Published in final edited form as:

J Biomech Eng. 2010 January ; 132(1): 011008. doi:10.1115/1.4000156.

Engineering Silicone Rubbers for *In vitro* Studies: Creating AAA Models and ILT Analogues with Physiological Properties

T.J. Corbett, B.J. Doyle, A. Callanan, M.T. Walsh, and T.M. McGloughlin¹

Centre for Applied Biomedical Engineering Research (CABER), MSSi, Department of Mechanical and Aeronautical Engineering, University of Limerick, Limerick, Ireland.

Abstract

Background—*In vitro* studies of abdominal aortic aneurysm (AAA) have been widely reported. Frequently mock artery models with intraluminal thrombus (ILT) analogues are used to mimic the AAA *in vivo*. While the models used may be physiological, their properties are frequently either not reported or investigated.

Method of Approach—This study is concerned with the testing and characterisation of previously used vessel analogue materials and the development of new materials for the manufacture of AAA models. These materials were used in conjunction with a previously validated injection moulding technique to manufacture AAA models of ideal geometry. To determine the model properties (stiffness (β) and compliance) the diameter change of each AAA model was investigated under incrementally increasing internal pressures and compared to published *in vivo* studies to determine if the models behaved physiologically. A FEA study was implemented to determine if the pressure – diameter change behaviour of the models could be predicted numerically. ILT analogues were also manufactured and characterised. Ideal models were manufactured with ILT analogue internal to the aneurysm region and the effect of the ILT analogue on the model compliance and stiffness was investigated.

Results—The wall materials had similar properties to aortic tissue at physiological pressures (E_{init} 2.22MPa and 1.57MPa (aortic tissue: 1.8MPa)). ILT analogues had similar Young's modulus to the medial layer of ILT (0.24 and 0.33MPa (ILT: 0.28MPa)). All models had aneurysm sac compliance in the physiological range ($2.62 - 8.01 \times 10^{-4}$ /mmHg (AAA *in vivo*: $1.8 - 9.4 \times 10^{-4}$ /mmHg)). The necks of our AAA models had similar stiffness to healthy aortas (20.44 – 29.83 (healthy aortas *in vivo*: 17.5 ± 5.5)). Good agreement was seen between the diameter changes due to pressurisation in the experimental and FEA wall models with a maximum error of 7.3% at 120mmHg. It was also determined that the inclusion of ILT analogue in the sac of our models could have an effect on the compliance of the model neck.

Conclusions—Ideal AAA models with physiological properties were manufactured. The behaviour of these models due to pressurisation was predicted using FEA, validating this technique for the future design of realistic, physiological AAA models. Addition of ILT analogues in the aneurysm sac was shown to affect neck behaviour. This could have implications for endovascular AAA repair due to the importance of the neck for stent-graft fixation.

Keywords

Abdominal aortic aneurysm; mock artery models; realistic properties

¹Corresponding author: Address: Professor Tim McGloughlin, MSG-014, MSSi Building, University of Limerick, Limerick, Ireland, tim.mcgoughlin@ul.ie.

INTRODUCTION

Abdominal aortic aneurysm (AAA) is defined as an abnormal localised dilation or bulge in the infrarenal aorta greater than 50% of its normal diameter [1]. It affects up to 5% of the population over the age of 55 years. Rupture of an AAA is responsible for 1.3% of deaths in males aged 65–85 years, accounting for 10,000 deaths annually in the United Kingdom [1–3]. Survival of ruptured abdominal aortic aneurysm (rAAA) may be as low as 10–20%.

Many investigators have undertaken *in vivo* studies into specific aspects of preoperative and post operative AAA's using silicone rubber or latex AAA models [4–13]. There are a number of important considerations when attempting to develop AAA models with physiological properties. *In vivo* the arteries are in a state of constant longitudinal tension [14]. As this is difficult to model *in vitro* it is usually not considered [4–12]. The abdominal aorta is surrounded by visceral organs, abdominal muscle and the spine which undoubtedly influence its deformation under pressure [15]. Because of these parameters creating materials with a similar stress-strain response to aortic tissue may not lead to models that behave similar to the vessel itself. Because of this, the compliance (or stiffness) of arteries has become the attribute which researchers attempt to mimic [10,16]. The manufacturing methods for creating both idealised and realistic AAA models have been previously reported [10,16–21]. O'Brien et al. [19] and Doyle et al. [20] have verified a lost wax manufacturing technique and reported that the variation in wall thickness for large vessel models manufactured using this technique was between 4–11%. We have previously described a rupture study in ideal AAA models and derived the material constants and mechanical properties of the materials used to construct these models [22]. The ultimate tensile strength of the material and the location of rupture of the models were the primary considerations in that study and as such we did not examine the compliance of the models themselves. Other studies have investigated model compliance but have not characterised the materials used to construct the models [10,16]. Those studies were based on AAA models created by brushing latex onto either a glass or plastic former. However, this manufacturing method leads to an uneven wall thickness which could affect observed compliance [23]. Here we characterise our materials using strain energy functions (SEF) and perform and validate a finite element analysis (FEA) investigation of our models so these materials may be used in the design realistic AAA models in future *in vitro* studies.

Some 75% of AAAs have been shown to have Intraluminal Thrombus (ILT) present in the aneurysm sac [24]. While many thrombus analogues ranging from gelatin to dough have been used in *in vitro* studies, a repeatable manufacturing method for creating physiological AAA models with integrated physiological thrombus analogues has not been fully described [11, 12,25]. Here we describe an injected silicone rubber based AAA model of ideal geometry with realistic vessel stiffness with a built in silicone rubber ILT analogue with realistic Young's modulus. The effect of changing the mechanical properties of the silicone rubber wall and the ILT analogue on the observed model stiffness and compliance is also investigated.

