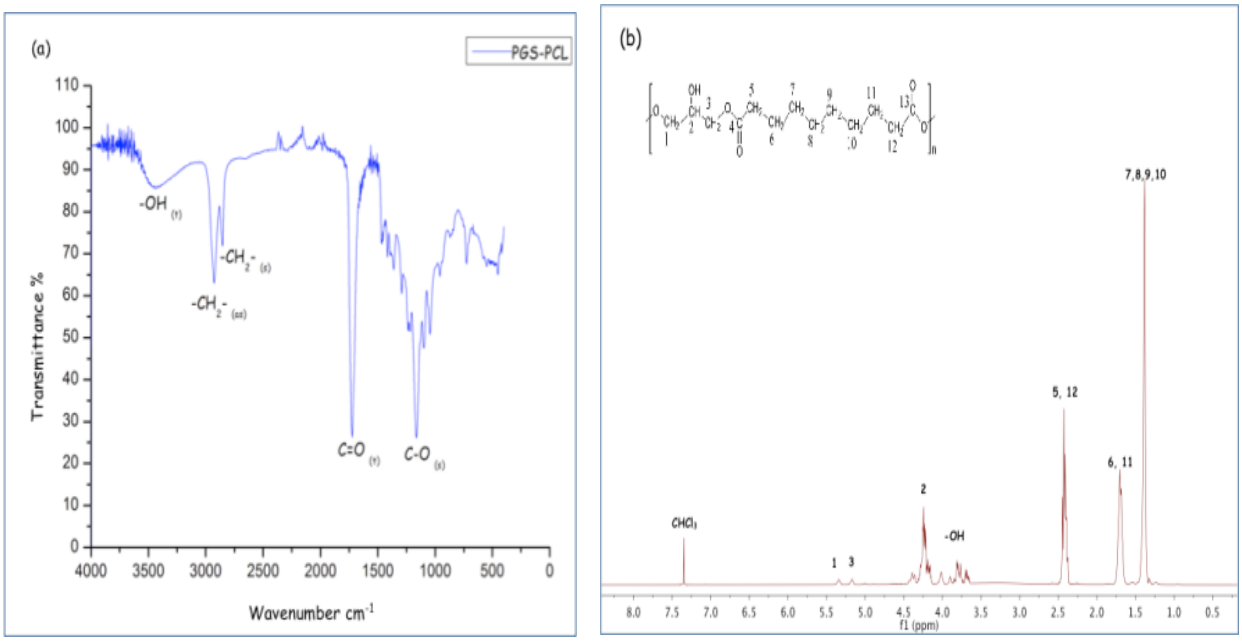

Figure 1. Structure of materials: (a) ATR-FTIR spectrum of o-PGS/PCL scaffold. (b) ^{1}HNMR spectrum of o-PGS.

A mixture of o-PGS and PCL, at a weight ratio of 2:1, dissolved in a mixture of DCM, THF and DMF, in a volume ratio of 7:2:1, formed homogeneous solutions. Polymers concentration was adjusted at 33% w/v for electrospinning.

The electrospinning injection flow modifies the fiber diameter and porosity of the scaffold, as shown in Fig 2. At high injection flow rate the mean fiber diameter and porosity were 2.6 nm and 80% respectively while at low rate they were 1.6 nm and 95%.

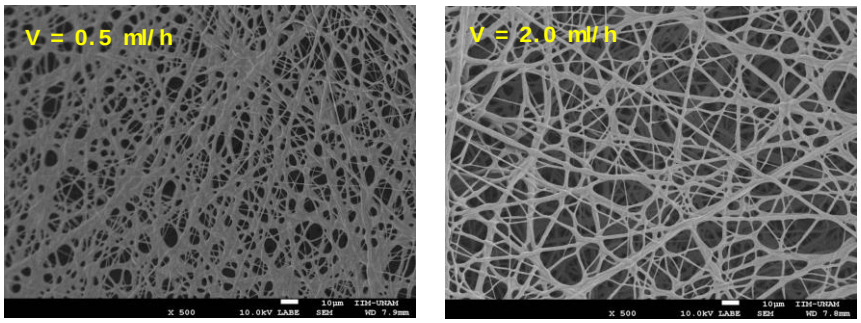

Figure 2. o-PGS/PCL scaffolds obtained at two injection flow rates: 2.0 ml/h. and 0.5 ml/h.

