Electrospun PHEA-PLA/PCL Scaffold for Vascular Regeneration: A Preliminary in Vivo Evaluation

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ABSTRACT

Background. There is increasing interest in the development of vessel substitutes, and many studies are currently focusing on the development of biodegradable scaffolds capable of fostering vascular regeneration. We tested a new biocompatible and biodegradable material with mechanical properties similar to those of blood vessels.

Methods. The material used comprises a mixture of α,β-poly(*N*-2-hydroxyethyl)-D,L-aspartamide (PHEA) and polylactic acid (PLA), combined with polycaprolactone (PCL) by means of electrospinning technique. Low-molecular-weight heparin was also linked to the copolymer. A tubular PHEA-PLA/PCL sample was used to create an arteriovenous fistula in a pig model with the use of the external iliac vessels. The flow was assessed by means of Doppler ultrasound examination weekly, and 1 month after the implantation we removed the scaffold for histopathologic evaluation.

Results. The implants showed a perfect leak-proof seal and adequate elastic tension to blood pressure. About ~3 weeks after the implantation, Doppler examination revealed thrombosis of the graft, so we proceeded to its removal. Histologic examination showed chronic inflammation, with the presence of foreign body cells and marked neovascularization. The material had been largely absorbed, leaving some isolated spot residues.

Conclusions. The biocompatibility of PHEA-PLA/PCL and its physical properties make it suitable for the replacement of vessels. In the future, the possibility of functionalizing the material with a variety of molecules, to modulate the inflammatory and coagulative responses, will allow obtaining devices suitable for the replacement of native vessels.

IN clinical practice, there is a strong need for alternatives to the use of autologous vascular grafts for vascular reconstructive surgery (eg, coronary bypass, bypass of the lower limbs, arteriovenous fistulas) [1,2]. Currently, autologous vessels, especially the saphenous vein, are the most widely used materials for the replacement of small arterial vessels [1–3]. Immunologic compatibility is one of the biggest advantages of using these patches; however, sometimes we cannot use human autologous as vascular substitutes and we have to resort to an alternate patch. The biologic patches used are limited to some prostheses and are

Funding: PRIN 2015: "Chirurgia rigenerativa nei difetti della parete addominale e nel deficit congenito o acquisito delle vie escretrici urinarie, biliari e dei vasi sanguigni: modellamento e crescita cellulare su scaffold planare e su scaffold tubulare. Studio sperimentale in vivo su modello suino" (2015X5AH3Z).

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0041-1345/17
http://dx.doi.org/10.1016/j.transproceed.2017.02.017

often represented by autologous pericardium, allogeneic or xenogeneic (fixed in glutaraldehyde). Unfortunately, these patches have well known limitations: They are subject to greater risk of infections and thrombosis, they tend to calcify, and often they cause foreign body reactions and do not guarantee an adequate growth potential [4–10].

Vascular grafts made from synthetic materials, such as polyester and expanded polytetrafluoroethylene, are routinely used to restore the blood flow in patients with various cardiovascular disorders. They still have many disadvantages, such as thrombogenicity, intimal hyperplasia, stenosis and occlusion (especially in the small caliber grafts), susceptibility to infections, formation of pseudoaneurysms, and lack of growth potential [3,11–13]. Therefore, a completely bioabsorbable vascular patch, capable of inducing the regeneration of a new vessel wall, may overcome the limitations of the current artificial patches, serving as architectural support for the development of the neotissue. Tissue engineering is focusing on the development of biomaterials capable of mimicking the biologic and mechanical functions of the extracellular matrix (ECM). In fact, it has been shown that tissue morphogenesis is strongly influenced by interactions between cells and the ECM [14–26].

The present study aimed to test a new material as a bioabsorbable substitute for blood vessels in a pig model. This material initially should behave like the common vascular prosthesis and, at a later stage, once degraded and reabsorbed by the host organism, should replace the graft in a new blood vessel with anatomic and functional characteristics similar to those of native vascular vessels.