MATERIALS AND METHODS

Materials Selection and Characterisation

Silicone rubbers have been widely used as aorta analogues in *in vitro* studies [4–7,9,10,19, 20,22]. Wacker Elastosil RT601, (Wacker-Chemie GMBH, Munich, Germany), was used by Doyle et al. [20] in a previous study to manufacture AAA models. This silicone rubber was employed as the base material for this study. Different quantities of Dow Corning 200/5CS silicone fluid (Midland, MI, USA), were added to this material to alter its material properties.

Ten Type 2 dumb bell samples of two different wall materials, 100%RT601 (hereafter “W1”) and 90%RT601 (hereafter “W2”) by mass, were created by injection moulding and tested in

accordance with BS ISO 37 on a uniaxial extensometer with a 1kN loadcell (Tinius Olsen, Surrey, UK). To ensure repeatable results and to minimise the Mullins effect the specimens were preconditioned by deforming the gauge length by 40% ten times before carrying out an actual test. This preconditioning serves to stabilise the stress-strain response of the material [26]. A video extensometer (MESSPHYSIK, Fürstenfeld, Austria) was used to measure the deformation of the specimen gauge length of the material during the test.

Material characterisation was carried out using previously described methods [22]. Briefly, the force extension data from the uniaxial tensile tests were converted to engineering stress and strain. Best fit polynomial trendlines were then fitted to the data for each material. Using these polynomials, twenty representative engineering stress-strain datapoints were calculated for engineering strains from 0–1 for each material and used to define the material in the finite element solver ABAQUS 6.7-1 (Dassault Systems, SIMULA, Providence, RI, USA). The uniaxial test was modelled in FEA with identical geometry to the test specimens. Boundary conditions mimicking the uniaxial test were used. Different strain energy functions were examined, and the most suitable strain energy function selected. To ensure that the optimum strain energy function had been selected, a comparison was made between experimentally determined true stress and strain and the true stress and strain predicted by the finite element analysis of the test specimen.

Silicone Rubber AAA model manufacture

Six idealised wall (AAA_w) models (Fig. 1.) were manufactured for this study (three manufactured from W1 and three from W2). The model dimensions were taken from the EUROSTAR database [27] and are an averaged set of dimensions based on a 3413 AAAs. This idealised AAA geometry has been used extensively in previous publications [19,22,28–30]. The lumen diameters of the ideal geometry are: neck: 24mm, aneurysm: 50mm legs: 12mm, the ideal AAA geometry has a bifurcation angle of 60° and wall thickness of 2mm.

The models were manufactured using the previously reported lost wax technique [19,20]. Briefly, a wax lumen was cast from an aluminium mould. This was placed within a wall mould with a 2mm cavity into which the silicone rubber wall material was injected. The wall material was allowed to cure at 40°C for 4 hours. After curing the wax was melted out of the wall model. The models were then cleaned to remove any remaining wax. The full method is outlined in appendix A.

Compliance Testing

Each model was placed in the pressurised air system shown in Fig. 2. A precision regulator (Norgren, Warwickshire, UK) was used to adjust the pressure inside the model. A digital manometer (Druck, Leicester, UK) was used to measure the pressure in the system. The models were pressurised from 20–160mmHg in steps of 20mmHg. A video extensometer (MESSPHYSIK, Fürstenfeld, Austria) was used to measure the change in diameter at each pressure increment.

The models were held at each pressure for 30 seconds before a diameter measurement was taken. The first location was in the proximal neck of the model, 30mm above the beginning of the aneurysm section and the second location was the area of maximum diameter of the aneurysm (Fig. 1.). The model response to pressurisation was investigated in two planes to allow for the mean compliance to be determined for each model, therefore minimising the effect of any variations in wall thickness on the results. These locations were chosen to determine the compliance not just of the aneurysm sac but also of the proximal neck of the model. The biomechanics in the proximal neck of abdominal aortic aneurysms are of interest as endovascular stent-grafts rely on the proximal neck for fixation within the vessel [31].

Therefore the behaviour in the proximal neck is directly related to the success or failure of EVAR. When the pressure diameter data was collected for all the models compliance was computed using Eq. 1. [32].

$$\text{Compliance} = \frac{(\Delta A)}{A_{\max}(\Delta P)} \quad (1)$$

Stiffness was then computed using Eq. 2. [33].

$$\text{Stiffness } \beta = \ln \left(\frac{P_{\max}}{P_{\min}} \right) \frac{D_{\min}}{\Delta D} \quad (2)$$

Where D is the outer diameter of the vessel, A is the cross sectional area of the vessel and P is the pressure within the vessel.

Finite Element Analysis

The FEA model was constructed in the nominal geometry of the experimental models. As these idealised models are symmetric about two planes a quarter model was sufficient to run the FEA study (Fig. 3.). Coefficients from the strain energy functions determined in the material characterisation study were used to define the material properties for the analysis. To simulate the connection of the AAA segment to the descending aorta and the iliac bifurcation, the model was constrained in all directions on the proximal and distal surfaces. A symmetry boundary condition was placed on surfaces A and B in Fig. 3. with another symmetry boundary condition placed on surface C. A uniform pressure was applied to the inside surfaces of the model and separate analyses were run for the pressures used in the experimental study. The model was meshed using C3D10MH ten node modified quadratic tetrahedron hybrid elements [34]. Mesh independence was performed by increasing the number of elements in the mesh until the difference in peak Von Mises stress was <2% of the result computed using the previous mesh [34].

