Cell Seeding of Decellularised Porcine Coronary Arteries; A Potential Tissue Engineered Blood Vessel
Evelyn M. Campbell,¹ Joseph N. Mackle,¹ Caitríona Lally,¹
triona.lally@dcu.ie
¹School of Mechanical & Manufacturing Engineering, Dublin City University, Dublin, Ireland

Introduction
Tissue engineered blood vessels (TEBV) offer potential solutions to the limitations of natural and synthetic grafts for coronary artery bypass grafting [1]. This study employs a short-term decellularisation technique to obtain a natural matrix scaffold suitable for repopulation with cells. Short-term decellularisation limits the alteration in the biomechanical properties and maintains the native architecture aiding the development of a potentially viable TEBV.

Materials and Methods
Decellularisation of 20 mm segments of coronary arteries isolated from porcine hearts was achieved using a modified protocol from Liu et al. [2]. Under constant agitation, the arteries were immersed in 0.1M sodium hydroxide (NaOH) for 3, 6 and 12 hours followed by 3 hours in 0.9% saline. Segments were processed for hematoxylin and eosin (H&E) and 4',6-diamidino-2-phenylindole (DAPI) staining.
To demonstrate repopulation, the luminal surfaces of 5 x 5 mm flat specimens were seeded with bovine aortic smooth muscle cells (BASMC, Coriell) in a 24-well plate in concentrations ranging from 5x10⁴ to 2.5x10⁵ cells/cm². In addition, a cylindrical specimen was rotated in BASMC-containing media for 24 hours. Flat specimens were analysed after 24 hours with H&E and DAPI to determine the optimum cell repopulation concentration. Sections of the cylindrical specimen were examined for cell growth and infiltration after 24 hours and 7 days in static conditions.

Results
H&E staining confirmed the removal of vascular cells and cellular debris after 6 hours of NaOH treatment, see Fig. 1(a). Cell repopulation concentration analysis confirmed that 1.25x10⁵ cells/cm² produced a confluent luminal monolayer on flat specimens after 24 hours, see Fig. 1(b). BASMCs attached to the luminal surface of the cylindrical specimen after 24 hours, whilst a confluent monolayer was established after 7 days, see Figs 1(c) and 1(d).

Discussion and Conclusions
Preliminary cell seeding and biomechanical tests of decellularised arteries illustrate their potential for use as TEBVs. Future work includes extensive biomechanical and histological characterisation of fresh, decellularised and repopulated arteries to include inflation, permeability and tensile tests and quantification of cell number and extracellular matrix.

References

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Disclosures
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