



HAL
open science

Factorial design optimization and in vivo feasibility of poly(ϵ -caprolactone)-micro- and nanofiber-based small diameter vascular grafts.

Benjamin Nottelet, Erman Pektok, Delia Mandracchia, Jean-Christophe Tille, Beat H. Walpoth, Robert Gurny, Michael Moeller

► To cite this version:

Benjamin Nottelet, Erman Pektok, Delia Mandracchia, Jean-Christophe Tille, Beat H. Walpoth, et al.. Factorial design optimization and in vivo feasibility of poly(ϵ -caprolactone)-micro- and nanofiber-based small diameter vascular grafts.. *Journal of Biomedical Materials Research Part A*, 2009, 89 (4), pp.865-75. 10.1002/jbm.a.32023 . hal-00416778

HAL Id: hal-00416778

<https://hal.science/hal-00416778>

Submitted on 12 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Factorial design optimization and *in vivo* feasibility of poly(ϵ -caprolactone)-micro- and nanofiber-based small diameter vascular grafts

B. Nottelet,^{1*} E. Pektok,^{2*} D. Mandracchia,^{1,3} J.-C. Tille,⁴ B. Walpoth,² R. Gurny,¹ M. Möller¹

¹Department of Pharmaceutics and Biopharmaceutics, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva 1211, Switzerland

²Cardiovascular Research, Service of Cardiovascular Surgery, University Hospital of Geneva, Geneva 1211, Switzerland

³Dipartimento Farmaco Chimico, Università di Bari, Via Orabona 4, Bari 70125, Italy

⁴Department of Clinical Pathology, University Hospital of Geneva, Geneva 1211, Switzerland

Abstract: Because of the severe increase of mortality by cardiovascular diseases, there has been rising interest among the tissue-engineering community for small-sized blood vessel substitutes. Here we present small diameter vascular grafts made of slow degradable poly(ϵ -caprolactone) nanofibers obtained by electrospinning. The process was optimized by a factorial design approach that led to reproducible grafts with inner diameters of 2 and 4 mm, respectively. Fiber sizes, graft morphology, and the resulting tensile stress and tensile strain values were studied as a function of various parameters in order to obtain optimal vascular grafts for implantation after γ -

sterilization. The influence of polymer concentration, solvent, needle-collector distance, applied voltage, flow rate, and spinning time has been studied. Consequently, an optimized vascular graft was implanted as an abdominal aortic substitute in nine rats for a feasibility study. Results are given following up a 12-week implantation period showing good patency, endothelialization, and cell ingrowth.

Key words: biodegradable polymers; polycaprolactone; vascular grafts; electrospinning; nanofibers; *in vivo* study

INTRODUCTION

Cardiovascular diseases are the leading cause of mortality in western countries and are becoming the main one in developing countries. In a single decade (1990–2000), the number of acute myocardial infarction increased about 6% for women and 13% for men across the world.^{1,2} Most ischaemic diseases like atherosclerosis require a revascularisation procedure. Normally, they are either treated by percutaneous coronary intervention with or without stent placement, or by bypass surgery.³ For bypass surgery, autologous graft material, mainly arteries, is preferable, since it leads to good long-term results.⁴ However, for cases with pre-existing vascular disease or prior surgery, alternative graft materials have to be used.⁵ While homografts can be used,

they are limited in number and only recommended for special indications.⁶ On the other hand, synthetic and biostable vascular grafts made of poly(tetrafluoro ethylene) (PTFE) or Gore-Tex[®] are widely used to overcome these limitations. Advantageously, they are shelf-ready and widely available for large blood vessels replacement, but they carry the severe risks of early thrombosis and late intimal hyperplasia, which are even more pronounced with small diameter grafts ($\emptyset < 6$ mm).⁷ Two main strategies were developed to avoid these problems. The first one tries to compensate the lack of tissue compatibility by coating biostable grafts with heparin⁸ or growth factors^{9,10} in order to create a non-thrombogenic or bio-active inner surface. Although this improved some cases, the use of biostable material is not preferable, since long-term residence of foreign material leads to late complications.^{11,12} The second approach uses biodegradable polymers for *in vitro* tissue engineering, that is a combination of a degradable scaffold with cells and mediators. Several approaches have been pursued, such as *in vitro* formation of a novel vessel.^{13–16} Unfortunately, this

*These authors contributed equally to this work.

Correspondence to: M. Möller; e-mail: Michael.Moeller@pharm.unige.ch

strategy relies on a personalized graft that must be “customized” with each patient’s own cells. As a consequence, this approach is inefficient, since many intermediate steps are required before implantation. Moreover, these grafts are neither shelf-ready nor cheap. For this reason, it is of great interest to develop an *in vivo* colonizable and simultaneously degradable polymeric graft for promoting tissue regeneration, which would be suitable for a direct implantation.

Different methods can be applied to prepare polymeric vascular grafts. However, the chosen method has a great influence on both the mechanical properties and the micro- and nanostructures of the resulting product. On the one hand, the ideal artificial graft should be mechanically compatible with the natural arteries and the surrounding tissue. On the other hand, it should also mimic the extracellular matrix morphology; it should have a nanoscale topography (~5 to 500 nm) with high porosity and adequate pore sizes (5–500 μm) in order to enhance cell attachment and proliferation for the regeneration of the natural tissues.^{17,18} One process which has attracted much attention in recent years is the use of melt or solution electrospinning. Melt or solution electrospinning allows the simple preparation of 3D highly porous polymeric scaffolds composed of nanofibers.^{19–23} Some natural degradable materials, like collagen,^{24,25} hyaluronic acid,^{26,27} sodium alginate,^{28,29} or fibrinogen,^{30,31} have been electrospun. Unfortunately, they show poor mechanical properties compared to synthetic degradable polymers and are most commonly used as coatings on biostable grafts. Several synthetic degradable polymers have been proposed, mainly aliphatic biodegradable polyesters like poly(lactic-co-glycolic acid) (PLGA),^{32,33} poly(lactic acid) (PLA),^{34–37} but also polycarbonate derivatives^{38,39} or polydioxanone,⁴⁰ since they are already in use in some biomaterial and drug delivery applications. The main problem encountered with these synthetic biodegradable polymers is their rapid loss of mechanical properties, which is due to an accelerated *in vivo* biodegradation of the nanofibers as a direct consequence of their very high surface/volume ratio. For example, nanofibers of PLGA have been reported to degrade within a few days,⁴¹ which is rather unsuitable for the application as vascular prosthesis. Furthermore, the nanostructure of the envisioned grafts can lead to an increased “pre-degradation” induced by the necessary sterilization. In contrast, poly(ϵ -caprolactone) (PCL) is well known for its very good mechanical properties, and the *in vivo* degradation of PCL occurs mainly by a very slow hydrolytic process. Some studies have shown a degradation time of several months for electrospun PCL fibers,^{42,43} which is reasonable for vascular applications.