Experimental Modelling of AAAs with ILT

In order to independently examine the effect of varying wall properties and varying ILT analogue properties on observed model compliance, two possible ILT analogues, 45%RT601 (hereafter “ILT1”) and 50%RT601 (hereafter “ILT2”) by mass, were also created and characterised as previously described. AAA models with ILT analogue (AAA_{ILT}) were manufactured which utilised these materials in the aneurysm sac. These models had lumen geometry identical to AAA_W models except in the aneurysm region where the lumen diameter was 38mm; the remaining volume consisted of the ILT analogue (Fig 4).

To manufacture the AAA_{ILT} models, 2 extra aluminium moulds were needed in addition to the two required for manufacturing the AAA_W models. The first of these was to manufacture the wax lumen which had a smaller diameter in the sac than the AAA_W model. The second was identical in geometry to the lumen mould for the AAA_W model. The AAA_{ILT} lumen was placed in this mould leaving a cavity in the aneurysm sac region. This mould had an inlet and vent in the aneurysm region to allow the ILT analogue to be injected easily into the mould. When the ILT analogue was cured the lumen + ILT analogue were placed inside the wall mould and the wall material injected as previously described. The full method is outlined in the appendix A.

Nine AAA_{ILT} models were manufactured and compliance tested for this study. Six were manufactured from W2, three of these had ILT1 analogue and the remaining three had ILT2

analogue in the sacs. The final three models were manufactured from W1 with ILT1 analogue in the sacs. The model specifics of all models created are summarised in table 1.

Dimensional Accuracy of AAA Models

While O' Brien et al. [19] and Doyle et al. [20] confirmed that the injection method produced models with acceptable variation in wall thickness the overall dimensional accuracy of the models was not investigated in these studies. To determine if the models were dimensionally accurate we measured the diameter of the models in the two locations of interest (Fig. 1.) and compared them to the nominal dimensions. In both these locations we measured the outside diameter of the model from the front and from the side.

RESULTS

The force extension data from the uniaxial tensile tests was converted to true stress (σ) and true strain (ε) using theory for large deformation of incompressible materials (Eq. 3. & Eq. 4.).

$$\sigma = \frac{f(1+\varepsilon)}{a_o} \quad (3)$$

$$\varepsilon = \ln \left(\left(\frac{\Delta l}{l_o} \right) + 1 \right) \quad (4)$$

Where f is force, a_o is undeformed cross sectional area of the test length of the dumb-bell test pieces and l is length.

The initial Young's modulus (E_{init}) [35] of the wall materials was calculated by fitting a best fit line to the linear portion of the stress-strain curves. It was determined that for the wall materials a sixth order reduced polynomial strain energy function was most suitable while for the ILT analogues a second order Ogden strain energy function was most appropriate. Due to the incompressible nature of these hyperelastic materials the reduced polynomial and Ogden strain energy functions take the form:

$$\text{Reduced Polynomial:} \quad W = \sum_{i=1}^N C_{i0} (\bar{I}_1 - 3)^i \quad (5)$$

$$\text{Ogden:} \quad W = \sum_{i=1}^N \frac{2\mu_i}{\alpha_i^2} \left(\bar{\lambda}_1^{\alpha_i} + \bar{\lambda}_2^{\alpha_i} + \bar{\lambda}_3^{\alpha_i} - 3 \right) \quad (6)$$

Where $\bar{I}$ is a unit matrix, $\lambda_1, \lambda_2, \lambda_3$ are the principle stretch ratios, C, μ and α are material constants.

Figure 5. shows a comparison of the stress-strain response of each material created. The wall analogue materials (W1 & W2) are seen to be much stiffer than the ILT analogues (ILT1 & ILT2). Figure 5 also shows the good agreement in true stress and true strain observed using the described numerical and experimental approaches. The initial Young's modulus and coefficients for the respective strain energy functions for each material are shown in Table 2.

Figure 6. shows a comparison of the experimental and numerical pressure-diameter changes in the AAA_W models. The maximum percentage error between the experimental and numerical model at the maximum pressure (160mmHg) was seen in the aneurysm region of the W2 models (10.66%) while at a pressure of 120mmHg the maximum percentage error was found in the neck of the W2 models (7.3%).

Figure 7. shows the experimental pressure diameter change curves for all the models in the aneurysm and neck regions. Using these curves and Eq. 1. & Eq.2. the mean stiffness and compliance of each set of models was computed for pressures of 120/80mmHg. These are shown in Fig. 8. and tabulated in Table 3 which also shows the range of stiffness and compliance calculated for each model set.

To determine if there was a significant difference in the observed compliance between different model sets, an independent samples T test was performed in the software SPSS 15.0 for windows (SPSS Inc. Chicago, IL, USA). The results of this statistical analysis are shown in table 4.

The mean and range of diameters of each model set measured in the locations shown in figure 3 is shown in table 5.

DISCUSSION

Many previous reports describe *in vitro* studies of pre and post operative abdominal aortic aneurysms [4–13]. The most important component in any *in vitro* study should be the AAA model itself. These models should be reproducible, have consistent material properties, consistent thickness and be physiological in behaviour. It has been proven that the injection method creates reproducible models with relatively consistent thickness [19,20]. However an investigation has not been undertaken into the properties of the materials used to create these models or the properties of the models themselves.

To address this we employed a previously used silicone rubber and performed mechanical testing. We also manufactured new wall and ILT analogue materials and performed similar tests. Previous studies have shown that the initial Young's Modulus for abdominal aortic aneurysm tissue lies in the range of 0.42 – 0.56MPa and that AAA tissue may be stiffer than healthy aorta [35,36]. Both of our wall materials have initial Young's modulus outside this range (2.22MPa (W1) & 1.57MPa (W2)).

