Title: Microfabrication and in vivo testing of small-diameter tissue-engineered vascular grafts

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Background
Vascular diseases affect million people worldwide, spanning from congenital arterial defects to atherosclerotic narrowing of arteries that perfuse the body's vital organs. The use of endovascular repair has increased threefold in recent years, however surgery is still a key therapeutic component in advanced vascular disease. Biological vascular grafts (like the saphenous vein) may fail due to restenosis and are not available in 10% of the patients. The reconstruction of small-diameter arteries, like the pulmonary branches in congenital heart defects and the popliteal artery in the adult is associated with the risk of early thrombus formation, infection and neointimal hyperplasia. Therefore, there is a need for novel vascular engineering design of responsive, living conduits, with properties similar to native tissues for small diameter arteries. Bristol has a unique multidisciplinary knowledge and expertise to generate novel tissue engineering solutions and to nurture a new generation of translational scientists that can champion the field. In this project, we propose that cellularised vascular matrices may be one of the most viable options for correction of small vessel defects.

Aims & Objectives (100 words)
We will build a novel TEVG composed of a multi-layered scaffold bioengineered with human endothelial cells (ECs), smooth muscle cells (SMCs) and adventitial progenitor cells (APCs).
Objective-1: Fabrication of the TEVG, with the student acquiring knowledge in physicochemical factors to improve or replace biological tissues
Objective-2: Endothelialisation of the TEVG, with the student acquiring knowledge in cellular and vascular biology.
Objective-3: Muscularization of the TEVG, expanding the knowledge on vascular cells and progenitor cells and acquiring expertise in innovative cell seeding.
Objective-4: In vivo testing of the cellularised TEVG in a large animal model, with the student participating to the work of a surgical team at the state-of-the-art facilities in Langford.

Methods (200 words)
Fabrication: We will use a novel electrospinning technique that generates a tubular graft consisting of two coaxial nanofiber mats. The outer layer is made of Polycaprolactone (PCL) and the porous internal structure of crosslinked gelatin (GL). The former provides mechanical properties to sustain blood pressure, while the GL layer favours the adhesion and survival of cells, as shown by us recently (Carrabba et al., Biofabrication. 2016). The objective is to create TEVGs compatible with the dimensions of small arteries (≦6 mm, internal diameter).
Cellularisation with endothelial cells: We will use a new rotating device that allows a stage of dynamic seeding followed by application of physiological flow. Once optimized for primary endpoints (ECs viability and molecular markers of endothelial function), the system will be upgraded to allow rotating seeding and flow to occur simultaneously.
Muscularization: This will consists of subsequent seeding of human SMCs and APCs onto the external surface of the endothelialized graft through ultrasonically focused bio-ink extrusion of an alginate-based cell suspension.
In vivo testing: At the Langford large animal infrastructure, we will test grafts engineered with porcine cells (available in the lab) in a pig model of carotid artery bypass.

Explanation of and justification for interdisciplinarity (150 words)
This project addresses the clinically relevant need of generating small vascular grafts for long-term correction of ischemic disease and congenital cardiac defects. We initially envision to

use the patient's own cells for tissue engineering. If the microfabrication system is valid and the autologous cell approach proves to be effective in the in vivo pig model, we contemplate widening the application to the use of haplotype-compatible banked vascular cells (including induced pluripotent cell-derived ECs, SMCs and APs) from human donors. The study is multidisciplinary as it comprises methodologies and expertise spanning cellular biology, device engineering design and tissue engineering. All the methodologies, ethical approvals, and infrastructures are in place thus making the project immediately feasible. Success of the study will open new avenues to the treatment of thousand patients with congenital or acquired vascular defects in the UK and abroad.

Field: Cardiovascular regeneration, tissue engineering, cell biology