To date, electrospinning studies of PCL have mostly focused on process parameters, in order to control the morphology of pure electrospun PCL

fibers and to evaluate their effect on degradability.^{43–46} A few other studies have focused on their histocompatibility using different type of cells,^{47–51} but without considering an identified final application. A very limited number of studies have been conducted to produce 3D scaffolds with an architecture mimicking the extracellular matrix of blood vessels. A sequential multilayering electrospinning has been described to form a bi-layered tubular scaffold, which is composed of an outer fibrous layer of stiff and oriented PLA and a fibrous inner layer of pliable and randomly oriented PCL.⁵² The same approach has been used for scaffolds made of an inner layer PCL microfibers and an outer layer of PCL nanofibers.⁵³ Nevertheless, all these studies focused and reported on *in vitro* studies only.

In this article we present the preparation of PCL-based small-diameter electrospun vascular grafts, optimized by a factorial design approach in regard to their final application being suitable for *in vivo* implantation in rats. Furthermore, we report the initial *in vivo* results of an optimized graft in the rat arterial circulation, with a follow-up of 12 weeks after implantation.

MATERIALS AND METHODS

Materials

Poly(ϵ -caprolactone) (PCL, $\overline{M}_n = 80,000$ g/mol) was purchased from Sigma/Aldrich (Buchs, Switzerland), acetone and ethanol 96% of pharmaceutical grades from Hanseler (Herisau, Switzerland), and chloroform from Riedel-de Haen (Seelze, Germany). All products were used as received. The electrospinning system used in this study is a homemade system (Custom Design & Fabrication, Virginia Commonwealth University: under the guidance of Prof. G. Bowlin) composed of a variable high voltage power supply (Spellman High Voltage Electronics, Ltd. Pulborough, West Sussex, UK); a syringe pump (Fresenius Vial SA, Brezins, France) suitable for different controlled flow rates; stainless steel mandrels used as collectors with diameters of 2 or 4 mm; and a stainless steel plate used as a second negative pole to stabilize the electric field. Pump compatible Omnifix[®] syringes of 20 and 50 mL were received from Braun (Melsungen, Germany) and used with a 21G, Sterican[®] stainless steel needle with an inner diameter (I.D.) of 1.27 mm.

Electrospinning and optimization of vascular grafts

At first nonwoven PCL vascular grafts were electrospun from different PCL solutions. The solvents used for the preparation were acetone (Ac), chloroform/acetone (CHCl_3/Ac) (7:3; v:v), and chloroform/ethanol ($\text{CHCl}_3/\text{EtOH}$) (7:3; v:v). PCL solutions of various concentrations were prepared by dissolving the desired amount in the solvent of choice. In a typical experiment, the prepared

PCL solution was ejected with a controlled flow by the syringe pump, while the positive pole of the high voltage power supply was connected to the needle, whereas the collector and the metal plate were both connected to the negative pole. A constant electric field was applied during the electrospinning process. The generated fibers were collected on a rotating (4500 rpm) and translating (200 movements/min, 4 cm amplitude) mandrel to form a nonwoven tubular PCL graft. The time of the graft preparation was adjusted to the PCL concentration to spin 360 mg of PCL. Vascular grafts having a length of 2 or 4 cm and an I.D. of 2 or 4 mm, respectively, were cut-off from the collector using a surgical scalpel before drying under reduced pressure at 37°C for 24 h. In addition, all 2-mm I.D. vascular grafts were sterilized by γ -radiation (2.5 MRad).

In the factorial design the following parameters solvents and solvents compositions (Ac, CHCl₃/Ac, CHCl₃/EtOH), PCL concentrations (5–15%; w:v), tip-collector distances (15–25 cm), applied voltages (15–25 kV), and flow-rates at which the PCL solution was ejected (12 or 24 mL/h), were studied for their influence on fiber size, bead and sheet occurrence, surface and 3D morphologies, and mechanical properties before and after γ -sterilization. Statgraphic Plus 5.1 software (Statistical Graphics Corp, Herndon, VA) was used for the factorial design supported optimization.

Characterizations of the vascular grafts

Fiber diameter and morphology of electrospun material were investigated using a scanning-electron microscope (JEOL JSM-6400, Jeol Ltd, Tokyo, Japan). SEM images were taken after depositing a conductive coating of gold on the graft sample with a sputter coater (Balzers SCD004, Oerlikon AG, Pfäffikon, Switzerland). ImageJ software (National Institutes of Health, USA) was used to measure the fiber size and to count the number of bead defects in the grafts. Fiber diameter mean values were calculated from 100 measurements.

Tensile stress and tensile strain were measured on complete vascular grafts using a mechanical testing bench from Schenck AG (Nänikon, Switzerland) using a cross-head speed of 10 mm/min at room temperature. The vascular grafts were fixed by clamps using 5 mm of each end of the graft, so that either a 1 or 3 cm length was used for the measurements corresponding for the 2 and 4 mm, respectively I.D. grafts.

Residual solvents were analyzed with a Headspace Sampler (HP7694, Hewlett-Packard, Palo Alto, CA) coupled to a gas-chromatography system (GC System, 6850 Series, Agilent Technologies, Santa Clara, CA) equipped with an HP-1 capillary column (Agilent Technologies, Santa Clara, CA). A 20 mg sample of the vascular graft was dissolved in benzyl alcohol containing a fixed amount of dioxane as an internal standard. The results were evaluated with the Agilent Chemstation software.

Feasibility of *in vivo* implantations using a dynamic model

The *in vivo* studies on rats were carried out in conformity with protocols approved by the local ethical commit-

tee (Protocol N°. 1081-3232-11 from the Veterinary Office of the Canton of Geneva, Experimental Ethics Committee Approval N°. 06/52 from the University of Geneva). Nine PCL-based vascular grafts having a 2-mm I.D. and a length of 1.3 cm were randomly selected out of a batch of 30 γ -sterilized, identical grafts. They were implanted in nine male Sprague-Dawley rats (350–400 g) under Isoflurane 2% mask anaesthesia after induction with Isoflurane 5%. The abdominal cavity was entered, and the infrarenal abdominal aorta was dissected by blunt and sharp dissection. PCL-based vascular grafts were implanted by simple sutures of 10/0 Nylon and standard microsurgical techniques under sterile conditions. Rats were followed up for 3, 6, and 12 weeks ($n = 3$ for each time point), and were sacrificed after digital subtraction angiography (DSA). For histological investigations, the whole graft was fixed in 4% formaldehyde for 24 h, embedded in paraffin, and 4- μ m sections were stained with hematoxylin-eosin.

RESULTS AND DISCUSSION

This study focused on the preparation of a functional vascular graft based on poly(ϵ -caprolactone) nanofibers by electrospinning. PCL was chosen due to its well known good mechanical properties as well as its slower degradation rate compared to the often proposed PLA, PLGA or PDS, which fail to maintain sufficient mechanical properties over the time period necessary for tissue reconstruction.^{54,55} The main aim was to optimize the mechanical and morphological properties of a PCL biodegradable prosthesis candidate to prepare a “reproducible”, “shelf-ready”, and “simple” alternative to biostable PTFE- or *in vitro* bio-engineered grafts. To achieve this goal, a factorial design for the process parameters optimization was set up, and the influence of the chosen parameters was evaluated in terms of mechanical and morphological properties on two different types of small-sized vascular grafts (2 and 4-mm I.D.) For the assessment of the applicability of the obtained optimized vascular prosthesis, *in vivo* implantations in rats were followed up for 12 weeks.