However Vallabhaneni et al. [37] reported that AAA tissue has a mean Young's modulus of 1.8MPa (0.16–4.52MPa) at stresses experienced *in vivo*. It is evident from the FE analysis that at physiological pressures the walls of the AAA models are in the linear phase of the silicone rubber stress-strain behaviour and are comparable to this published data.

ILT has been found experimentally to be a material consisting of three separate layers (luminal, medial and abluminal). The properties of these layers were investigated by Wang et al. [38] They found that the Young's moduli for the luminal and medial layers was 0.54 MPa and 0.28MPa. The abluminal layer was so degraded that they could not perform mechanical tests on the material [38]. In this study we used single layer wall models due to manufacturing constraints. We also considered the ILT as a single layer due to these constraints. This approach is consistent with previously published experimental modelling of AAA [11,12,19,20]. From the uniaxial tensile testing we determined that our ILT analogues had Young's modulus of 0.24Mpa (ILT1) and 0.33MPa (ILT2) which is similar to values for the medial layer found by Wang et al. [38].

The materials were characterised using previously reported techniques to obtain material coefficients that describe the stress-strain response of each particular material. A good comparison was seen in the pressure - diameter change behaviour between the experimental and FEA AAA_W models with a maximum difference in diameter change of <11%. This variance was less significant at lower pressures with a maximum error between the FEA and experimental models of 7.3%. This validation of our FEA model and material properties could allow for improved design of patient specific AAA models which are usually given an arbitrary wall thickness [19,20]. Using characterised materials, AAA models could be carefully designed utilising the finite element method to include varying local compliance by varying local wall thickness. This variation in stiffness could be investigated clinically by utilising improved imaging techniques.

Mock artery models have also been used to predict rupture location and to identify the areas of highest stress on the AAA wall [22,28]. The existing methodology for creating patient specific or realistic AAA models from CT images does not allow for the fact that the models are at physiological pressures during imaging. The model is usually designed with the dimensions straight from the CT image and therefore the resulting silicone model has pressurised dimensions when there is no pressure within the model. Therefore when such a model is pressurised it may not be completely realistic and this may inaccurately predict rupture location or deformation behaviour of the aneurysm. With the assistance of a verified FEA model and manufacturing technique it is possible to alter the design of the AAA model during the design stage so that it will have realistic diameters when pressurised.

We examined the stiffness in two locations in our models for physiological pressures and compared them to values from literature for *in vivo* studies of actual abdominal aortas and aneurysms. Sonnesson et al. [33] reported that the stiffness of the aorta in healthy, non-smoking individuals of age ~69 years was 17.5 ± 5.5 . While the stiffness values for all our W1 models are outside this range, the neck region of all the W2 models and the aneurysm region of the W2 models without thrombus are within this range. Even though the W1 models have stiffness outside the range determined by Sonnesson et al. [33] it is possible that they may be still physiological. This is because frequently the necks of AAA are often heavily calcified and therefore the neck is stiffer than healthy aorta [39].

Vorp et al. [32] reported that the compliance of AAAs with ILT of similar sized to our models was $1.8 - 9.4 \times 10^{-4} / \text{mmHg}$ (mean $4 \times 10^{-4} / \text{mmHg}$). All our models had aneurysm compliance within this range. However in the 8 patients that Vorp et al. [32] studied there were two distinct groups. Six of the patients had aneurysms that had compliances between 1.8 and $3.6 \times 10^{-4} / \text{mmHg}$ while the remaining two patients had aneurysm compliances of 6 and $9.4 \times 10^{-4} / \text{mmHg}$. All our models are within the former range having compliances between $2.62 - 3.5 \times 10^{-4} / \text{mmHg}$. These values also are comparatively close to the mean of the range outlined by Vorp et al. [32]. The models without ILT analogue are within the latter range with compliances of 6.12 and $8.01 \times 10^{-4} / \text{mmHg}$.

In our models there was a significant difference between compliance in the aneurysm region in models with and without ILT analogue, which is to be expected. However the presence of the ILT analogue also had an effect on the stiffness of the neck. There was a significant difference between the compliance of the neck of W1 (AAA_W) and W1/ILT1 (AAA_{ILT}) models and between the neck of W2 (AAA_W) and W2/ILT2 (AAA_{ILT}) models. There was not a significant difference between the neck compliance of W2 (AAA_W) and W2/ILT1 (AAA_{ILT}) models. It is unclear whether ILT offers support to the proximal neck in AAAs *in vivo* as the authors are unaware of any study which investigates this hypothesis. It may be an important consideration because endovascular grafts often rely on the ability of the proximal neck to withstand excessive deformation under the radial force of a stent to provide fixation [31]. It

has already been shown that ILT present in an AAA reduces the risk of graft migration by acting normal to the drag force of the graft [40]. If the ILT provides structural support to the proximal neck it could also be a positive factor in the prevention of stent-graft migration.

From the pressure - diameter change results it is clear that model geometry has a pronounced effect on the deformation characteristics of the model. The pressure - diameter change behaviour of the necks is almost linear while there is a nonlinear pressure diameter change relationship in the aneurysm region, owing to the fact that the area around the inflection points in the aneurysm region undergoing greater strain than the area at the max diameter [28]. This has the effect of opening the radius of curvature of the aneurysm region at low pressures, preventing a noticeable diameter change. In the aneurysm region, the models without ILT analogue also seem to initially contract when viewed from the front. This is not apparent when the models are viewed from the side. A possible reason for this is the effect of the geometry of the bifurcation and the effect of changes in geometry here during pressurisation of the model. This serves to reinforce the point that care should be taken when attempting to ascertain mechanical properties of tissue based on imaging techniques alone without attempting to determine the effect of shape on the deformation behaviour of the vessel in question.

