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Original Article

In vitro experiments and in vivo implants to evaluate a new silicone-based polyurethane material for replacement of small vessels

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THE IDEA UNDERSCORING OUR PROPOSED development is to take advantage of the good properties of both polyurethanes (PU) and silicones (PDMS). The attributes which make polyurethanes attractive as materials for biomedical applications are their excellent physical–chemical properties, and their relatively good biocompatibility. Against their use is the phenomenon of biodegradation that occurs after long-term implantation. Silicones, on the other end, are known to have long-term biostability and good haemocompatibility subsequent to their use in several biomedical settings.

Our new material, named PU-PDMS, is made by an aromatic PU which can contains different percentages of PDMS. Before synthesising the material, we used a soxhlet apparatus to process the PU, and the solvents in which the polymer is dissolved in order to obtain a material of very great purity. The reaction was carried out in a three-neck flasks under mechanical agitation and flow of nitrogen for 6 hours at 82° centigrade.

The specific objectives of our programme of research are as follows:

- to evaluate the use of the PU-PDMS material to construct vascular grafts of small diameter
- to evaluate its value for coating metallic intravascular stents and Dacron® vascular grafts
- to evaluate the haemocompatibility and biostability of the various biomedical devices.

Because it is well known that synthetic grafts cannot be used to replace vessels having an internal diameter of less than 6 millimetres,[1] we have focussed in this review on the evaluation of the performance of PU-PDMS grafts having an internal diameter of less than 5 millimetres by circulating blood through them in an in vitro system, and by in vivo implants.

We used the spray machine to produce the PU-PDMS vascular grafts, this equipment allows the production of microporous or dense structures by varying the parameters of fabrication of the material deposited onto a rotating mandrel. We used the phase-inversion principle when preparing the polymer solutions.[2] The PU-PDMS grafts were manufactured with an internal porous layer, shown by our previous studies to be an highly compatible with exposure to blood,[3,4] and an external dense layer that confers resistance and elasticity (Fig. 1). The haemocompatibility of the grafts in relation to their content of silicone was investigated using an in vitro circulation system, as shown in Figure 2. The entire system is maintained at 37° centigrade in a thermostatic bath for the duration of the experiment.[5] Before assembling the circuit, the grafts were sterilised by sonication for 30 minutes in dilute hydrochloric acid. They were then thoroughly rinsed in sterile distilled water. Finally, the grafts were coated externally with a highly concentrated solution of PU-PDMS solution, about 13% weight to volume, to make them impermeable to the circulating blood. Human blood was drawn

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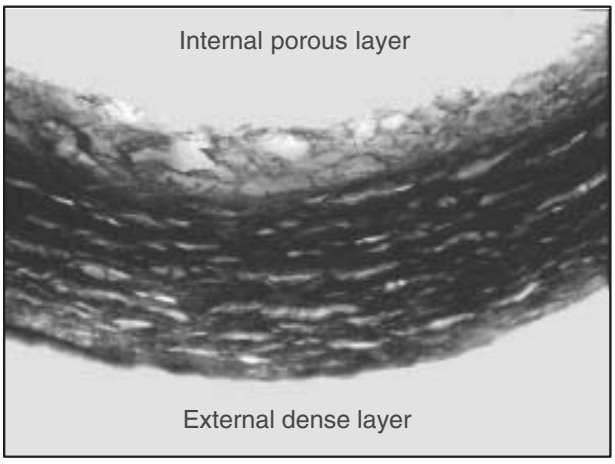

Figure 1.
Section of a two-layer PU-PDMS graft.

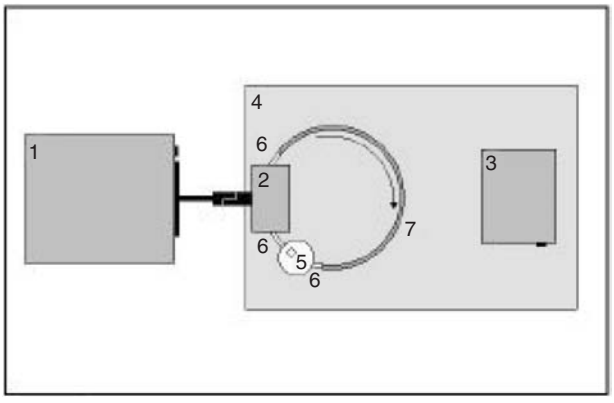

Figure 2.
The in vitro circuit: (1) roller pump, (2) pump head, (3) heating element, (4) water bath, (5) reservoir in silicone medical grade, (6) 30-centimetre long silicone medical grade tube, and (7) 50-centimetre long vascular grafts.

from healthy, non-smoking, donors who had not taken medication for at least two weeks prior to the study. Prior to each experiment, we measured the haematocrits, and these were within the normal range for all subjects. Blood was collected in acid citrate dextrose anticoagulant, at proportions of 1 to 10 by volume, from the antecubital vein using a 19-gauge butterfly needle to minimise activation of platelets.

