The handle [http://hdl.handle.net/1887/29721](http://hdl.handle.net/1887/29721) holds various files of this Leiden University dissertation.

**Author:** Steenhoven, Timothy Jason van der  
**Title:** On prevention of second hip fracture surgery: epidemiological and biomechanical aspects of elastomer femoroplasty  
**Issue Date:** 2014-11-11
CHAPTER 9
Thrombogenicity of a new injectable biocompatible elastomer for aneurysm exclusion, compared to expanded polytetrafluoroethylene in a human ex vivo model

T.J. van der Steenhoven
W.M.P.F. Bosman
C. Tersteeg
M.J. Jacobs
F.L. Moll
P.G. de Groot
J.M.M. Heyligers

European journal of Vascular and Endovascular surgery June 2012
ABSTRACT

Objectives

Customized Aortic Repair (CAR) is a new concept for endovascular aortic aneurysm repair in which a non-polymerised elastomer is injected to fill the aneurysm sac around a balloon catheter. Amongst other variables, the thrombogenicity of the elastomer should be tested, before further clinical experiments can take place. The aim of this human ex vivo study was to measure the thrombogenicity of the elastomer and to compare it to expanded polytetrafluoroethylene (ePTFE).

Design and materials

In a validated ex vivo model, non-anticoagulated blood was drawn from the antecubital veins of 10 healthy donors with a 19-gauge needle. It was drawn through elastomer tubes and through ePTFE Gore-Tex vascular grafts, both 60 cm long and with an inner diameter of 3 mm.

Methods

Fibrinopeptide A (FPA) and P-selectin expression was measured in blood samples, collected at the end of the grafts. After the experiments, the deposition of platelets and fibrin onto the grafts was visualized by scanning electron microscopy.

Results

For these graft types, a progressive increase in FPA production was observed in time. No significant difference was observed between the elastomer and ePTFE grafts (p > 0.05). No increase in P-selectin expression, and thereby no platelet activation, was observed in the perfusate of either grafts (p > 0.05). By scanning electron microscopy, numerous platelet aggregates were observed on the ePTFE grafts, whereas just a few adhered platelets and no aggregates were observed in the elastomer grafts.

Conclusions

The elastomer in its current formulation has a low thrombogenicity, comparable to ePTFE, making it an ideal substance for endovascular aneurysm sac filling. Further research should clarify the feasibility of CAR in vivo.
INTRODUCTION

Endovascular aneurysm repair (EVAR) of abdominal aortic and other arterial aneurysms has become a well-established treatment modality [1, 2]. However, there are still several drawbacks to EVAR. Endoleaks, endotension, stent migration and stent failure are complications that might lead to re-interventions, prolonged follow-up or even rupture after treatment [3, 4]. Furthermore, EVAR has anatomical restrictions. In the literature, up to 27% of aneurysms are considered to be unsuitable for EVAR because of insufficient neck length, large neck diameter or severe angulation [5].

To overcome these anatomical disadvantages, Customized Aortic Repair (CAR, a concept formerly known as Aortic Customize) was developed as a new approach for aneurysm repair (Fig. 1) [6-8].

With this concept, the lumen of the aneurysm is excluded by one or more endovascular balloon(s), and a non-polymerized liquid elastomeric solution (polydimethylsiloxane, PDMS) is used to fill the aneurysm sac around the balloon catheter. After the in situ polymerization and balloon deflation, an endoluminal mould with a patent lumen excludes the aneurysm sac.

One of the key attributes of this newly engineered elastomer is its low viscosity, enabling injection into the aneurysm sac through small profile (7Fr) endovascular catheters, resulting in a fully percutaneous, rapid and easily accessible technique. Filling the cavity of the aneurysm sac with an injectable biocompatible elastomer reduces wall stress and thereby will probably reduce the chance of rupture risk, as aneurysm rupture occurs when the local wall stress exceeds the local wall strength [6, 9, 10]. Extensive in vitro and preliminary in vivo porcine experiments have shown the feasibility and potential of CAR [6, 7, 11]. The elastomer has the physical properties necessary for endovascular injection and successful aneurysm exclusion.