The neck diameters of all models were acceptably close to the nominal dimension of 28mm with a mean neck diameter of 27.85mm and a maximum variation in diameter of 2.14%. Likewise, the aneurysm sections of the AAA_w models were acceptably close to the nominal dimension of 54 mm with a mean diameter of 54.18mm and a maximum variation of 1.48%. The introduction of the ILT analogue did appear to have an effect on the dimensional accuracy of the models. The mean diameter in the aneurysm region was 55.39mm and the maximum variation in diameter was 5.56%. The primary limitation of this study is the idealisation of considering the ILT and the artery wall as single layers. While this is a gross assumption, it is less significant than using ILT analogues without investigating their mechanical properties, as previously reported [11,12,41]. We believe that our simplified one layer ILT analogue is more representative than materials such as dough or gelatin. The materials were evaluated in ideal AAA geometries. It is possible that in realistic geometries there may be a difference in observed stiffness of the models due to shape effects. However it is probable that these differences would not be significant and therefore the silicone rubber models would remain physiological in their behaviour. The use of our AAA wall and ILT analogues will be examined in realistic AAA geometries in future studies in our group. Furthermore this manufacturing approach can be extended to model other arterial structures such as plaque laden carotid arteries [42].

CONCLUSION

Silicone Rubber based models of ideal AAA geometries were manufactured with physiological characteristics. New artery and ILT analogue materials were developed and characterised. A methodology has been developed for creating models with different properties from one set of moulds. Manufactured models without ILT analogue were dimensionally accurate to 2.14%. Introduction of ILT analogue affected the dimensional accuracy of the aneurysm region of the models. We have validated a finite element analysis of our wall models which will allow for careful design of realistic AAA geometries in the future.

Acknowledgments

The authors would like to thank (i) The Irish Research Council for Science Engineering and Technology (IRCSET) Grant# RS/2005/27 (T. Corbett) and Grant # RS/2005/340 (B. Doyle) and NIH Grant # R01-HL-060670 from the United States National Heart Lung and Blood Institute (ii) Liam Morris (Galway and Mayo Institute of Technology) for initial model development, (iii) Stephen Broderick (CABER) for assistance with image rendering and diagrams.

APPENDIX A – Detailed Procedure for AAA Model Manufacture

The models were manufactured as follows:

1. All mould halves were cleaned thoroughly using methanol.
2. Silicone release agent (Ambersil formula 6) was sprayed onto the surfaces of all moulds.
3. The lumen mould halves were bolted together tightly, placed in an oven and heated to 55°C
4. A casting wax (Castylene B581, Remet Corporation, The Netherlands) was melted on a hotplate to a temperature of ~ 150°C
5. The Lumen Moulds were tilted at 45 degrees to horizontal and the molten wax poured into the models. Tilting of the moulds helped to minimise splashing and prevented air being trapped inside the casting. The mould was returned to a vertical position to allow for complete filling of the mould.
6. When the mould was full it was tapped with a mallet for several minutes to allow for any air bubbles in the molten wax to rise out of the mould.
7. As the wax cooled and solidified the mould was topped up with molten wax to prevent the casting drastically losing dimensional accuracy.
8. When the wax was fully solidified (after cooling for ~2 hours) the mould halves were unbolted and the wax model carefully removed. Any flash as a result of the casting process was shaved off with a sharp knife.

Follow steps 9 – 17 for manufacturing AAA models with ILT: For models without ILT proceed to step 18:

9. The ILT lumen casting was placed in the ILT mould and placed in an oven at 45°C. The ILT mould was rotated one quarter turn every ten minutes to ensure that the wax did not sag inside the mould.
10. The components of the thrombus analogue were placed in a plastic beaker in the following order: 1: Wacker Elastosil RT601 Part A, 2: Wacker Elastosil RT601 Part B, 3: Dow Corning 200/5CS Silicone Fluid. The components were mixed with a glass rod for two minutes.
11. The lid was then placed on the beaker and the mixture shaken vigorously for a further 60 seconds. The beaker was then placed in an oven at 45°C.
12. To ensure that no separation of the mixture occurred, the mixture was mixed with a glass rod for one minute and shaken vigorously 5 times at 10 minute intervals.
13. The liquid ILT mixture was drawn into a syringe and injected into the mould cavity. An M8 bolt was inserted into the inlet port of the mould and an M3 bolt was inserted into the vent of the mould to prevent leakage of the ILT mixture.
14. The mould was rotated one quarter turn every 10 minutes to prevent separation of the ILT mixture and to prevent sagging of the ILT Lumen casting.
15. Once the mould had been at 45°C for 40 minutes it was removed from the oven, allowed to cool and opened. The ILT lumen and ILT analogue (henceforth ‘the lumen’) were carefully removed from the mould.
16. The wall mould was cleaned thoroughly using methanol

17. The lumen was placed inside the wall mould, the mould halves were bolted tightly together.
18. The wall material was prepared in a beaker with the components of the wall mixture added in the same order as before and stirred with a glass rod for two minutes.
19. As the liquid wall material was far more viscous than the ILT material the beaker was placed in a freezer at -20°C for 4 hours to stop the curing process and allow bubbles due to mixing to rise out of the mixture.
20. The wall material was then drawn into a 60mm catheter tipped syringe and injected into the wall mould. For this step the mould was injected from the bottom up with the inlet at the aorta end of the AAA model and the vents at the iliac legs.
21. The inlet port was again sealed with an M8 bolt.
22. The wall model was placed in a freezer for 4 hours to allow any bubbles due to injecting to rise to the ends of the iliac legs, away from the regions of interest.
23. The mould was then transferred to an oven at 40°C and the silicone rubber was allowed to cure for 5 hours.
24. The mould was removed from the freezer, opened and the wax lumen with silicone wall removed.
25. The model was placed in an oven at 100°C to melt out the wax lumen leaving the silicone rubber model behind.

