# Manufacturing of an Artificial Artery Graft and In-Vitro Testing Model for Cardiovascular Disease (2019)

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**Introduction:** Typical cardiovascular disease (CVD) pathology can lead to coronary artery occlusion and as a consequence, cause serious health problems such as myocardial infarction (1). Late stage treatment of coronary artery occlusion involves coronary artery bypass graft (CABG) surgery, suturing a vessel around an arterial blockage to revascularize downstream tissues. CABG can prove successful, however, limited availability of autologous vessel grafts, and functional limitations of synthetic constructs, have initiated the development of tissue engineered vascular grafts (TEVGs) (2). TEVGs improve upon the biocompatibility of synthetic constructs, through the implementation of biological cells, whilst providing a more available alternative to autologous constructs. The production of tubular TEVGs currently comprises three main methods: "sheet rolling, tubular moulding, and direct scaffolding" (3). This study aims to develop a simple and accessible direct-scaffolding technique, employed by Tabriz, which involves 'dip-coating' a steel rod to produce a natural polymer TEVG (3, 4). The process comprises submerging a steel rod into an alginate solution and subsequently submerging the rod into a barium chloride crosslinking agent solution, in order to improve its structural properties. The rod is then removed, leaving behind a vessel with a representative vascular lumen (4).

**Aims:** The aim of this study was to develop the graft fabrication method used by Tabriz to produce alginate vessels resembling endogenous coronary arteries' geometric and structural properties. The study also analyses the necessity for constructs to include a base layer to help with subsequent layer adhesion to the dipping rod, as has been seen in other works (4). The storage potential for grafts in physiologically relevant conditions is also examined. Further, a luminal perfusion testing rig was produced in order to assess the fabricated constructs' response to luminal flow. These aims were set out as TEVGs are more likely to perform better when used in-vivo when they resemble the geometry, strength, layer profile and thermal stability of endogenous vessels (3).

**Methods:** To produce the vessels, 4.4% alginate solutions were prepared, mixing alginic acid sodium salt and deionized water. This concentration showed more uniform thickness and strength when compared with vessels fabricated from larger or smaller alginate concentration values. Three vessel types with different layering profiles were then produced with 5 samples produced per vessel type (Table 1). One submersion into alginate solution followed by rapid transfer into crosslinking agent solution corresponded to one layer in each vessel type. Base layers were submerged for 5 seconds in the crosslinking agent whilst subsequent layers were submerged for 2 minutes. The dipping rod was submerged in both solutions to a 10cm depth, forming a 10cm alginate vessel (Figure 1). This vessel is then removed from the 4mm-diameter dipping rod, leaving behind a similarly sized vessel lumen. Two 2.5cm samples are then excised from the central region of the original 10cm vessel. The samples are dried and measured for mass and outer diameter. Samples are then incubated at 37°C in PBS/CaCl (0.01mM/1.8mM)

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storage media. Mass and outer diameter values were then gathered after 24-, 96- and 168hrs of incubation time.

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A 3D-printed ABS-polymer testing rig was produced which incorporated detachable inner perfusion portals to allow easier attachment of vessels to the testing rig (Figure 2). The testing rig was then attached to a perfusion circuitry system to enable flow of blue dyed distilled water through the secured vessels and allow assessment of system leakages. The pressure equation ($P=\rho gh$) was used with a raised reservoir and peristaltic pump to acquire an approximate pressure in the perfusion system of 70mmHg. Flow rate was calculated by using a collection reservoir and a stopwatch. The structural integrity of the vessel segment under luminal perfusion could then be assessed over time at a pressure and flow rate comparable to coronary vasculature.

Table 1: Alginate vessel structure types to be fabricated during a simple dip-coating procedure. The vessel types have been assigned arbitrary letters and their associated number of alginate base layer and subsequent layers is indicated.

| Vessel type | Vessel Structure | |
|---|---|---|
| | Base Layer | Subsequent Layers |
| A | 1 | 3 |
| B | 1 | 1 |
| C | 0 | 1 |