The direct contact with blood requires a low thrombogenicity of the elastomer to prevent occlusive thrombosis or embolization. Regular PDMS has proven its low thrombogenic properties in the past when compared to expanded polytetrafluoroethylene (ePTFE) [12]. The elastomer used in CAR is very similar to regular PDMS. However, there are no data available on the thrombogenicity of this particular elastomer. In advance of any in vivo animal experiments or human clinical procedures, further basic research on the thrombogenicity must be performed.

The aim of this human ex vivo study was to measure the thrombogenicity of the elastomer in healthy young volunteers and compare it to the thrombogenicity of ePTFE grafts, one of the most used materials in synthetic arterial grafts for aneurysm repair and peripheral bypasses.
MATERIAL AND METHODS

Ex vivo model

In a validated and earlier described ex vivo model, non-anticoagulated blood was drawn from the antecubital veins of 10 healthy donors with a 19-gauge needle (Fig. 2) [13].
A vascular graft with a length of 60 cm and a diameter of 3 mm was connected to the needle. Using a syringe pump, blood was aspirated with a constant flow rate of 20 ml min$^{-1}$, for 6 min. The combination of this diameter and flow rate resulted in a shear rate of 74 s$^{-1}$, which reflects venous flow conditions and favours fibrin-rich clot formation. A cuff was wrapped around the upper arm to ensure a constant pressure of 45 mmHg, resulting in a continuous blood flow through the graft during the experiment.

All volunteers were healthy male subjects, who had no vascular history and had no coagulopathy. The median age of the volunteers was 24.3 (range: 22—26) years. They denied taking any medication 2 weeks before the experiment and gave informed consent. The experiments were approved by the local medical ethics committee (UMCU, Utrecht, the Netherlands). Every volunteer served as his own control. Randomization of the grafts for first and second run took place. When the elastomer graft was used for the first run, the ePTFE graft was used for the second run. This second run was performed within half an hour after the first run and blood was drawn from the contralateral arm.

**Figure 2.** *Ex vivo* perfusion model using a cuff at a constant pressure of 45 mmHg to ensure blood flow. Blood is aspirated through the graft with a constant flow of 20 mL/ min, resulting in a shear rate of 74/s.

**Grafts**

The elastomer tubes were cast using a custom-made mould (Department of Fine Mechanics, Leiden University Medical Center, Leiden, the Netherlands). The elastomer...
was a two-component room-temperature addition-cure liquid silicone formulation, obtained from Viazym BV (ViaZym BV; Delft, the Netherlands) [8]. The two components consisted of:

1. A platinum-containing vinyl-terminated PDMS, surface-treated amorphous silica and vinyl Q,
2. A methylhydro-dimethyl-siloxane copolymer containing vinyl-terminated PDMS, surface-treated amorphous silica and vinyl Q.

The two components of the elastomer were mixed using a static mixer and injected in the mould; creating solid elastomer tubes 60 cm long with an inner diameter of 3 mm. Standard ePTFE Gore-Tex vascular grafts (Gore, Flagstaff, AZ, USA) with an internal diameter of 3 mm and a length of 60 cm were used for comparison.

**Blood samples and assays**

Blood samples (900 μl) were collected at the end of the graft, starting directly after connection to the vein and thereafter every minute for a total of 4 min. After 4 min, samples were collected every 30 s until the end of the perfusion (total perfusion time was 6 min for each arm). The samples were mixed immediately with 100 μl of 0.5 M ethylenediamine tetra acetic acid (EDTA) and centrifuged at 3500 rpm for 5 min, and aliquots of plasma were stored at -20 °C until assayed.

Fibrinopeptide A (FPA) and P-selectin levels were determined in the blood samples. FPA is the product from the transformation of fibrinogen in fibrin, which is a process activated by thrombin release. P-selectin is a cell-adhesion molecule on the surfaces of activated endothelial cells, that line the inner surface of blood vessels, and activated platelets. When endothelial cells are activated by molecules such as thrombin or histamine during injury or inflammation, P-selectin moves from an internal cell location to the endothelial cell surface. Therefore FPA- and P-selectin-positive platelets are an adequate method to measure the advance of the activated coagulation process.