REFERENCES

1. Sakalihasan N, Limet R, Defawe OD. Abdominal Aortic Aneurysm. *Lancet* 2005;365(9470):1577–1589. [PubMed: 15866312]
2. Li Z, Kleinstreuer C. Fluid-Structure Interaction Effects on Sac-Blood Pressure and Wall Stress in a Stented Aneurysm. *Journal of Biomechanical Engineering* 2005;127(4):662–671. [PubMed: 16121537]
3. Office of Population Censuses and Surveys. Mortality Statistics, England and Wales. London HMSO; 1989.
4. Chong CK, How TV, Black RA, Shortland AP, Harris PL. Development of a Simulator for Endovascular Repair of Abdominal Aortic Aneurysms. *Annals of Biomedical Engineering* 1998;26(5):798–802. [PubMed: 9779952]
5. Chong CK, How TV, Gilling-Smith GL, Harris PL. Modelling Endoleaks and Collateral Reperfusion Following Endovascular AAA Exclusion. *Journal of Endovascular Therapy* 2003;10(3):424–432. [PubMed: 12932151]
6. Chong CK, How TV. Flow Patterns in an Endovascular Stent Graft for Abdominal Aortic Aneurysm Repair. *Journal of Biomechanics* 2004;37(1):89–97. [PubMed: 14672572]
7. Chong CK, How TV, Harris PL. Flow Visualization in a Model of a Bifurcated Stent-Graft. *Journal of Endovascular Therapy* 2005;12(4):435–445. [PubMed: 16048375]
8. Metha M, Veith FJ, Ohki T, Lipsitz EC, Cayne NS, Darling RC. Significance of Endotension, Endoleak, and Aneurysm Pulsatility After Endovascular Repair. *Journal of Vascular Surgery* 2003;37(4):842–846. [PubMed: 12663987]
9. Gawenda M, Jaschke G, Winter S, Wassmer G, Brunkwall J. Endotension as a Result of Pressure Transmission Through the Graft Following Endovascular Aneurysm Repair – an *In Vitro* Study. *European Journal of Vascular and Endovascular Surgery* 2003;26(5):501–505. [PubMed: 14532877]
10. Gawenda M, Knez P, Winter S, Jaschke G, Wassmer G, Schmitz-Rixen T, Brunkwall J. Endotension is Influenced by Wall Compliance in a Latex Aneurysm Model. *European Journal of Vascular and Endovascular Surgery* 2004;27(1):45–50. [PubMed: 14652836]

11. Chaudhuri A, Ansdell LE, Grass AJ, Adiseshiah M. Intrasc Pressure Waveforms After Endovascular Aneurysm Repair (EVAR) are a Reliable Marker of Type I Endoleaks, but not Type II or Combined Types: An Experimental Study. *European Journal of Vascular and Endovascular Surgery* 2004;28(4):373–378. [PubMed: 15350558]
12. Chaudhuri A, Ansdell LE, Grass AJ, Adiseshiah M. Aneurysmal Hypertension and its Relationship to Sac Thrombus: a Semi-Qualitative Analysis by Experimental Fluid Mechanics. *European Journal of Vascular and Endovascular Surgery* 2004;27(3):305–310. [PubMed: 14760601]
13. Corbett TJ, Callanan A, Morris LG, Doyle BJ, Grace PA, Kavanagh EG, McGloughlin TM. A Review of The *In Vivo* and *In Vitro* Biomechanical Behaviour and Performance of Postoperative Abdominal Aortic Aneurysms and Implanted Stent-Grafts. *Journal of Endovascular therapy* 2008;15(4) 498-484.
14. Nichols, WW.; O' Rourke, MF. McDonald's Blood Flow in Arteries. London: Edward Arnold; 1998. Chap. 4; p. 73
15. Williams, PL.; Warwick, R.; Dyson, M.; Bannister, LH. Gray's Anatomy. New York: Churchill Livingstone; 1989. Chap. 6; p. 766-767.
16. Walker RD, Smith RE, Sherriff SB, Wood RF. Latex Vessels with Customized Compliance for use in Arterial Flow Models. *Physiological Measurement* 1999;20(3):277–286. [PubMed: 10475581]
17. Chong CK, Rowe CS, Sivanesan S, Rattray A, Black RA, Shortland AP, How TV. Computer Aided Design and Fabrication of Models for *In Vitro* Studies of Vascular Fluid Dynamics. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine* 1999;213(1):1–4.
18. Friedman MH, Kuban P, Schmalbrock K, Smith K, Altan T. Fabrication of Vascular Replicas from Magnetic Resonance Images. *Journal of Biomechanical Engineering* 1995;117(3):364–365. [PubMed: 8618391]
19. O' Brien T, Morris L, O' Donnell M, Walsh M, McGloughlin T. Injection-Moulded Models of Major and Minor Arteries: the Variability of Model Wall Thickness Owing to Casting Technique. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine* 2005;219(5):381–386.
20. Doyle BJ, Morris LG, Callanan A, Kelly P, Vorp DA, McGloughlin TM. 3D Reconstruction and Manufacture of Real Abdominal Aortic Aneurysms: from CT Scan to Silicone Model. *Journal of Biomechanical Engineering* 2008;130(3):034501-1–034501-5. [PubMed: 18532870]
21. Chaudhuri A, Ansdell LE, Richards R, Adiseshiah M, Grass AJ. Non-Axisymmetrical (Life-Like) Abdominal Aortic Aneurysm Models: A Do It Yourself Approach. *Journal of Endovascular Therapy* 2003;10(6):1097–1100. [PubMed: 14723579]
22. Doyle, BJ.; Callanan, A.; Corbett, TJ.; Cloonan, AJ.; O'Donnell, MR.; Vorp, DA.; McGloughlin, TM. The Use of Silicone Materials to Model Abdominal Aortic Aneurysm Behaviour. In: McNally, G.; Czuba, L., editors. *Proc. 1st European Conference on Medical Polymers*; Northern Ireland: Belfast; 2008. p. 115-119.
23. Morris, LG. Ph.D. Thesis. Limerick, Ireland: University of Limerick; 2004. Numerical and Experimental Investigation of Mechanical Factors in the Treatment of Abdominal Aortic Aneurysms.
24. Harter LP, Gross BH, Callen PW, Barth RA. Ultrasonic Evaluation of Abdominal Aortic Thrombus. *Journal of Ultrasound in Medicine* 1982;1(8):315–318. [PubMed: 7166768]
25. Timaran CH, Ohki T, Veith FJ, Lipsitz EC, Gargiulo NJ, Rhee SJ, Malas MB, Suggs WD, Pacanowski JP. Influence of Type II Endoleak Volume on Aneurysm Wall Pressure and Distribution in an Experimental Model. *Journal of Vascular Surgery* 2005;41(4):657–663. [PubMed: 15874931]
26. Mullins L. Softening of Rubber by Deformation. *Rubber Chemistry and Technology* 1969;42(1):339–362.
27. Laheij R, Van Marrewijk C, Buth J. Progress Report on the Procedural and Follow up Results of 3413 Patients who Received Stent-Graft Treatment for Infra-Renal Abdominal Aortic Aneurysms for a Period of 6 Years. EUROSTAR Data Registry Centre. 2001
28. Morris L, O'Donnell P, Delassus P, McGloughlin T. Experimental Assessment of Stress Patterns in Abdominal Aortic Aneurysms Using the Photoelastic Method. *Strain* 2004;40(4):165–172.
29. O' Brien T, Morris L, McGloughlin T. Evidence Suggests Rigid Aortic Grafts Increase Systolic Blood Pressure: Results of a Preliminary Study. *Medical Engineering and Physics* 2007;30(1):109–115. [PubMed: 17360221]