Anticoagulated whole blood had already been circulated in the perfusion system for 2 hours at flows of 98 millilitres per minute. In every experiment, samples of blood were collected from the reservoir to measure the adhesion of platelets after 30, 60 and 120 minutes circulation. In the same way, a solution of fibrinogen at 300 milligrams per decilitre, this being the concentration as in human plasma, was circulated for 3 hours at the same rate, and samples were collected

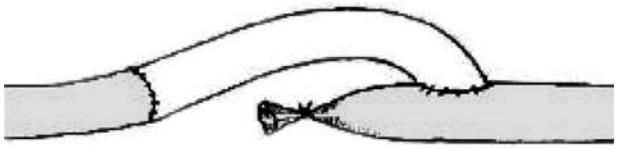

Figure 3.
The techniques used to create the end-to-end and end-to-side anastomoses.

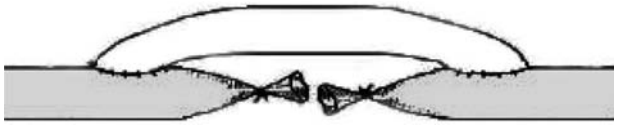

Figure 4.
The techniques for the double end-to-side anastomosis.

every half an hour. The decrease in fibrinogen was measured by a spectrophotometer.

We evaluated concentrations of silicone at 20%, 40%, 60% and 100%, and the haemocompatibility was determined as described above. The parameters used to evaluate the in vitro haemocompatibility were chosen according to the standard ISO 10993-4 (Biological evaluation of medical devices – Section of tests for interactions with blood), which are related to the haemocompatibility rather than the thrombogenicity of the grafts in vivo.[6] The in vitro experiments indicated that, with the content of silicone varying from 20 to 40%, there was low adhesion of platelets and adsorption of fibrinogen when compared to lower or higher value, so we used grafts of these kinds for the subsequent in vivo implants.

We implanted the grafts first in the porcine carotid artery, testing the surgical feasibility of by-pass grafting by acute experiments lasting up to 24 hours, and second, in the ovine carotid artery, testing the long-term performance of the grafts by using chronic experiments lasting up to one year. The grafts were implanted either using an "end-to-end" anastomosis proximally and an "end-to-side" anastomosis distally, or by a double end-to-side anastomosis (Figs 3 and 4, respectively).[7]

The grafts implanted in the porcine model (Fig. 5) permitted no bleeding, so that pre-clotting was not required. They proved easy to suture, showed excellent elastic properties with no kinking, and revealed good mechanical resistance, with no failure in six experiments. The grafts implanted in the ovine model (Fig. 6) retained their patency, with an initial deposition of a thin, uniform fibrinous layer of platelets and red cells. They also maintained their elasticity, had a pressure-compliant external layer which was semi-permeable, and were well integrated into the host, with formation of neointimal neoadventitial

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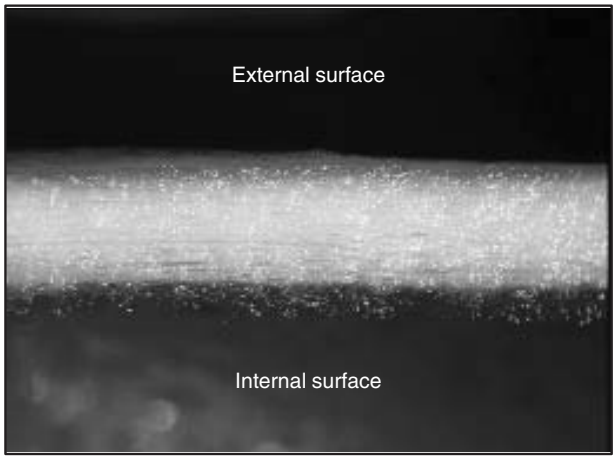

Figure 5.
A PU-PDMS graft implanted in the pig model for 24 hours shows a thin fibrinous layer on the internal surface.