For the FPA measurements, a commercially available enzyme-linked immunosorbent assay was used as an indicator of fibrin formation (Zymutest FPA; Hyphen Biomed, Andresy, France). P-selectin expression on perfused platelets, as an indicator of activation of platelets, was determined in EDTA anti-coagulated whole blood before plasma centrifugation. Platelet activation was assayed by flow cytometric analysis using a phycoerythrin (PE)-conjugated monoclonal antibody, following the instructions for use of the manufacturer (BD Pharmingen, San Diego, CA, USA, Catalog No 555524).

**Scanning electron microscopy**

The deposition of platelets and fibrin onto the grafts was visualized by scanning electron microscopy. The distal end of the graft was cut into small pieces (5x5 mm), fixed in 2%
glutaraldehyde, and then dehydrated through increasing concentrations of ethanol (80—100%). The samples were dried with the use of hex-amethyldisilazane. Next, the graft pieces were sputter-coated with a thin layer of platinum/palladium and analyzed with scanning electron microscopy (Philips XL30; Eindhoven, the Netherlands).

**Statistical analysis**

The results were analyzed with a paired t test, as implemented in the statistics program Statistical Package for the Social Sciences (SPSS) 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The results were expressed as the mean ± standard error of the mean (SEM). A P value < 0.05 was considered significant.

**RESULTS**

In the current ex vivo model, non-anticoagulated blood was drawn directly from the antecubital veins over either Gore-Tex® ePTFE grafts or elastomer grafts (Fig. 2). All 10 perfusions were performed without any technical or subject-related complications.

FPA concentration was measured in plasma samples taken at different time points. For both graft types, a progressive increase in FPA production was observed in time. No significant difference (P > 0.05) was observed between elastomer and ePTFE grafts (Fig. 3, Table 1).

The activation of platelets during blood drawing was analyzed by measuring the percentage of P-selectin-positive platelets. No increase (P > 0.05), and thereby no platelet activation, was observed in the perfusate of either graft (Fig. 4, Table 1).

We also analyzed the results by using each volunteer as his own control, as one arm was used for an ePTFE graft and the other arm for an elastomer graft. Fig. 5 shows the mean differences (delta values) in FPA- and P-selectin levels per patient. These delta values were obtained by subtracting the ePTFE measurements from the elastomer values.

Using scanning electron microscopy, numerous platelet aggregates were observed on the ePTFE grafts whereas only a few adhered platelets and no aggregates were observed in the elastomer grafts (Fig. 6).

**DISCUSSION**

The measurements of FPA concentration and of P-selectin-positive platelets show that the elastomer, designed and tailored for CAR, has a low thrombogenicity. We found no statistically significant difference in thrombogenicity compared to ePTFE, one of the most used materials in synthetic arterial grafts for aneurysm repair and peripheral bypasses (Figs. 3 and 4, Table 1).
The differences in measured FPA levels and P-selectin percentages per patient were minimal as shown in Fig. 5. Individuals who had a ‘strong’ thrombogenic response to ePTFE also had a ‘strong’ thrombogenic response to the elastomer. In the same manner, individuals who had a ‘moderate’ response to ePTFE, had the same response to the elastomer.

The electron microscopic analysis showed a decrease in platelet adhesion and aggregation on the elastomer samples compared to ePTFE grafts (Fig. 6). Unfortunately, it was not possible to quantify these results. The extremely smooth surface of the elastomer graft compared to the porosity of the ePTFE graft could have contributed to this difference in the adhesion of platelets. As the use of ePTFE grafts in an aortic position has a low thrombosis rate, similar low thrombosis rates can be expected with the use of the elastomer.