30. O' Brien, TP.; Walsh, MT.; Morris, LG.; Grace, PA.; Kavanagh, EG.; McGloughlin, TM. Biomechanical Systems Technology. Singapore: World Scientific Publishing Co; Numerical and Experimental Techniques for the Study of Biomechanics in the Arterial System Chap. 7; p. 233-270.
31. Chuter TAM. Stent-Graft Design: The Good, the Bad and the Ugly. Cardiovascular Surgery 2002;10(1):7-13. [PubMed: 11790568]
32. Vorp DA, Mandarino M, Webster MW, Gorcsan J. Potential Influence of Intraluminal Thrombus on Abdominal Aortic Aneurysm as Assessed by a New Non-Invasive Method. Cardiovascular Surgery 1996;4(6):732-739. [PubMed: 9013001]
33. Sonesson B, Lanne T, Vernersson E, Hansen F. Sex Difference in the Mechanical Properties of the Abdominal Aorta in Human Beings. Journal of Vascular Surgery 1994;20(6):959-969. [PubMed: 7990192]
34. Doyle BJ, Callanan A, McGloughlin TM. A Comparison of Modelling Techniques for Computing Wall Stress in Abdominal Aortic Aneurysms. Biomedical Engineering Online 2007;6:38. [PubMed: 17949494]
35. Raghavan ML, Webster MW, Vorp DA. Ex Vivo Biomechanical Behaviour of Aabdominal Aortic Aneurysm: Assessment Using a New Mathematical Model. Annals of Biomedical Engineering 1996;24(5):573-582. [PubMed: 8886238]
36. He CM, Roach MR. The Composition and Mechanical Properties of Abdominal Aortic Aneurysms. Journal of Vascular Surgery 1994;20(1):6-13. [PubMed: 8028090]
37. Vallabhaneni SR, Gilling-Smith GL, How TV, Carter SD, Brennan JA, Harris PL. Heterogeneity of Tensile Strength and Matrix Metalloproteinase Activity in the Wall of Abdominal Aortic Aneurysms. Journal of Endovascular Therapy 2004;11(4):494-502. [PubMed: 15298501]
38. Wang DHJ, Makaroun M, Webster MW, Vorp DA. Mechanical Properties and Microstructure of Intraluminal Thrombus from Abdominal Aortic Aneurysm. Journal of Biomechanical Engineering 2001;123(6):536-539. [PubMed: 11783723]
39. Fairman RM, Velazquez OC, Carpenter JP, Woo E, Baum RA, Golden MA, Kritpracha B, Criado F. Midterm Pivotal Trial Results of the Talent Low Profile System for Repair of Abdominal Aortic Aneurysm: Analysis of Complicated Versus Uncomplicated Aaortic Necks. Journal of Vascular Surgery 2004;40(6):1074-1082. [PubMed: 15622358]
40. Li Z, Kleinstreuer C. Analysis of Biomechanical Factors Affecting Stent-Graft Migration in an Abdominal Aortic Aneurysm Model. Journal of Biomechanics 2006;39(12):2264-2273. [PubMed: 16153654]
41. Hinnen JW, Rixen DJ, Koning OH, van Bockel JH, Hamming JF. Development of Fibrinous Thrombus Analogue for *In Vitro* Abdominal Aortic Aneurysm Studies. Journal of Biomechanics 2007;40(2):289-295. [PubMed: 16516895]
42. Finn, R.; McGloughlin, T.; Delassus, P.; Morris, L. An Experimental Evaluation of the Deformation During and Post Stenting on a Realistic Coronary Artery Phantom. In: McGloughlin, T.; Walsh, M., editors. Proc. 15th Annual Conference of the Section of Bioengineering of the Royal Academy of Medicine in Ireland; Ireland: Limerick; p. 10

Fig. 1.
AAA model viewed from front and side, arrows show where measurements were taken during compliance testing.