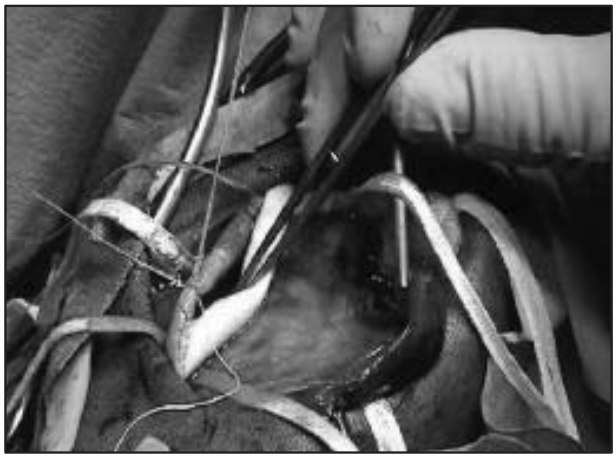

Figure 6.
The Silcrothane® graft implanted in the sheep carotid artery by-pass model.

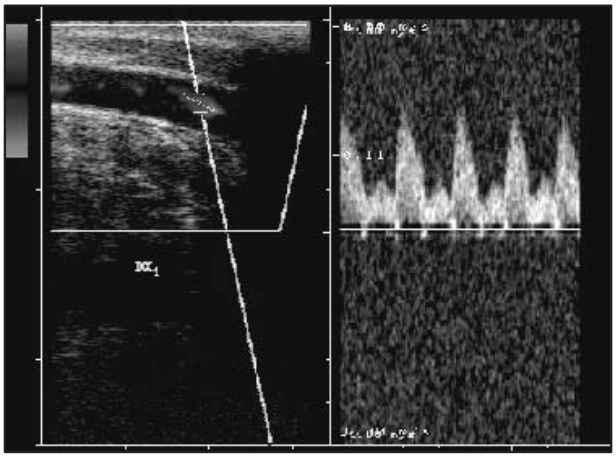

Figure 7.
Echo-Color-Doppler of a PU-PDMS graft as implanted in a sheep.

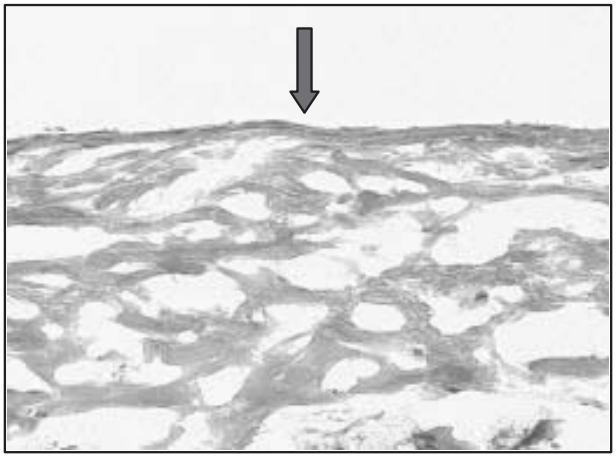

Figure 8.
Cellularisation at distal anastomosis after 21 days of implantation in a sheep (haematoxylin–eosin stain; original magnification 2003).

layers on the scaffold of the graft material. Patency during the experiments was monitored using echo-Color-Doppler examination (Fig. 7).

One chronic experiment was interrupted after 21 days of implantation because of the death of the sheep caused by abortion. The graft was explanted and stained using haematoxylin and eosin. This revealed cellularisation on the luminal surface of the graft near to the anastomosis (Fig. 8). The other chronic experiments are still in progress.

Up to now, therefore, our grafts have shown consistent experimental results, good patency and stability, with the possibility of growth according to the type and proportions of materials, absence of toxicity, degeneration, calcification, aneurysmal dilation or neointimal hyperplasia. They have been completely integrated into the host to form a neo-viable vessel. Preliminary results have confirmed their versatility, and enhanced the perspectives of further research in this field. Additional experiments are now required to verify our previous results, and to develop new techniques and applications, such as insertion in growing sheep, evaluation of grafts of different sizes, and implantation in the venous position, such as the jugular vein.

We now anticipate the potential use of our material during the three stages of palliation of the functionally univentricular heart. In the first stage, a graft with an internal diameter of 3 to 4.5 millimetres could be used as a systemic-pulmonary or ventriculo-pulmonary arterial conduit. The material can also be used to reconstruct the aortic arch. In the second stage, creation of the cavo-pulmonary connection, the graft can be used as needed to enlarge the pulmonary arteries. In the third stage, completion of the total cavo-pulmonary connection, our material could be used to create a

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venous conduit, with an internal diameters between 14 and 18 millimetres, placed between the inferior caval vein and the pulmonary arteries. If such a graft is used in the third stage, we anticipate that there will be no need to use larger conduits, since grafts prepared using our material will have the capacity to grow. In this way, we can avoid mismatch between the patient and the prosthesis. Moreover, we emphasise that anticoagulation or antiplatelet therapy is not required at any time should our new material be used as a graft.

Acknowledgements

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