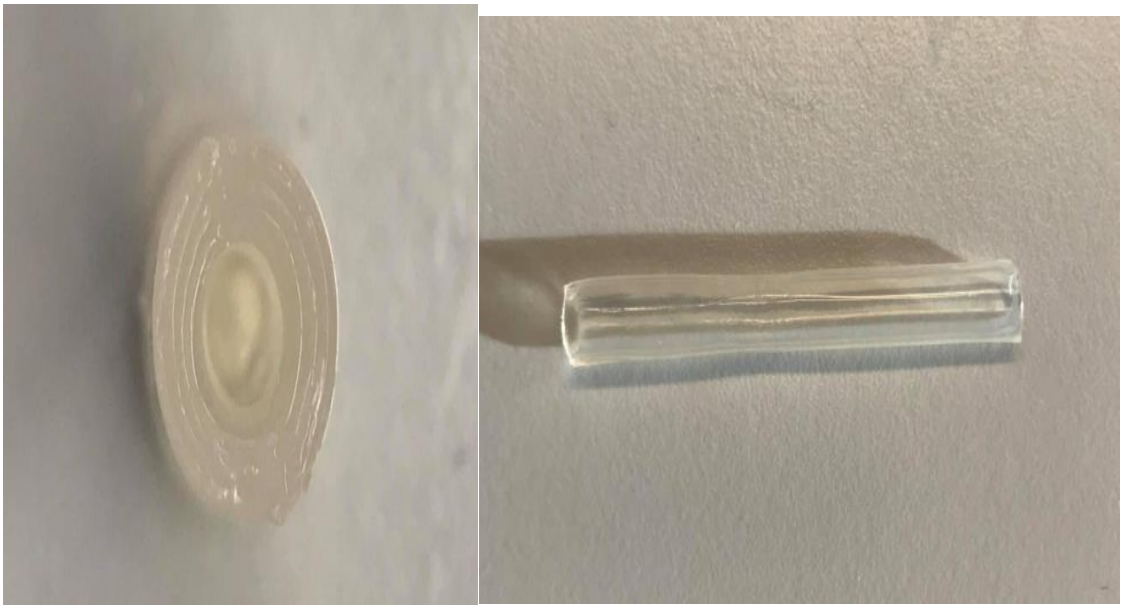

***Figure 1: Artificial alginate vessel.*** *The vessel was imaged immediately after fabrication with a simple dip-coating protocol, using 4.4% alginate solution and 200mM barium chloride, with the one base layer and 3 subsequent layer type (A) photographed along its axis (left) and the single 'subsequent' layer type (C) after removal from the dipping rod (right).*

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As incubation time increased, after the 24hrs incubation point, alginate vessel mass degradation was insignificant for each vessel type ($P<0.01$). Larger vessel types appeared to have less mass stability with incubation than smaller vessel types. Furthermore, during incubation, larger vessel types displayed larger inter-sample mass variability than vessel types with less layers. Alginate segment outer diameter changes were insignificant for each vessel type, during an incubation period of 168hrs ($P<0.001$). Also, there was no significant inter-sample variability for outer diameter measurements within each vessel type ($P<0.001$). Therefore, all vessel types showed appreciable incubation stability. However, the single layer vessels appeared the most stable in mass and geometry. This has verified the potential for alginate vascular grafts to be stored with little degradation and represents a progression towards an 'off the shelf' vascular graft solution.
All vessel types were capable of withstanding testing rig applied luminal perfusion; however, ruptures were observed in some samples. Further, ruptures through manipulation and luminal perfusion appeared to increase with incubation time. In future studies, mechanical compressive and tensile testing in both the longitudinal and radial planes of the vessel would be beneficial, in order to examine alginate vessels' resemblance to endogenous vessel mechanical characteristics (3, 5). A possible method of strengthening vessels may involve more intense crosslinking, however, this can reduce graft biocompatibility through reducing graft porosity and increasing toxicity to endogenous cells (5).