Solvent, PCL concentration, and flow rate optimization

Commonly used PTFE vascular grafts with an inner diameter less than 6 mm carry severe risks of early thrombosis and late intimal hyperplasia. Vascular grafts based on biocompatible and -degradable polymeric nanofibers with suitable properties may overcome these problems. For this reason, 4-mm I.D. PCL-based vascular grafts were prepared by the electrospinning process in order to evaluate this preparation method and their *in vivo* application feasibility. In a first series of experiments, the suitability

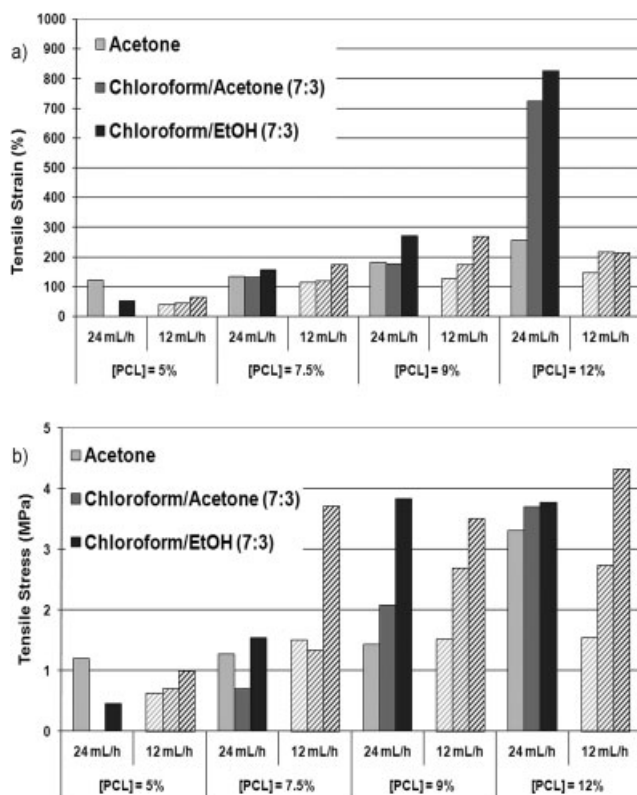


Figure 1. Influence of the solvent, PCL concentration and flow rate (dashed histograms = 12 mL/h; plain histograms = 24 mL/h) onto (a) tensile strain values and (b) tensile stress values of PCL electrospun vascular grafts (inner diameter = 4 mm; length = 3 cm).

of different solvents was evaluated. The use of acetone,^{56,57} CHCl₃/acetone,⁴⁶ and CHCl₃/EtOH⁴² to electrospin PCL fibers has been reported in the literature, and these solvents were chosen here for their relative ease of removal, their weak or absence of toxicity, and their low price compared to more frequently used fluoro-compounds.^{40,58} Vascular grafts having an inner diameter of 4 mm and a length of 4 cm were prepared using each of the above mentioned solvent and solvent mixtures and studied in terms of their mechanical and morphological properties.

Considering the *in vivo* application of vascular grafts, the properties of the prepared prosthesis should be ideally of equal value to the ones found in human, or accordingly higher to account for loss of

mechanical properties with the *in vivo* degradation. In native human arteries, tensile stress and strain were found to be 1.4 MPa⁵⁹ and around 100%,⁶⁰ respectively. In this study, the highest tensile stress and tensile strain were achieved with the CHCl₃/EtOH (7:3; v:v) solvent mixture followed by CHCl₃/acetone (7:3; v:v) and pure acetone (Fig. 1). The desired value was hardly reached with pure acetone, while almost all grafts prepared with CHCl₃/EtOH had maximum tensile stress above 1.5 MPa, independent of the flow rate or PCL concentration. From the morphological point of view, SEM pictures of the grafts showed that these solvents did not appear to have great influence on the fiber sizes, or number and size of beads, which are considered to be a defect structure in the fibers. In fact, the dominant factor was the PCL concentration. Because of these observations, the solvent mixture CHCl₃/EtOH (7:3; v:v) was chosen for all the following experiments.

For the factorial design supported optimization of vascular grafts, a randomized rotatable central composite design with three repetitions at the center of the domain was carried out. The flow rate (12 and 24 mL/h) and PCL concentration (5–15% (w:v)) were the variables, while the tip-collector distance (20 cm) and the voltage (20 kV) were fixed. The obtained surface response diagrams, as well as the interdependence between the two variables, PCL concentration and flow rate, are presented in Table I and Figure 2. From the R^2 adjusted to the degree of freedom, it can be concluded that in all cases the proposed mathematical treatment of the data is in good agreement with the observed experimental results, except for the size of the beads, which can occur as defects in the fibers.

Regarding the mechanical properties, the tensile strain values increased with the PCL concentration from about 100 to 700% [Fig. 2(a)]. A slight interdependence was found for the flow rate and PCL concentration on strain values. High flow rates and high PCL concentration lead to the highest strain values, whereas high flow rates and low PCL concentration lead to the lowest strain values. The maximum tensile stress should theoretically be obtained with a PCL concentration around 11% (w:v). Higher values of tensile stress were also obtained with lower flow rates [Fig. 2(b)]. These results confirm that the good inherent mechanical properties of PCL ensure suffi-

TABLE I
Optimization of PCL Vascular Grafts (4 mm I.D.)

Parameters	Fiber Diameters	Bead Sizes	Bead Densities	Tensile Stress	Tensile Strain
[PCL]/Flow rate interaction	No	No	No	Weak	Yes
R^2 adjusted to the degree of freedom	90.5	36.5	92.4	65.6	94.0

Summary of the central composite design carried out with PCL concentration in the range 5–15% (w:v) and a flow rate in the range 12–24 mL/h.