Fig. 2.
Pressure – diameter change measurement setup for investigating model compliance

Fig 3.
Quarter model used in finite element analysis

Fig 4. Manufacturing sequence for manufacturing AAAILT models, Left: wax lumen, middle: ILT analogue injected around wax lumen, right: wall material injected + allowed to cure before wax is melted out

Fig. 5.
Comparison of experimentally calculated and FEA true stress and strain in a dumb bell sample for wall and ILT materials

Fig. 6. Comparison of mean experimental and FEA predicted pressure – diameter change curves for wall models in the neck (left) and the aneurysm (right) regions

Fig. 7. Mean pressure – diameter change curves for each set of models in the neck (left) and aneurysm (right) regions ($n = 3$ for all cases)

Fig. 8.

Mean model stiffness in the neck (left) and aneurysm (right) for each model set ($n = 3$ for all cases)

Table 1

List of AAA models manufactured for compliance study

Wall	Wall only (AAA _w)	With ILT (AAA _{ILT})	
		ILT1	ILT2
W1	3 (Model set: W1)	3 (Model set: W1 + ILT1)	0
W2	3 (Model set: W2)	3 (Model set: W2 + ILT1)	3 (Model set: W2 + ILT2)

Table 2

Coefficients for strain energy functions and initial Young's modulus for each material

Material	E_{init} (MPa)	SEF Type	Coefficients for strain energy functions
W1	2.22	R. Poly (6 th order)	$C_{10} = 0.2259$, $C_{20} = 0.1821$, $C_{30} = -0.3302$, $C_{40} = 0.3071$, $C_{50} = -0.1178$, $C_{60} = 0.01788$
W2	1.57	R. Poly (6 th order)	$C_{10} = 0.2813$, $C_{20} = .2263$, $C_{30} = -0.4017$, $C_{40} = 0.3829$, $C_{50} = -0.0148$, $C_{60} = 0.0255$
ILT1	0.24	Ogden (2 nd order)	$\mu_1 = 6.49\text{e-}4$, $\mu_2 = 9.0562$, $\alpha_1 = 0.0622$, $\alpha_2 = 2.9419$
ILT2	0.33	Ogden (2 nd order)	$\mu_1 = 5.09\text{e-}4$, $\mu_2 = 7.3653$, $\alpha_1 = 0.0804$, $\alpha_2 = 1.8246$

Table 3

Mean stiffness and compliance for each model set at neck and aneurysm regions (range shown in parentheses)

Model Set	β_{neck}	β_{aneurysm}	C_{neck} ($\times 10^{-4}/\text{mmHg}$)	C_{aneurysm} ($\times 10^{-4}/\text{mmHg}$)
W1	25.39 (23.77 – 26.95)	32.38 (31.76 – 33.36)	7.79 (7.31 – 8.29)	6.12 (5.94 – 6.23)
W1 + ILT1	29.83 (29.12 – 30.23)	77.78 (77.17 – 78.74)	6.63 (6.53 – 6.79)	2.62 (2.58 – 2.63)
W2	20.44 (19.64 – 21)	23.26 (22.05 – 23.6)	9.61 (9.38 – 9.97)	8.01 (8.01 – 8.9)
W2 + ILT1	21.19 (20.78 – 21.53)	57.06 (56.49 – 57.57)	9.27 (9.14 – 9.45)	3.5 (3.47 – 3.54)
W2 + ILT2	22.88 (22.31 – 23.21)	64.15 (63.33 – 64.94)	8.62 (8.52 – 8.83)	3.19 (3.16 – 3.2)

Table 4

Statistical difference in stiffness and compliance between model sets

Yes = p < 0.05					
		W1 + ILT1	W2	W2 + ILT1	W2 + ILT2
	W1	Yes	Yes	Yes	No
Neck	W1 + ILT1	-	Yes	Yes	Yes
	W2		-	No	Yes
	W2 + ILT1			-	Yes
Sac	W1	Yes	Yes	Yes	Yes
	W1 + ILT1	-	Yes	Yes	Yes
	W2		-	Yes	Yes
	W2 + ILT1			-	Yes

Table 5

Mean diameter of neck and aneurysm region measured from front and side (range in parentheses)

All dimensions in mm				
Model Set	D _{neck} (front)	D _{neck} (side)	D _{aneurysm} (front)	D _{aneurysm} (side)
W1	27.98 (27.9 – 28.05)	28.23 (28.2 – 28.3)	54.48 (54.2 – 54.8)	54.72 (54.62 – 54.8)
W1 + ILT1	28.03 (28 – 28.1)	28.41 (28.3 – 28.5)	55.65 (55.15 – 56.5)	56.65 (56.4 – 57)
W2	27.66 (27.4 – 27.9)	27.9 (27.85 – 27.95)	53.68 (53.3 – 54.1)	53.85 (53.6 – 54.05)
W2 + ILT1	27.71 (27.65 – 27.8)	28.03 (27.75 – 28.2)	55.13 (54 – 56.2)	56.35 (55.5 – 56.85)
W2 + ILT2	27.65 (27.6 – 27.7)	27.95 (27.95 – 27.95)	53.61 (53.1 – 54)	54.94 (54.2 – 55.45)