An o-PGS-PCL 3D tubular scaffold is shown in fig. 3(a). Figures 3(b) and 3(c) reveal that the walls of the tubular scaffold are build up by concentric porous layers, due to the effect of the rotating collector. Wall thickness depends mainly on the time of fiber collection.

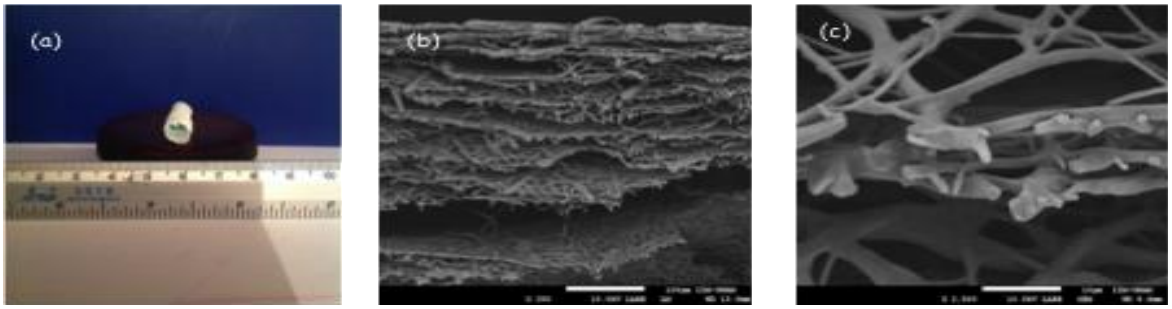

Figure 3. (a) o-PGS/PCL tubular scaffold of 0.6 cm inner diameter. Crosscut of a tubular scaffold wall at (b) 500X and (c) 2500X.

The Young`s modulus of the scaffolds measured at two injection flow rates are shown in Table 1; at a low rate (0.5 ml/h) this modulus is 5 fold superior than the observed in a sane human adult aorta, Elsie et al [6]. Since the scaffold is biodegradable it is necessary to equilibrate the gradual loss of properties of the implanted scaffold with the rate of regeneration of the native extracellular matrix. Thus, it will be convenient to test *in vivo* biodegradable scaffolds with initial elastic modulus above that of the native blood vessel [7].

Table 1. Elastic modulus of PGS-PCL scaffolds at two injection flows.

Sample	Elastic modulus (MPa)	Standard error	Injection flow rate ml/h
PGS-PCL	7.6125	0.3121	0.5
PGS-PCL	13.046	1.614	2.0

The o-PGS/PCL scaffolds were completely hydrophilic (0° water contact angle). These features should enhance cell attachment, protein deposition, cellular affinity, and degradation rate [8].

The fibrous structure and porosity obtained at a high injection flow as well as the scaffold hydrophilicity are factors that contribute to cell adhesion and growth as well as homogeneous diffusion of nutrients and waste metabolites. It has been shown that an increase in porosity and pore size are positive for the vascularisation of tissues [5].

Both o-PGS and PCL are semicrystaline (~ 15%), as determined by XRD and DSC. The crystalline peaks of o-PGS appear at 2Θ (Bragg angle) of 19.2° and 23.1°, and for PCL appear at 21.27° and 23.69°, as shown in Fig. 4(a). However, crosslinked PGS is 100% amorphous [8]. The crystallinity of the o-PGS/PCL scaffolds was about the same. Figure 4(b) clearly shows both the melting peak of o-PGS and PCL in the scaffold, indicating that the crystalline phases remain unaltered after the electrospinning process.