Table 1. P-values of differences in FPA and P-selectin levels measured at different time points. The results were analyzed with a paired t test.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>FPA</th>
<th>P-selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.72</td>
<td>0.44</td>
</tr>
<tr>
<td>1</td>
<td>0.94</td>
<td>0.58</td>
</tr>
<tr>
<td>2</td>
<td>0.88</td>
<td>0.74</td>
</tr>
<tr>
<td>3</td>
<td>0.97</td>
<td>0.48</td>
</tr>
<tr>
<td>4</td>
<td>0.90</td>
<td>0.53</td>
</tr>
<tr>
<td>4.5</td>
<td>0.33</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>0.26</td>
<td>0.74</td>
</tr>
<tr>
<td>5.5</td>
<td>0.71</td>
<td>0.28</td>
</tr>
<tr>
<td>6</td>
<td>0.66</td>
<td>0.60</td>
</tr>
</tbody>
</table>

**Figure 3.** Fibrinopeptide A measurements show a progressive increase in fibrin formation in time during ex vivo perfusions. No significant differences were found between the two types of grafts. Error bars indicate standard error of the mean.
Thrombogenicity of a new elastomer compared to ePTFE

Figure 4. P-selectin expression as a measure of platelet activation during ex vivo perfusion. No significant increase was observed between the two types of grafts. Error bars indicate standard error of the mean.

Figure 5. Graph depicting the difference in the levels of FPA and P-selectin levels in each patient (Elastomer value –ePTFE value). Error bars show SD. The delta values were obtained by subtracting the ePTFE measurement from the elastomer measurement in each patient.

Figure 6. Scanning electron microscopy of grafts from one single donor. The circles show platelet adhesion and aggregation on the grafts after 6 minutes of perfusion with non-anticoagulated blood. On the left, the ePTFE specimen, on the right, the elastomer specimen. Pictures are representative for all healthy volunteers.
**Limitations of the study**

To measure the thrombogenicity of the elastomer, a previously validated human *ex vivo* set-up was used [13]. The main advantage of this *ex vivo* set-up is that the blood of the volunteer does not come in contact with surfaces other than the materials investigated. Furthermore, in this set-up, every volunteer was his own control. However, we appreciate the fact that every *ex vivo* experiment is a simplification of the complex *in vivo* situation. The small diameter (3 mm) of the grafts we used is not comparable to the diameter of the lower abdominal aorta (±19 mm). However, increased thrombogenicity and vessel occlusion is especially a problem occurring in small-diameter vessels as shear stress increases and therefore platelet activation and deposition increase. When a small, 3-mm-diameter graft shows only moderate platelet aggregation or fibrin depositions, it is likely that a wider lumen (e.g., 10—20 mm) will not show increased thrombogenicity.

Another difference with the *in vivo* situation was the average age (24.3 years) of the test subjects, while the average age of an aneurysm patient is higher (average age 60—80 years). The use of this young age group creates an unreal scenario, with regard to the thrombogenicity in the targeted patient group. A young age group was however chosen to be sure that the subjects were free of coagulopathic diseases and were not on medications that possess thrombogenic properties. For that same reason, male subjects were chosen, as they certainly do not take oral contraceptives. The high thrombogenicity in this group of young, healthy volunteers would be a major drawback of the new technique and would directly limit the use of the elastomer *in vivo*. Furthermore, the use of this age group as test subjects is a validated method in comparable thrombogenicity studies [13]. It would certainly be interesting for future experiments to repeat the experiments in a more senior age group.

A potential shortcoming of the set-up might be the hypothetical activation of the coagulation cascade when the first run of sample collection takes place. This might influence the measurements during the second run. In this study and in the earlier study with the same *ex vivo* model, no difference was shown in baseline FPA levels in both runs, indicating that systemic coagulation activation by the procedure is unlikely [13]. In addition, the randomization of the grafts used in the first and second run should have corrected for this potential bias.

**Customized Aortic Repair**

CAR has been developed to overcome the shortcomings of EVAR, as stated above. In an *in vitro* set-up, it has proven to significantly reduce aneurysmal wall stress [6]. When endovascular balloons become available in different forms and configurations, in theory, any aneurysm with a deviant anatomy will be treatable with endovascular techniques using elastomer sac filling.
Although new EVAR grafts (e.g., the Endurant and the Aorfix) have been launched, which prove the ability to treat severe angulations [14, 15], with many of the current EVAR techniques, severe angulations may still lead to kinking of the graft material and eventually to migration of the graft [16-18]. As no additional supporting graft material is used, these problems are not likely to occur with CAR. The fluidity of the non-polymerized elastomer inherently causes adjustment to the geometry of the aneurysm, not only by filling the large cavity of the aneurysm sac but also by diffusing into all irregularities and side holes. The elastomer mould will attach itself as it customizes itself to the form of the abdominal aorta aneurysm (AAA) sac.