METHODS

The scaffold we tested was created in the Biocompatible Polymer Laboratory of the Department of Science and Molecular and Biomolecular Technology (STEMBIO) of the University of Palermo. It consists of electrospun fibers based on synthetic polymers. Electrospinning is a technique that uses a high voltage source for biasing a polymer solution or a polymer melt, which is then accelerated toward an opposite polarity collector. Through electrospinning, one can obtain the 3-dimensional scaffold composed of micronanometer polymeric fibers, interconnected to form a microporous structure [27,28]. α,β-Poly(*N*-2-hydroxyethyl-D,L)-aspartamide (PHEA) is the starting polymer; we used it for the production of copolymers for our scaffold. PHEA is a biocompatible synthetic polymer, soluble in water, with a similar structure to human amino acids. The use of PHEA as carriers of drugs and as a starting material for many other biomedical and pharmaceutical applications has already been reported [29,30]. PHEA was linked with polylactic acid (PLA) and subsequently electrospun in a mixture with polycaprolactone (PCL) [31,32]. Scaffolds obtained from the PHEA-PLA/PCL mixture had fibers with diameters from 500 nm to 1 μm, similar to the ECM. In fact, the adhesion, proliferation and differentiation of cells are strongly influenced by size, geometry, and density of the pores and the surface properties [33–40]. From a study carried out previously by our group, PHEA-PLA/PCL was found to be a biocompatible material, elastic, and possessing great mechanical strength [41]. The data obtained from in vivo experiments showed that the material has good biocompatibility, evoking an inflammatory response of modest degree, which has a prime role in the reabsorption of the material and processes of tissue regeneration. Subsequently, the scaffold was made tubular in a structure with a diameter of ~5 mm and a length of a few centimeters. The material was also bound to heparin by covalent bonding to reduce the risk of thrombosis [42].

We performed an arteriovenous fistula (AFV) between the external left iliac artery and the external left iliac vein in a 4-month-old pig model weighing 40 kg. Our experiments were conducted according to the provisions of Legislative Decree no 26 of March 4, 2014, implementing Directive 2010/63/EU on the protection of animals used for scientific purposes, after regulatory approval of the project by the Italian Ministry of Health. All animal experiments were in compliance with the Animal Research: Reporting of In Vivo Experiments guidelines and were carried out in accordance with the EU Directive 2010/63/EU guidelines for animal experiments, the National Institutes of Health guide for the care and use of laboratory animals (legislative decree no 26, March 4, 2014) and the Organismo Preposto al Benessere Animale of the "A. Mirri" Zooprophylactic Institute.

The anesthetic protocol included premedication (6.3 mg/kg zolazepam tiletamine + 2.3 mg/kg xylazine), induction (0.5 mg/kg propofol), and maintenance (isoflurane + pancuronium, 12:7 mg/kg). After obtaining access to "J" in the left iliac fossa, we proceeded to the isolation of the iliac vessels and packaging of secondary arteriovenous fistula between the left external iliac artery and the vein ipsilateral homologous, with laterolateral anastomosis on the arterial side and terminolateral anastomosis on the venous side; this was carried out by means of double hemicontinuous suture of 180° with the use of a suture thread of a nonabsorbable Prolene type 8-0 (Fig 1). At the moment of the arterial unclamping, we were able, macroscopically, to appreciate a good pulse rate on the prosthetic surface. After surgery, the animal was subjected to a liquid diet for the first 24 hours and antibiotic treatment with the use of oxytetracycline (20 mg/kg/d for 3 days). The flow within the AFV was assessed by means of Doppler ultrasound examination with the use of a microconvex probe of 4–7.5 MHz. The study of the flow was performed by means of Doppler ultrasound every week. In the 1st 2 weeks, the Doppler checks revealed the patency of our AFV (Fig 2). After 1 month, seeing the Doppler scaffold thrombosis responses, we proceeded to en bloc scaffold removal for histopathologic examination. The histologic sections of the surgical specimen were analyzed, after fixing in a 10% formalin solution, with the use of hematoxylin-eosin staining.

RESULTS

The bioresorbable tubular prosthesis proved to be easy to handle and resistant to traction during the anastomoses. The anastomosis proved to be leak proof, but subject to an arterial pressure regime, as evidenced by the absence of signs of intraoperative bleeding and early complications.