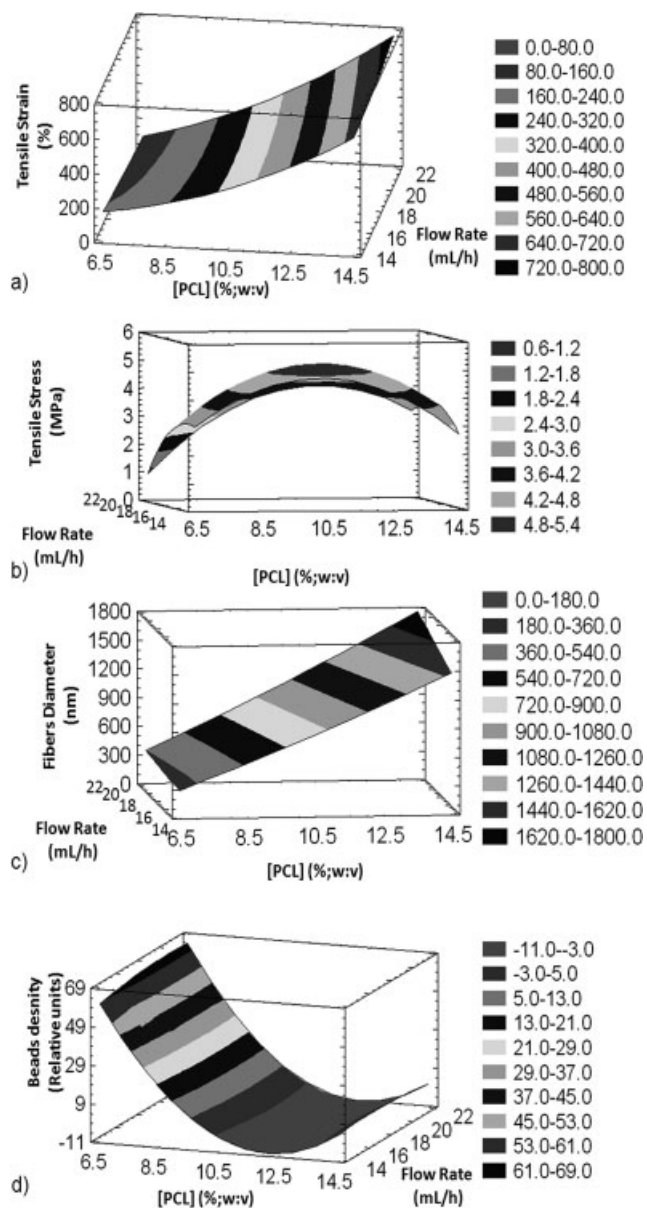


Figure 2. Response surfaces of (a) tensile strain, (b) tensile stress, (c) fiber diameter, and (d) number of beads, of PCL electrospun vascular grafts (inner diameter = 4 mm; length = 3 cm) as a function of the flow rate and the PCL concentration using a rotatable composite centered design.

cient strength to the prosthesis despite the nanosize of the fibers. In terms of morphology, the fiber diameter increased from 200 to 1800 nm with an increasing PCL concentration from 5 to 15% (w:v), but was independent of the flow rate [Fig. 2(c)]. Both the number and the size of beads (starting from ca. 10 μm) decreased with increasing PCL concentration up to 11% (w:v); for higher concentrations no more fiber defects could be detected. Again this was independent from the flow rate [Fig. 2(d)], which is in agreement with data from the literature for PCL nanofibers.^{43,45,53}

In summary, the results obtained with this randomized rotatable central composite design applied

for 4-mm I.D. vascular grafts showed that the fiber sizes are controllable and depend mainly on the used PCL solution and proved the feasibility of electrospinning vascular grafts with defined properties. In a next step, smaller 2-mm I.D. vascular grafts were investigated for *in vivo* studies in rats.

Implantable 2-mm I.D. vascular grafts optimization

The 2-mm I.D. implantable grafts were prepared using conditions, which previously led to the best tensile stress values, namely, $\text{CHCl}_3/\text{EtOH}$ (7:3; v:v) as the solvent at a flow rate of 12 mL/h. A second factorial design was set up to study the additional parameters electric field density, needle-collector distance and spinning time (see Table II). All grafts were γ -sterilized prior to any tensile stress and strain measurement to ensure that implanted grafts would have sufficient mechanical properties under real application conditions. It is well known that sterilization can influence the mechanical properties of polymer devices, which could have a dramatic impact in the case of vascular grafts *in vivo*. The mechanical properties of all tested sterilized grafts are summarized in Figure 3. As observed for the 4-mm I.D. grafts, only the PCL concentration influenced the tensile strain of the 2-mm I.D. grafts [Fig. 3(a)]. The tensile strain increased from about 200% for concentrations of 7.5 and 9% (w:v) to values between 750 and 1200% for the 12 and 15% (w:v) PCL solutions. Results were slightly different for the tensile stress, as shown in Figure 3(b). Many of the prepared grafts had tensile stress values of around 4 MPa after γ -sterilization, which is far above the minimum desired value of 1.4 MPa. Only a few grafts prepared with the lowest PCL concentration of 7.5% and a longer spinning time showed lower tensile stress values. Secondly, and surprisingly, it appeared that increasing the spinning time, and thus the total amount of PCL, weakened the prostheses. Although it is not fully understood yet, this effect was mainly pronounced for the lower PCL concentrations, which underwent a 30–50% decrease. All other parameters did not show a significant influence in the studied parameter ranges, and thus the only relevant parameters for the tensile stress are the PCL solution concentration and the spinning time. Figure 4 shows the mean stress and strain values as a function of the mean fiber sizes for vascular grafts with a tensile stress superior to 2 MPa. The same tendencies were observed as found for the 4-mm I.D. grafts. The highest stress values are obtained from grafts with fibers having a diameter of about 2 μm , which are mainly obtained by electrospinning with a PCL concentration of 12% (w:v). For the strain values, a lower elongation before rupture is found for thin

TABLE II
2 mm I.D. PCL Vascular Grafts Optimization

Parameters	Grafts n°																																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32							
[PCL] (% w:v)	7.5									9									12									15											
Spinning time ^a (min)	12			24			1.33			1			1.33			1			1.33			1			1.33			1			1.33			1			1.33		
Electric field density (kV/cm)	1			1.33			1			1.33			1			1.33			1			1.33			1			1.33			1			1.33					
Needle-collector distance (cm)	15			20			15			20			15			20			15			20			15			20			15			20					

Studied parameters using CHCl₃/EtOH (7:3; v:v) as solvent at a 12 mL/h flow rate.

^aThe spinning time was adjusted as a function of the PCL solution concentration. For each type of graft two spinning time have been tested. The first equals that of the 4 mm I.D. grafts and was the time necessary for the spinning of 360 mg of PCL. The second equalled half this time.

fibers, around 250%, while thicker fibers show elongations of around 900%.

Finally, the influence of the studied parameters on the morphological properties of the vascular grafts having tensile stress values above 2 MPa after sterilization was studied. Results are shown in Figures 5 and 6. As expected, the PCL concentration was the main parameter influencing the fiber sizes, with mean diameters of 500 nm, 1 μm, 1.9 μm, and 2.5 μm for 7.5, 9, 12, and 15% (w:v) PCL solutions, respectively. No beads were observed by SEM-analysis for grafts prepared with the two highest concentrations. These results correspond to the reported behaviors for the electrospinning process.^{61,62} Fiber sizes in the micrometer range seem to be quite thick for electrospun fibers, but could be of advantage for good cell adherence. The spinning time influenced