The melting (Tm), crystallization (Tc) and glass transition (Tg) temperatures of the materials and the scaffolds are shown in Table 2. It can be observed that the melting temperatures of the o-PGS and PCL of the scaffold are slightly lower than those of the o-PGS and of the PCL before mixing.

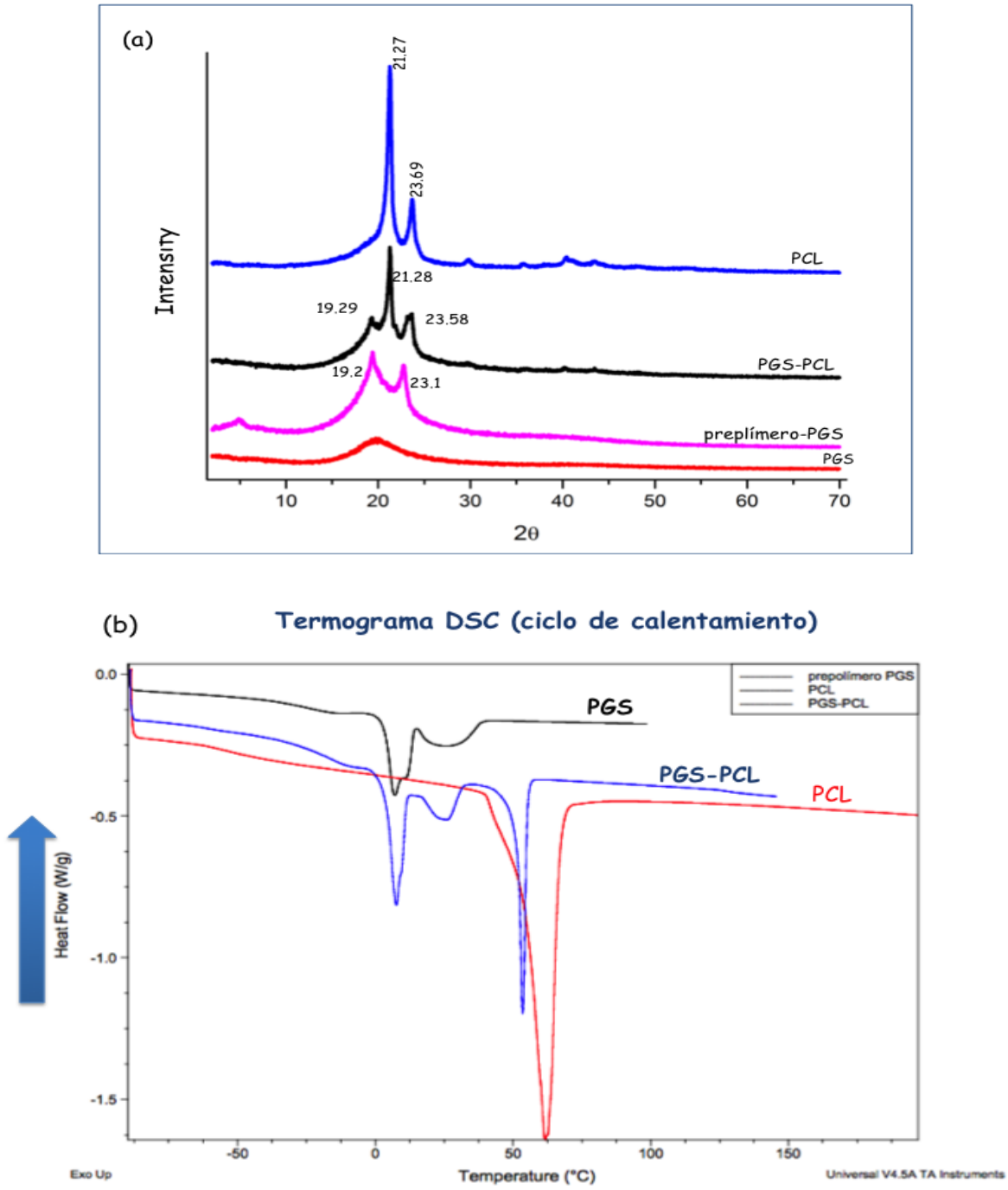

Figure 4. (a) X-ray diffraction spectrum of the scaffold. (b) DSC thermogram (heating cycle).