Angulation and occlusive disease of the iliac arteries are an important exclusion criterion for EVAR, and tortuosity may form a serious obstacle for a successful EVAR procedure. A minimal diameter of 12—22 Fr is often needed to access the bulky and rigid delivery sheath. To fill the sac with the biocompatible elastomer, a catheter of only 7 Fr is needed. Preliminary in vivo porcine experiments have shown that this concept is technically feasible [7].

Besides the treatment of AAAs, CAR may prove a possible treatment modality for thoracic-aorta aneurysms, peripheral aneurysms and iliac aneurysms. With the current EVAR techniques, iliac aneurysms can sometimes be difficult to treat due to short distal sealing areas, leading to coiling and overstenting of the internal iliac artery. With the CAR concept, there is no necessity for a long landing area, and this may prevent the necessity to overstent the iliac artery, thereby preventing the introduction of buttock claudication and ischemia.

Beside the stand-alone treatment concept, the elastomer injection technique is more broadly applicable. The elastomer can already be used as an adjuvant with current stent grafts when problems of endoleak or migration occur. In these cases, the elastomer can be used to fill up the aneurysm sac and secure the endovascular stent graft [11, 19].

The CAR concept is not the only aneurysm treatment concept that addresses aneurysm cavity filling. Early in vivo human results with the Nellix have been recently published and show promising results [20]. The Nellix concept uses ‘bag-filling’ to fixate its 20 Fr grafts with endo-bags in the aneurysm cavity. Although it may seem very similar, it is a different concept than the concept presented in the current manuscript, as it always needs a bulky graft for aneurysm exclusion. Furthermore, the polymer is injected in a closed endo-bag instead of ‘free-range’, thereby limiting its ‘customizing’ capabilities. Nevertheless, the Nellix graft has very promising results and may prove to be an excellent addition to the abdominal aneurysm treatment modalities.

**The elastomer**

The elastomer used in this study was designed for aneurysm sac filling in CAR [6-8, 11]. It consists of PDMS that has a widespread use in multiple in vivo applications. PDMS
is a biocompatible elastomer, and grafts coated with PDMS have shown less graft stenosis compared with other grafts [12, 21-25]. The current formula is non-toxic and the product cross-links isothermally in the presence of blood, without the release of toxic by-products. Viscosity of the compound allowed infusion rates of up to 2 ml s\(^{-1}\) using a standard angiographic pump with an injection pressure up to 1200 pounds per square inch. The substance has an average polymerization time of approximately 5 min. After curing, the material had a yield stress of approximately 400 kiloPascal (kPa), failing at more than 20% elongation. The density of the cured elastomer is 1.0167 g crrT\(^3\). More details about the development of the current elastomer can be found in an earlier publication [7].

Clinical relevance

CAR may prove to be an exciting new endovascular treatment modality to exclude different types of aneurysms. As mentioned above, the concept of aneurysm sac filling is feasible in several \textit{in vitro} and \textit{in vivo} set-ups [6, 7, 11, 19]. Before clinical applications can take place, the biocompatibility, biostability and the thrombogenicity have to be indisputable. The current study showed that the elastomer has, according to FPA- and P-selectin measurements (Figs. 3 and 4), a thrombogenicity comparable to ePTFE, the preferred material in synthetic (endo-)vascular grafts. The elastomer tubes seem to be superior to the ePTFE tubes with regard to adhesion of platelet aggregates (Fig. 6).

CONCLUSIONS

In conclusion, the elastomer in its current formulation has a low thrombogenicity, comparable to the thrombogenicity of ePTFE, in an \textit{ex vivo} human model. This property makes it an ideal substance for endovascular aneurysm sac filling. Further research should clarify the thrombogenicity of the elastomer \textit{in vivo}, as well as the feasibility of the novel treatment concept ‘Customized Aortic Repair’ \textit{in vivo}. 
REFERENCES

8. de Vries, A.C., Willemstad, NL patent 20040209998. Composition for in vivo vessel repair.