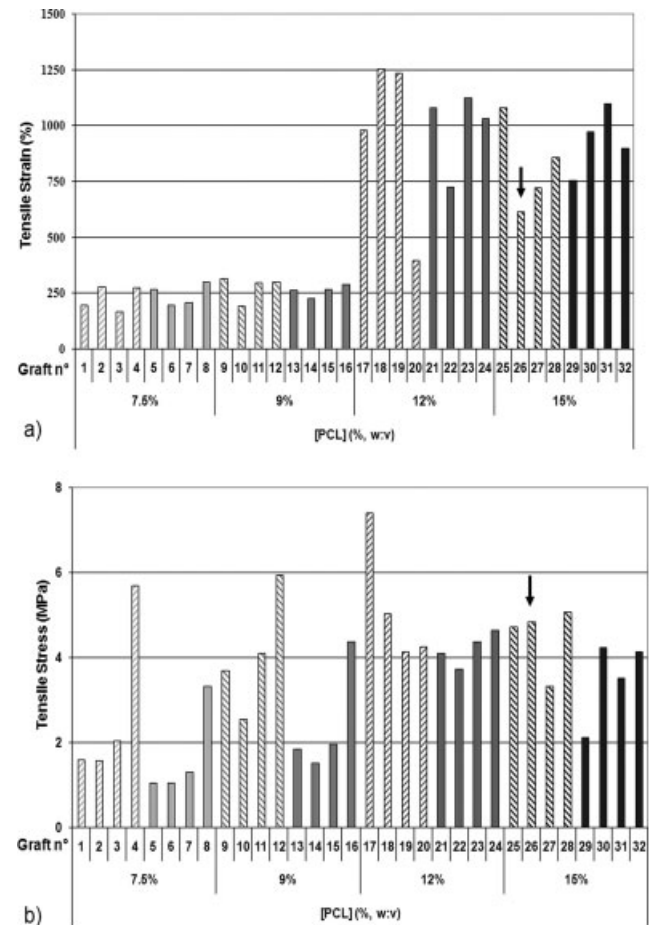


Figure 3. (a) Tensile strain values and (b) tensile stress values of sterilized PCL electrospun vascular grafts (inner diameter = 2 mm; length = 1 cm) as a function of the PCL solution concentration, the spinning time (plain histogram = longer time, dashed histogram = shorter time), the electric field density, and the tip-collector distance. Parameters used for each graft are listed in Table II. The black arrow identifies the graft which was used for the *in vivo* studies.

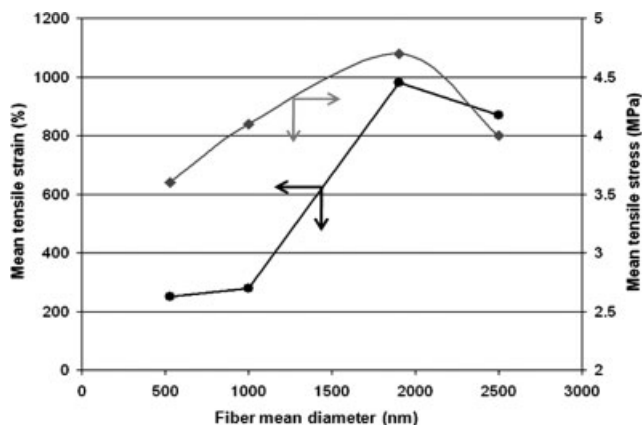


Figure 4. Mean tensile stress (◆) and strain (●) values as a function of the fiber mean diameters of γ -sterilized PCL electrospun vascular grafts (inner diameter = 2 mm; length = 1 cm).

the size of the fiber diameters, which increased with the spinning time when other parameters were kept constant (Fig. 6). It appeared, that in most cases, a higher electric field density led to thinner fibers, which is easily understood considering that the jets ejected out of the needle tip were subjected to higher stretching stress when the electric field increased.

As a result of this optimization process, the best PCL-based graft was selected for *in vivo* implantations. Thirty identical grafts were prepared by using a 15% (w:v) PCL solution in $\text{CHCl}_3/\text{EtOH}$ (7:3; v:v), a 6-min spinning time, a 12 mL/h flow rate, a 20-cm tip-collector distance, and a 20 kV voltage (see Table II, Figs. 3 and 6, graft n°26). They were free of beads, had a fiber diameter of 1.90 μm , a tensile stress of 4.8 MPa, and a tensile strain value of 600% after γ -sterilization. Prior to implantation, three sterile grafts were tested for residual solvent traces using a headspace-coupled GC detection technique. None of these controls contained chloroform or ethanol traces.

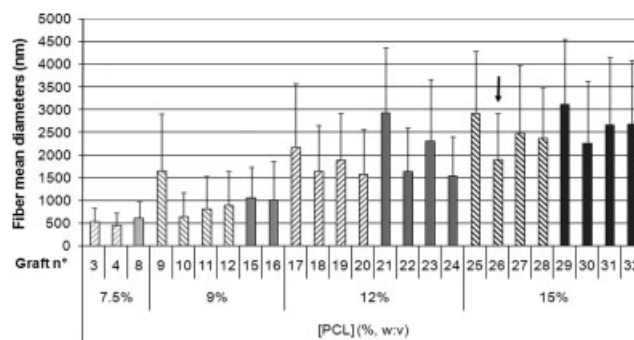


Figure 6. Fiber mean diameters of γ -sterilized PCL electrospun vascular grafts (inner diameter = 2 mm; length = 1 cm and tensile stress value >2 MPa) as a function of the PCL solution concentration, the spinning time (plain histogram = longer time, dashed histogram = shorter time), the electric field density, and the tip-collector distance. Parameters used for each graft are listed in Table II. The black arrow identifies the graft, which was used for the *in vivo* pilot studies.

Pilot *in vivo* studies in rats

To evaluate the practical feasibility of the optimized graft in terms of surgical handling, functionality, and biocompatibility, identical grafts were implanted in rats following the experimental setup as described in the materials and methods part. The desired function of such a biodegradable graft requires the absence of blood leakage, of acute thrombogenicity, of inflammatory response, of intimal hyperplasia, and the colonization of the scaffolds by cells to regenerate natural tissues, and finally the degradation and disappearance of the graft at a rate compatible with the blood vessel reconstruction.

Out of the batch of the 30 identical grafts, nine grafts were picked randomly by the surgeon and implanted into the abdominal aortas of Sprague-Dawley rats. These grafts had good surgical handling and suture retention properties, and led to successful implantations. None of the nine vascular

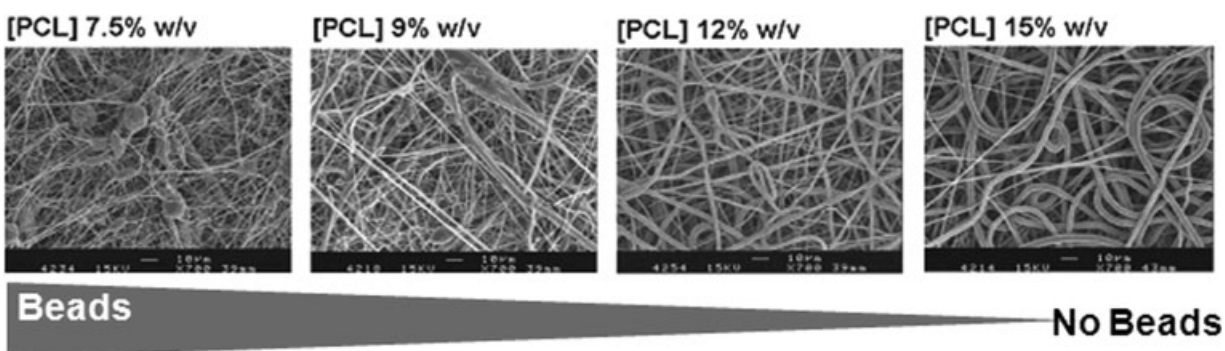


Figure 5. SEM pictures of γ -sterilized PCL electrospun vascular grafts inner surface at a $\times 750$ magnification showing the influence of the PCL solution concentration on the fiber sizes and the presence of beads.