The scaffold developed by the STEMBIO laboratory also seemed to accord with the suture needle passage, making it, from a surgical point of view, particularly suited to clinical applications. After this procedure, the scaffold remained perfectly intact and impermeable to blood serum. The scaffold showed great flexibility, allowing it to pulsate in the same manner as the adjacent arteries. Doppler examination

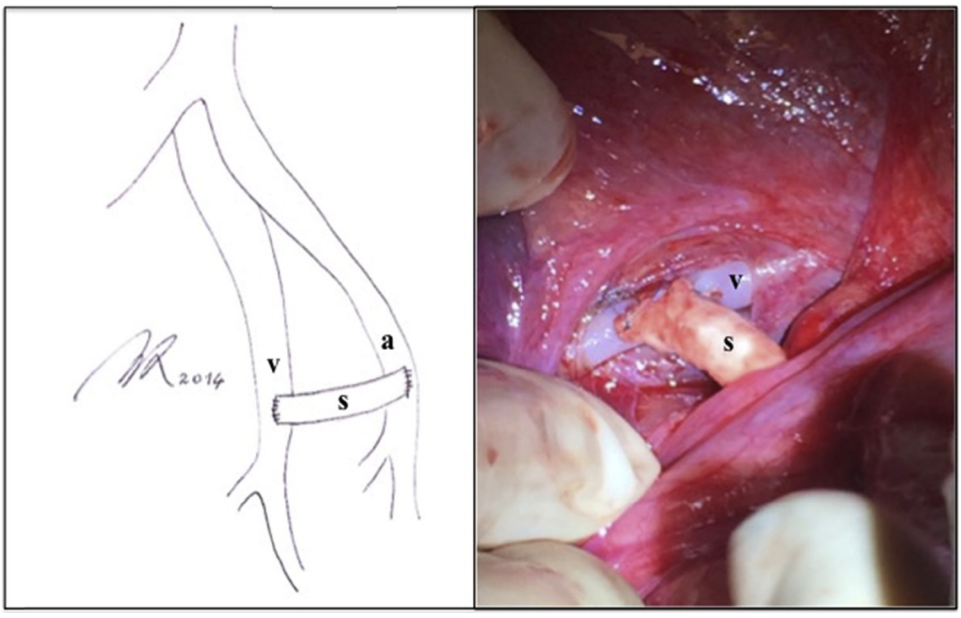

Fig 1. Bridge fistula (*s*) between the left external iliac artery (*a*) and the ipsilateral homologous vein (*v*).

performed immediately after surgery showed the patency of the anastomosis, with a typically turbulent flow (Fig 2). Subsequent ultrasound evaluations revealed the patency of the graft in the 1st 2 weeks and a thrombosis during the Doppler control in the 3rd week.

Histologic examination showed a chronic granulomatous "foreign body" inflammation with giant cells; this was associated with a fibrotic stromal reaction and multiple small newly formed vessels. It was possible to still see the presence of as yet unabsorbed material in the context of granulomatous inflammation (Fig 3).

DISCUSSION

The goal of tissue engineering is to obtain a biofunctional and bioabsorbable scaffold, capable of fostering the regeneration of native tissue [43,44]. The possibility of producing bioengineered vessels is the subject of several experimental studies [45–66]; however, the material to be proposed as a "scaffold ideal" has not yet been found [35–40,67–80]. The scaffold created in the STEMBIO laboratories proved to be biocompatible, as evidenced by our preliminary studies in the mouse model and as confirmed by the present experiment with the use of the pig model. Our scaffold differs from others used so far, because it seems to present the structural characteristics that make it suitable for the

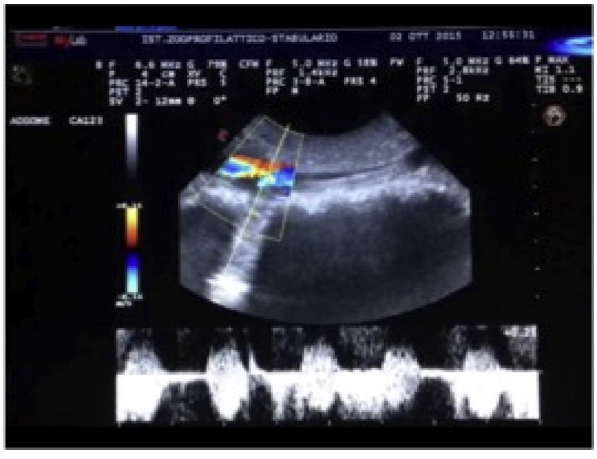

Fig 2. Doppler ultrasound performed immediately after surgery, showing the patency of the anastomosis, with a typically turbulent flow.