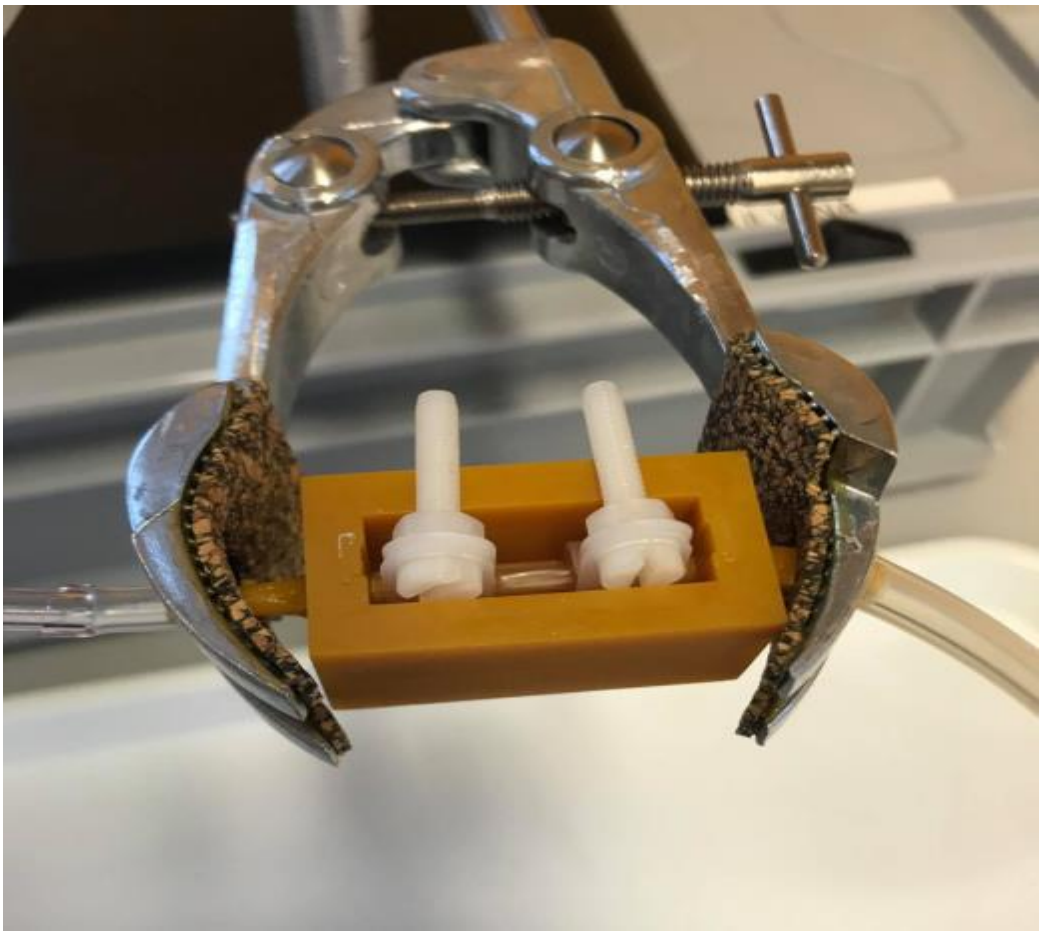

***Figure 2: Single layer alginate vessel undergoing 250ml/min perfusion media luminal flow.*** *The vessel is attached to the version two artificial artery in-vitro testing rig via the inner*

*perfusion portals and 5mm P-clips. On the testing rig's outer perfusion portals, perfusion tubing is connected from the perfusion system. The vessel was fabricated using 4.4% alginate solution, 200mM barium chloride solution and a simple dip-coating fabrication technique. The vessel was able to endure luminal flow of perfusion media without observable vessel wall rupture.*

Samples were secured successfully to the testing rig and subjected to a 250ml/min flow rate via the perfusion circuitry system without experiencing leakages (Figure 2). However, developments on specific fluid dynamic application to vessels and adaptability of the testing rig are necessary to better mimic in-vivo conditions. This improvement in in-vitro modelling may help to improve translation of in-vitro CABGs, or drug eluting stents, to in-vivo cardiovascular treatments, thereby, reducing the requirement for pre-clinical animal modelling (2).

**Conclusion:** The study demonstrated that a simple dip-coating fabrication technique can be used to reliably produce crosslinked alginate hydrogel vascular grafts, displaying similar vessel luminal diameters, wall thicknesses, and tubular shapes to endogenous coronary arteries. Further, a range of alginate vessel types have demonstrated geometric and mass stability when incubated over a period of 1 week in physiological conditions. This has verified the potential for alginate vascular grafts to be stored with limited degradation and represents a progression towards an 'off the shelf' vascular graft solution.

The 3D printed artificial artery testing rig developed during this study has demonstrated its capability to attach and perfuse a range of artificial artery grafts with luminal flow resembling that of endogenous coronary arteries. Introducing physiologically relevant luminal flow to grafts has produced an in-vitro testing model which better resembles in-vivo conditions for CVD treatment options such as vascular grafts, endovascular stents and pharmacologics.

Although this study offers developments in vascular graft fabrication and CVD in-vitro modelling techniques, further vascular graft research is necessary to improve fabricated vessel mechanical performance, bio-integration, non-immunogenicity and functionality in comparison to endogenous blood vessels, in order to improve CVD treatments such as CABG surgery and PCI.

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