Figure 7. Typical angiography of abdominal aortic PCL electrospun vascular graft in a rat after 12 weeks showing good patency, no stenosis, and no dilatation. White arrows point out the proximal and distal anastomoses on the abdominal aorta.

grafts showed thrombosis or aneurysm formation at the three different time points. Figure 7 illustrates a typical DSA image of an implanted graft after 12 weeks of implantation, showing good patency, no anastomotic stenosis, and no aneurysmatic dilatation.

Histological analyses of grafts revealed endothelial coverage limited to the endoluminal graft surface near the distal and proximal anastomoses after 3 weeks. Graft endothelialization was almost complete after 6 weeks with the exception of a small area in the middle of all three grafts. Full endothelialisation with some intima hyperplasia formation was observed in all grafts after 12 weeks (Fig. 8). Compared to previous grafts, these PCL grafts showed faster endothelial coverage within 6 to 12 weeks than what has been reported in the literature for ePTFE grafts. Zhang et al. reported a slower endothelialization rate with 30 μm ePTFE grafts in a similar rat model, where only less than half of the graft surface was covered after 8 weeks.⁶³ This might be a consequence of the fiber and pore size of the prosthesis, and the biocompatibility of the material. An increasing cellular infiltration of the graft from the adventitia through the graft body was observed for the three time points (Fig. 8). This infiltration is composed of macrophages and fibroblasts, and accompanied by new capillary formation. No chronic lymphocytic reaction was observed. A layer of multinucleated giant cells was present on the adventitial surface of the PCL grafts at all time points [Fig. 8(b)]. The adventitial part of the graft and then the middle part showed some degradation by macro-

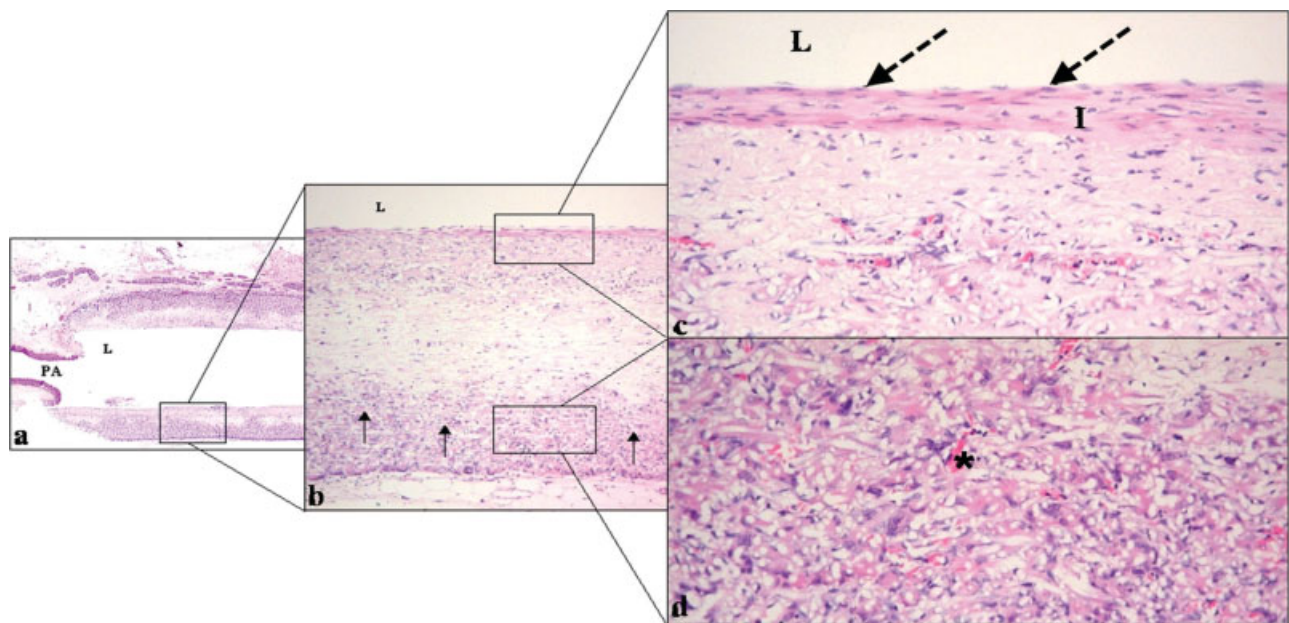


Figure 8. Typical histological cut of an abdominal aortic PCL electrospun vascular graft in a rat, 12 weeks after implantation (hematoxylin-eosin staining, HE). (a) $\times 20$, (b) $\times 100$, (c) and (d) $\times 200$ magnification. A confluent layer of endothelium with some neo-intima formation can be observed on the luminal side of the graft (a, b, and c). Cellular infiltration was prominent from the adventitial side towards the graft matrix (a and b). Further magnification revealed that this infiltration was mainly composed of macrophages and fibroblasts, and was accompanied with new capillary formation in the matrix (d). The graft material was surrounded by a layer of multinucleated giant cells on the adventitial side (b). PA, Proximal anastomosis; L, Lumen; I, Intima, arrows in b: cellular infiltration; dotted arrows in c: endothelium, asterisk: capillary formation.

phages, as well as collagen deposition by fibroblasts, but without multinucleated giant cells [Fig. 8(c)].

These first promising results with *in vivo* cell colonization confirm the feasibility of electrospun biodegradable, porous, nano- and microfiber-based PCL vascular grafts. In future studies these PCL-based grafts need to be evaluated in comparison with conventional grafts over a whole clinical application time period.

CONCLUSION

In this article we present the successful preparation of biofunctional vascular grafts obtained by electrospinning of PCL solutions. Optimization of the electrospinning parameters led to small-diameter vascular grafts with reproducible mechanical and morphological properties. The control of the mean diameter of the fibers, ranging from 500 to 2500 nm, is mainly dependent on the PCL concentration of the electrospun solution. In addition, grafts could be prepared having tensile stress values higher than 2 MPa, and up to 7.4 MPa, and tensile strain values in the range of 200–1200% after γ -sterilization. These values are above those for natural human blood vessels (1.4 MPa and 100%), which facilitate the *in vivo* use as vascular prostheses. In a pilot study on nine rats nine identical optimized PCL-based vascular grafts (fiber mean diameter = 1.9 μ m, tensile stress = 4.8 MPa, tensile strain = 600%) were implanted into the rats arterial circulation as an abdominal aortic substitute. In addition to easy surgical-handling of the grafts, this study showed, that all implanted grafts were fully patent up to 12 weeks after implantation, and none of the nine vascular grafts at the three different time points (3, 6, and 12 weeks) demonstrated thrombosis or aneurysmal dilatation. Histological analyses revealed a homogeneous cellular infiltration associated with polymer degradation and extracellular matrix deposition, and a complete endothelialisation with little intimal hyperplasia. These results show that the combination of PCL and the optimized electrospinning procedure enables the preparation of biodegradable, shelf-ready vascular grafts. However, *in vivo* healing and degradation characteristics of the electrospun PCL grafts need to be studied more extensively and over a longer time period, in comparison with standard vascular prostheses as controls, before any clinical application.

The authors thank Stéphanie Corboud for the help on some grafts electrospinning, Nathalie Boulens and Catherine Siegfried for the SEM pictures and Unn Lutzen for the preparation of the histological cuts.