Table 2. Transition temperatures of PGS, PCL and PGS-PCL scaffolds

Materials	Tm ºC	Tc ºC	Tg ºC	References
o-PGS	16.0	-14.0	-	This work
PGS	15.0	-10.1	-	[5]
PCL	58-60	25.3	-60.0	[5]

o-PCL	61.7	-	-55.2	This work
PGS-PCL scaffolds				
o-PGS	8.5	-10.2		This work
PCL	56.9	28.0		This work
PGS	12.2	-11.4		[5]
PCL	55.8	29.51		[5]

CONCLUSIONS

The o-PGS, obtained by a polycondensation reaction, is a linear polymer with a Mn of 13,600. The mixture of o-PGS and PCL, at a weight ratio of 2:1, formed homogeneous solutions when it was dissolved in a mixture of DCM, THF and DMF, in a volume ratio of 7:2:1. The toxicity of these solvents is 10 fold below those reported elsewhere. The solutions o-PGS/PCL were processed easily by electrospinning to obtain 3D scaffolds of different inner diameters and wall thickness. The wall of the tubular scaffolds was built-up in layers to produce constructs of different pore size and fibers of different inner diameter. Typical electrospinning parameters for preparing the tubular scaffolds at room conditions were: voltage 17.5 kV, needle-collector distance 20 cm and, relative humidity 30-35%, flow injection 0.5 to 2.0 ml/h.

The morphology and physical properties of electrospun PGS/PCL tubular scaffolds show useful features such as fibers arrangement and nanometric diameter, scaffold porosity, hydrophilicity, mechanical properties, and thus, potentiality for adhesion and growth of living cells. The initial mechanical properties of the biodegradable scaffolds are better than those made of natural grafts; the Young's modulus ranged from 7.6 to 13.0 MPa, depending on electrospinning process parameters. The initial mechanical properties of the biodegradable scaffolds are better than those made of natural grafts. These features are in the range of biocompatible and biodegradable constructs used in vascular tissue engineering.

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REFERENCES

1. G. Koullias, R. Modak y M. Tranquilli, Journal of Thoracic and Cardiovascular Surgery **130**, 677 (2005).
2. Y. D. Wang, G. A. Ameer, F. J. Sheppard, Nat. Biotechnol. **20**(6), 602 (2002).
3. S. Sant, D. Iyer, Akhilesh, K. Gaharwar, A. Patel, A. Khademhusseini, Acta Biomaterialia **9,** 5963 (2013).

4. E. Y. Gómez-Pachón, F. M. Sánchez-Arévalo, F. Sabina, A. Maciel-Cerda, R. Montiel Campos, N. Batina, I. Morales-Reyes, R. Vera-Graziano, Journal of Materials Science **48,** 8308 (2013).
5. S. Sant, A. Khademhosseini, Conf. Proc. IEEE Eng. Med. Biol. Soc. **1**, 3546 (2010).
6. E. S. Palce, J. H. Gerge, C. K. Williams and M. M. Stevens. Synthetic polymer scaffolds for tissue engineering, Critical Review, DOI:10.1039/b811392k (2009).
7. Q.-Z. Chen, A. Bismarck, U. Hansen, S. Junaid, M. Q. Harding, N. N. Ali, A. R. Boccaccini, Biomaterials **29,** 47 (2008).
8. S. R. Vallabhaneni, G. L. Gilling-Smith, T. V. How, S. D. Carter, J. A. Brennan, and P. L. Harris, J. Endovasc. Ther. **11**, 494 (2004).