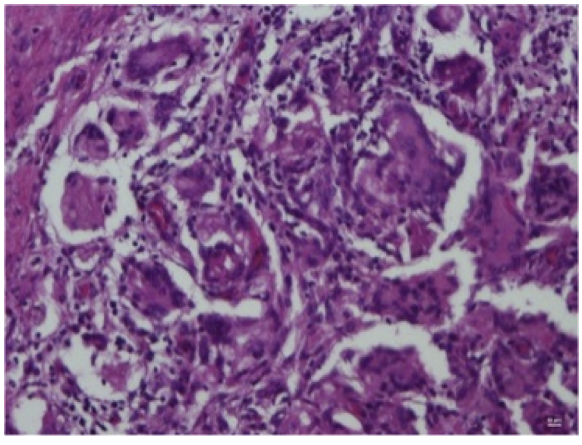

Fig 3. Chronic granulomatous "foreign body" inflammation with giant cells, associated with a fibrotic stromal reaction and multiple small newly formed vessels.

temporary replacement of blood vessels, without being affected by the early hydrolysis phenomena which too quickly degrade the other materials proposed so far. The histologic results highlighted how the time required for the degradation of our scaffold is >1 month. However, we were able to observe the start of a regeneration process, as evidenced by the presence of neovascularization with the formation of an intricate vascular network associated with characteristic cellular elements of chronic inflammation.

The electrospinning of PHEA-PLA in a mixture with PCL resulted in a scaffold with excellent elasticity, probably related to the regularity and dimensional uniformity of the fibers and the absence of a merger between them; these morphologic characteristics are similar to those of the native ECM. In addition, our vascular substitute showed good mechanical properties, allowing easy handling during the experiments conducted. After the AVF had been created, arterial unclamping showed that the graft presented good compliance, responding to changes in blood flow and arterial pulse waves. The tensile properties and the microporous structure are responsible for 2 other fundamental results of our research: the complete absence of bleeding or serous leakage through the walls, and excellent resistance to the needle suture passage, with a consequent perfect seal of the anastomosis. The ultrastructure of the scaffold, with an intricate plot of nanofilaments distributed over a thickness of ~0.5 mm, enables the cells to colonize the scaffold and, at the same time, prevents fluid from exuding from the surface. It is these same characteristics that guarantee that this tissue remains intact during the needle passage from the suture, because the fibers are spread apart and are not in any way damaged or severed by the mechanical puncture. This resistance to tension guarantees the perfect seal of the anastomosis, avoiding blood loss at the point of suture. This characteristic resistance to puncture may, prospectively, allow the use of such devices for vascular access use, and, in particular, for the performing bridge fistulas.

Polymer thrombogenicity was partially resolved by functionalizing it with heparin molecules covalently bonded to the nanostructure of the scaffold. Although the scaffold was pervious only for a month, we had no evidence of early thrombosis phenomena, despite the complete absence of anticoagulant therapy. Heparin also had an important role in guiding vascular endothelialization and by stimulating numerous growth factors [42,81]. This would allow the circulating stem cells to engraft to the implanted tissue and promote the regeneration of a fabric (regarding mechanical and biologic characteristics) similar to that of the host organism. Three weeks after implantation, the graft showed thrombosis phenomena; we think that one of the major causes of thrombosis may have been an inappropriate postoperative anticoagulant regimen. Probably, the absence of flow within the vascular graft did not allow effective colonization, particularly in its central portion, an event that contributed to only partial resorption of the material, especially in the part farthest from the anastomosis area.

CONCLUSION

The “ideal scaffold” boasts certain fundamental properties: It must be made from biocompatible material, having a degradation rate that corresponds to the production of new ECM by the host; and it must possess the ability to interact with host cells, mechanical properties, and ultrastructural characteristics that match the target anatomic site.

The biocompatibility of PHEA-PLA/PCL and its physical properties make it suitable in the replacement of vessels. The main disadvantage is that its thrombogenicity is only partially solved by adding heparin to the scaffold. To resolve this issue and obtain a new vessel from a synthetic substitute, we will need to conduct further studies in which individualized anticoagulation treatments will be used; these will be associated with the use of PHEA-PLA/PCL scaffolds that could be further functionalized with growth factors to modulate the inflammatory response and coagulation.

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