References

1. Avendano M, Kunst AE, Huisman M, Lenthe FV, Bopp M, Regidor E, Glickman M, Costa G, Spadea T, Deboosere P, Borrell C, Valkonen T, Gisser R, Borgan JK, Gadeyne S, Mackenbach JP. Socioeconomic status and ischaemic heart disease mortality in 10 western European populations during the 1990s. *Heart* 2006;92:461–467.
2. Ounpuu S, Anand S, Yusuf S. The impending global epidemic of cardiovascular diseases. *Eur Heart J* 2000;21:880–883.
3. Rodengen JL, Stephensen LW. Coronary artery disease and treatment options. In: Rodengen JL, Stephensen LW, editors. *The Practical Guide to Your Heart and Heart Surgery*. Fort Lauderdale, FL: Write Stuff Enterprises Inc.; 1999. pp 110–129.
4. Tomizawa Y. Vascular prostheses for aortocoronary bypass-grafting—A review. *Artif Organs* 1995;19:39–45.
5. Emery RW, Mills NL, Teijeira FJ, Arom KV, Baldwin P, Petersen RJ, Joyce LD, Grinnan GLB, Sussman MS, Copeland JG, Ochsner JL, Boyce SW, Nicoloff DM. North American experience with the perma-flow prosthetic coronary graft. *Ann Thorac Surg* 1996;62:691–695.
6. Brockbank KGM, McNally RT, Walsh KA. Cryopreserved vein transplantation. *J Cardiac Surg* 1992;7:170–176.
7. Teebken OE, Haverich A. Tissue engineering of small diameter vascular grafts. *Eur J Vasc Endovasc* 2002;23:475–485.
8. Walpoth BH, Rogulenko R, Tikhvinskaia E, Gogolewski S, Schaffner T, Hess OM, Althaus U. Improvement of patency rate in heparin-coated small synthetic vascular grafts. *Circulation* 1998;98:II319–II323.
9. Walpoth BH, Zammaretti P, Cikirikcioglu M, Khabiri E, Djebaili K, Pache J-C, Kalangos A, Hubbell JA, Zisch AH. VEGF¹²¹ induced enhancement of cellular coverage of ePTFE grafts in the chronic pig model. Joint Meeting of the Tissue Engineering Society International and the European Tissue Engineering Society. 2004.
10. Crombez M, Chevallier P, Gaudreault RC, Petitclerc E, Mantovani D, Laroche G. Improving arterial prosthesis neo-endothelialization: Application of a proactive VEGF construct onto PTFE surfaces. *Biomaterials* 2005;26:7402–7409.
11. Suma H, Wanibuchi Y, Terada S, Fukuda S, Takayama T, Furuta S. The right gastroepiploic artery graft—Clinical and angiographic midterm results in 200 patients. *J Thorac Cardiovasc Surg* 1993;105:615–623.
12. Stock UA, Wiederschain D, Kilroy SM, Shum-Tim D, Khalil PN, Vacanti JP, Mayer JE, Moses MA. Dynamics of extracellular matrix production and turnover in tissue engineered cardiovascular structures. *J Cell Biochem* 2001;81:220–228.
13. Kadner A, Zund G, Maurus C, Breymann C, Yakarisik S, Kadner G, Turina M, Hoerstrup SP. Human umbilical cord cells for cardiovascular tissue engineering: A comparative study. *Eur J Cardiothorac Surg* 2004;25:635–641.
14. McFetridge PS, Bodamyali T, Horrocks M, Chaudhuri JB. Endothelial and smooth muscle cell seeding onto processed *ex vivo* arterial scaffolds using 3D vascular bioreactors. *ASAIO J* 2004;50:591–600.
15. Nasser BA, Pomerantseva I, Kaazempur-Mofrad MR, Sutherland FWH, Perry T, Ochoa E, Thompson CA, Mayer JE, Oesterle SN, Vacanti JP. Dynamic rotational seeding and cell culture system for vascular tube formation. *Tissue Eng* 2003; 9:291–299.
16. Williams C, Wick TM. Perfusion bioreactor for small diameter tissue-engineered arteries. *Tissue Eng* 2004;10:930–941.
17. Lanza RP, Langer R, Vacanti JP. *Principles of Tissue Engineering*. San Diego: Academic Press; 2000.
18. Stevens MM, George JH. Exploring and engineering the cell surface interface. *Science* 2005;310:1135–1138.
19. Ashammakhi N, Ndreu A, Yang Y, Ylikauppila H, Nikkola L, Hasirci V. Tissue engineering: A new take-off using nano-fiber-based scaffolds. *J Craniofac Surg* 2007;18:3–17.

