# Polymeric 3D printed tubular tissue-engineering scaffolds for advanced pre-clinical testing

Anna Johnston[1,2,3], James O'Connor[3], Viviane Chiaradia[3,4], Andreas Heise[3,4], Karen Redmond[3], Sally-Ann Cryan[1,2,3]

[1]School of Pharmacy and Biomolecular Sciences, RCSI, Dublin, Ireland, [2]Tissue Engineering Research Group, RCSI, Dublin, Ireland, [3]CÚRAM, Research Ireland Centre for Medical Devices, Galway, Ireland [4]Department of Chemistry, RCSI, Dublin Ireland

**Background:** Three-dimensional (3D) printing is a popular technique for fabricating scaffolds for tissue engineering applications. We are developing a 3D printed tubular scaffold for tracheal tissue engineering, using both polycaprolactone (PCL) and a novel polyester ($P_1$), that has previously shown higher biocompatibility than PCL. The initial prototype developed was much smaller than anatomical sizes, and we have been scaling up the fabrication to produce scaffolds that are more relevant in size in order to carry out advanced pre-clinical testing in a 20kg porcine (ex vivo lung perfusion) EVLP model[1].

20kg pig cartilaginous tracheas have an inner diameter (ID) between 12.7 and 17.4mm, and an outer diameter (OD) between 23.0 and 25.4mm (varied along the length and cross-sectional direction of the trachea)[2].

**Methods:** The previously developed 3D printed backbones had an ID of 9.6mm, an OD of 12mm, and a length of 10mm. The printed scaffolds were combined with collagen and hyaluronic acid (CHyA) phase using a custom freeze-drying process, with a CHyA film lining the inside of the scaffold. The scaffolds were then manufactured using a custom stainless-steel mould consisting of 7.8mm wide pegs and 14mm wide holes.

To create scaffolds of a size compatible with the porcine model, tubular backbones were 3D printed with an ID of 13mm and an OD of 15mm, and combined with a CHyA phase and film using the same freeze-drying method. For these larger scaffolds, another custom stainless-steel mould with 12mm pegs and 16mm holes was used.

3D printed backbones were compression tested to determine their mechanical properties. Completed scaffolds were inserted into a porcine EVLP model using lungs from 20kg pigs. EVLP was carried out for 24 hours, with leakage and gas composition recorded hourly over.

**Results:** Scaffolds were successfully produced with the new mould. These larger PCL scaffolds had similar mechanical properties to the smaller prototypes produced in each respective material. Producing large 3D printed scaffolds using $P_1$ proved more challenging, due to batch variability requiring the printing parameters for each batch to be carefully selected. $P_1$ scaffolds were also significantly more brittle than their PCL counterparts, meaning more care needed to be taken when handling them.

**Conclusions:** Previous work by this group has shown that $P_1$ is more favourable to cell growth than PCL. While $P_1$ is more time consuming and challenging to obtain and process, this is a promising result that warrants further development. The next steps for the development of these scaffolds is to 3D print backbones using a blend of PCL and $P_1$ to attempt to balance the favourable mechanical properties of the PCL with the more bioactive $P_1$.

1. Prasad, N. K. *et al.* Ex vivo lung perfusion: Current achievements and future directions: Current achievements and future directions. *Transplantation* **105**, 979–985 (2021).
2. Han, M.-N., Kim, J.-H. & Choi, S. H. Evaluation of biomechanical properties and morphometric structures of the trachea in pigs and rabbits. *In Vivo* **36**, 1718–1725 (2022).