20. Li D, Xia YN. Electrospinning of nanofibers: Reinventing the wheel? *Adv Mater* 2004;16:1151–1170.
21. Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Compos Sci Technol* 2003;63:2223–2253.
22. Chew SY, Wen Y, Dzenis Y, Leong KW. The role of electrospinning in the emerging field of nanomedicine. *Curr Pharm Design* 2006;12:4751–4770.
23. Xu CY, Inai R, Kotaki M, Ramakrishna S. Electrospun nanofiber fabrication as synthetic extracellular matrix and its potential for vascular tissue engineering. *Tissue Eng* 2004;10:1160–1168.
24. Boland ED, Matthews JA, Pawlowski KJ, Simpson DG, Wnek GE, Bowlin GL. Electrospinning collagen and elastin: Preliminary vascular tissue engineering. *Front Biosci* 2004;9:1422–1432.
25. Buttafoco L, Kolkman NG, Engbers-Buijtenhuijs P, Poot AA, Dijkstra PJ, Vermes I, Feijen J. Electrospinning of collagen and elastin for tissue engineering applications. *Biomaterials* 2006;27:724–734.
26. Remuzzi A, Mantero S, Colombo M, Morigi M, Binda E, Camozzi D, Imberti B. Vascular smooth muscle cells on hyaluronic acid: Culture and mechanical characterization of an engineered vascular construct. *Tissue Eng* 2004;10:699–710.
27. Arrigoni C, Camozzi D, Imberti B, Mantero S, Remuzzi A. The effect of sodium ascorbate on the mechanical properties of hyaluronan-based vascular constructs. *Biomaterials* 2006;27:623–630.
28. Lee JH, Kim WG, Kim SS, Lee JH, Lee HB. Development and characterization of an alginate-impregnated polyester vascular graft. *J Biomed Mater Res* 1997;36:200–208.
29. Lu J-W, Zhu Y-L, Guo Z-X, Hu P, Yu J. Electrospinning of sodium alginate with poly(ethylene oxide). *Polymer* 2006;47:8026–8031.
30. Wnek GE, Carr ME, Simpson DG, Bowlin GL. Electrospinning of nanofiber fibrinogen structures. *Nano Lett* 2003;3:213–216.
31. Morton T, Nikkola L, Ashammakhi N, Wolbank S, Peterbauer A, van Griensven M, Redl H. Electrospun fibrinogen and fibrin nanofibers for angiogenesis in vitro. *Wound Repair Regen* 2007;15:A48.
32. Miller DC, Thapa A, Haberstroh KM, Webster TJ. Endothelial and vascular smooth muscle cell function on poly(lactic-co-glycolic acid) with nano-structured surface features. *Biomaterials* 2004;25:53–61.
33. Iwai S, Sawa Y, Ichikawa H, Taketani S, Uchimura E, Chen GP, Hara M, Miyake J, Matsuda H. Biodegradable polymer with collagen microsphere serves as a new bioengineered cardiovascular prosthesis. *J Thorac Cardiovasc Sur* 2004;128:472–479.
34. Boland ED, Pawlowski KJ, Barnes CP, Simpson DG, Wnek GE, Bowlin GL. Electrospinning of bioresorbable polymers for tissue engineering scaffolds. *ACS Sym Ser.* 2006;918 (Polymeric Fibers):188–204.
35. Li WJ, Cooper JA, Mauck RL, Tuan RS. Fabrication and characterization of six electrospun poly(α -hydroxy ester)-based fibrous scaffolds for tissue engineering applications. *Acta Biomater* 2006;2:377–385.
36. Patel HJ, Su SH, Patterson C, Nguyen KT. A combined strategy to reduce restenosis for vascular tissue engineering applications. *Biotechnol Progr* 2006;22:38–44.
37. Bacakova L, Lapcikova M, Kubies D, Rypacek F. Adhesion and growth of rat aortic smooth muscle cells on lactide-based polymers. *Adv Exp Med Biol* 2003;534:179–189.
38. Buttafoco L, Boks NP, Engbers-Buijtenhuijs P, Grijpma DW, Poot AA, Dijkstra PJ, Vermes I, Feijen J. Porous hybrid structures based on P(DLLA-co-TMC) and collagen for tissue engineering of small-diameter blood vessels. *J Biomed Mater Res B* 2006;79B:425–434.
39. Zhang J, Qi HX, Wang HJ, Hu P, Ou LL, Guo SH, Li J, Che YZ, Yu YT, Kong DL. Engineering of vascular grafts with genetically modified bone marrow mesenchymal stem cells on poly (propylene carbonate) graft. *Artif Organs* 2006;30:898–905.
40. Cikirikciouglu M, Bowlin G, Cikirikciouglu YB, Djebaili K, Tatar T, Kalangos A, Walpoth BH. Biodegradable poly-dioxanone grafts for replacement of the abdominal aorta in the rat model. *Br J Surg* 2005;92:915.
41. Zeng J, Chen XS, Liang QZ, Xu XL, Jing XB. Enzymatic degradation of poly(L-lactide) and poly (epsilon-caprolactone) electrospun fibers. *Macromol Biosci* 2004;4:1118–1125.
42. Huang ZM, He CL, Yang A, Zhang Y, Han XJ, Yin J, Wu Q. Encapsulating drugs in biodegradable ultrafine fibers through co-axial electrospinning. *J Biomed Mater Res A* 2006;77A:169–179.
43. Bolgen N, Menciloglu YZ, Acatay K, Vargel I, Piskin E. In vitro and in vivo degradation of non-woven materials made of poly(ϵ -caprolactone) nanofibers prepared by electrospinning under different conditions. *J Biomater Sci Polym Ed* 2005;16:1537–1555.
44. Linnemann B, Ali R, Gries T, Grafahrend D, Klee D, Moeller M, Roth G. Electrospinning: Nanofiber from polycaprolactone (PCL). *Chem Fibers Int* 2005;55:370–372.
45. Jeun JP, Lim YM, Nho YC. Study on morphology of electrospun poly(caprolactone) nanofiber. *J Ind Eng Chem* 2005;11:573–578.
46. Hsu CM, Shivkumar S. Nano-sized beads and porous fiber constructs of poly(ϵ -caprolactone) produced by electrospinning. *J Mater Sci* 2004;39:3003–3013.
47. Li WJ, Danielson KG, Alexander PG, Tuan RS. Biological response of chondrocytes cultured in three-dimensional nanofibrous poly(epsilon-caprolactone) scaffolds. *J Biomed Mater Res A* 2003;67A:1105–1114.
48. Li WJ, Tuli R, Huang XX, Laquerriere P, Tuan RS. Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold. *Biomaterials* 2005;26:5158–5166.
49. Venugopal J, Ma LL, Yong T, Ramakrishna S. In vitro study of smooth muscle cells on polycaprolactone and collagen nanofibrous matrices. *Cell Biol Int* 2005;29:861–867.
50. Venugopal JR, Zhang YZ, Ramakrishna S. In vitro culture of human dermal fibroblasts on electrospun polycaprolactone collagen nanofibrous membrane. *Artif Organs* 2006;30:440–446.
51. Serrano MC, Pagani R, Vallet-Regi M, Pena J, Ramila A, Izquierdo I, Portoles MT. In vitro biocompatibility assessment of poly(epsilon-caprolactone) films using L929 mouse fibroblasts. *Biomaterials* 2004;25:5603–5611.
52. Vaz CM, Van Tuijl S, Bouten CVC, Baaijens FPT. Design of scaffolds for blood vessel tissue engineering using a multilayering electrospinning technique. *Acta Biomater* 2005;1:575–582.
53. Pham QP, Sharma U, Mikos AG. Electrospun poly(epsilon-caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: Characterization of scaffolds and measurement of cellular infiltration. *Biomacromolecules* 2006;7:2796–2805.
54. Zong XH, Ran SF, Fang DF, Hsiao BS, Chu B. Control of structure, morphology and property in electrospun poly (glycolide-co-lactide) non-woven membranes via post-draw treatments. *Polymer* 2003;44:4959–4967.
55. Im JN, Kim JK, Kim HK, In CH, Lee KY, Park WH. In vitro and in vivo degradation of synthetic absorbable biocomponent monofilament suture prepared with poly(p-dioxanone) and its copolymer. *Polym Degrad Stabil* 2007;92:667–674.
56. Williamson MR, Black R, Kieley C. PCL-PU composite vascular scaffold production for vascular tissue engineering: Attachment, proliferation and bioactivity of human vascular endothelial cells. *Biomaterials* 2006;27:3608–3616.

57. Yarin AL, Kataphinan W, Reneker DH. Branching in electrospinning of nanofibers. *J Appl Phys* 2005;98:064501/1-064501/12.
58. McManus MC, Boland ED, Koo HP, Barnes CP, Pawlowski KJ, Wnek GE, Simpson DG, Bowlin GL. Mechanical properties of electrospun fibrinogen structures. *Acta Biomater* 2006;2:19-28.
59. Valenta J. *Clinical Aspects of Biomedecine*. Amsterdam: Elsevier Science; 1993.
60. Evans FG. *Strength of Biological Materials*. Baltimore: Williams & Wilkins; 1980.
61. Fong H, Chun I, Reneker DH. Beaded nanofibers formed during electrospinning. *Polymer* 1999;40:4585-4592.
62. Deitzel JM, Kleinmeyer J, Harris D, Tan NCB. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer* 2001;42:261-272.
63. Zhang Z, Wang Z, Liu S, Kodama M. Pore size, tissue ingrowth, and endothelialization of small-diameter microporous polyurethane vascular prostheses. *Biomaterials* 2004;25:177-187.