Highly controlled mass production of collagenous tissues of variable size and shape

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Introduction

The RAFT™ system uses proprietary reagent and consumable kits to generate cellular or acellular collagen gels, which are subsequently submitted to plastic compression (Brown *et al*., 2005) using special absorbers, whose function is to gently remove liquid from the hyperhydrated collagen gel. Within an hour, up to 96 dense, collagenous tissues are produced in parallel. The resulting tissue properties, including thickness, collagen concentration and surface topography depend intimately on the absorber properties, including choice of material, porosity, pore size and surface modification. To support expansion of the RAFT technology we have carried out an extensive study which investigated various absorbent materials with respect to the above properties.

Methods

Absorbers for use with the RAFT system were produced from different classes of materials (incl. cement, ceramic, polymer and fibre), of different surface modification, porosity and pore size.

For the various absorbers which were produced, we investigated the effect of surface modification and porosity by gravimetric methods. For the effect of pore size, scanning electron micrographs were captured of both the absorber working face and the resulting tissue surface (after fixation in 1.5% glutaraldehyde and dehydration in increasing concentrations of alcohol through absolute, followed by a final dehydration step with hexamethyldisilazane; Sigma-Aldrich, St. Louis, MO).

The absorbers were used in the RAFT system **(Figure 1)** to produce circular tissues within multi-well plates of one or more formats: 96 × 6 mm; 24 × 10 mm; 24 × 16 mm; and 12 × 22 mm. In brief, the RAFT system consists of the TAP Plate Heater, the RAFT Reagent kit and the RAFT Plate kit. From the Reagent kit, collagen type I is mixed with nutrient media along with base to neutralise the mix accurately and precisely. At this stage, cells may be added of the type and concentration of choice, and this cell-collagen mix is then dispensed into the wells of a multi-well plate, and the plate placed onto the Heater at 37 ºC; the combination of elevated temperature and pH causes the collagen to spontaneously cross-link, forming a hyperhydrated collagen gel. The RAFT plate is then placed over the multi-well plate, making each gel come into contact with one absorber, initiating a slow and gentle absorption of water from the gel, leaving behind cells and collagen in a dense, tissue-like structure.

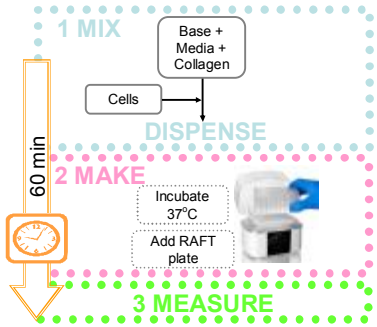

Figure 1
The RAFT system
Up to 96 tissues are made in parallel in less than one hour.

The thicknesses of the produced tissues were routinely measured with a novel, non-invasive optical measuring system manufactured by Lein Applied Diagnostics (Reading, UK). A sub-set of tissues were also measured *in situ* during compression in real-time.

Results

All absorbers exhibited a porous structure under SEM visualisation **(Figure 2)** with distinct differences in pore size in the different formulations of each material group (relative within each group only: large/medium/small pore size). The produced absorbers were also used successfully as RAFT system components to produce dense collagenous tissues. Typified by absorbers from the fibre group of relatively large, medium or small pore size, tissues had relatively rough, smooth or very smooth surface topography on the meso-scale, respectively **(Table 1)**. The thickness was measured in real-time **(Figures 3-4)** and at the last timepoint measured as 180, 120 and 100 µm, respectively.

One absorber material group, of a specific porosity, pore size and surface modification, was produced in four formats, and tissues produced using the RAFT system maintaining other parameters to scale. These tissues, which were produced from a starting gel height of 6500 µm, measured inside the 128-147 µm range in thickness **(Figure 5)**.

Conclusion

We can now control liquid removal, which allows the process to be streamlined, standardised and simplified. Tissue properties can be fine-tuned according to the requirements, with tissues reproducibly and conveniently produced in parallel in standard multi-well plates or in a range of shapes and sizes for tissue engineering and regenerative medicine therapies

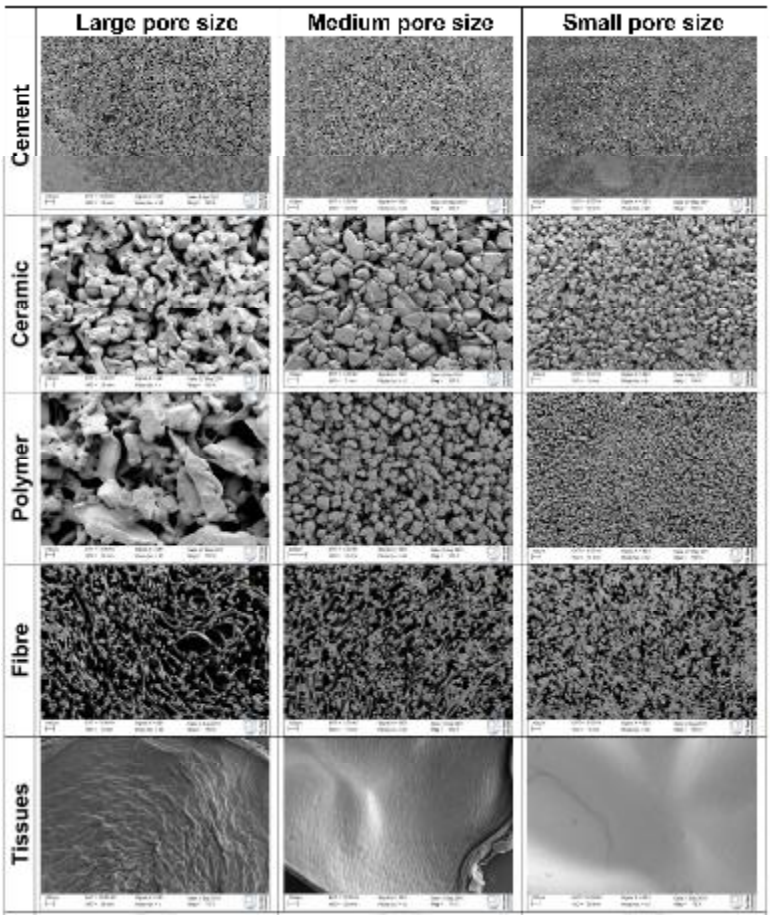

Figure 2
Scanning electron micrographs of RAFT absorbers of different materials and pore sizes and the resulting engineered tissues as exemplified by those produced by the fibre-based absorbers
Different materials including cement, ceramic, polymer and fibre-type were produced, each with a controlled porosity and pore size. Depending on the choice of material, porosity and pore size, different tissue characteristics could be engineered including meso-scale surface roughness/smoothness, tissue thickness and collagen concentration.

	Large pore size	Medium pore size	Small pore size
Properties	Absorber capillarity: Low	Absorber capillarity: Medium	Absorber capillarity: High
	Tissue meso-scale surface: Rough	Tissue meso-scale surface: Smooth	Tissue meso-scale surface: Very smooth
	Tissue thickness: 180 µm	Tissue thickness: 120 µm	Tissue thickness: 100 µm

Table 1
Generalisation of RAFT absorber effect on engineered tissue
The pore size of the absorber has an influence on absorber capillarity which in turns determines how much water it is able to remove from the collagen gel during the RAFT process, giving rise to tissues of different thickness. These thicknesses relate to the tissues produced by the fibre-based absorbers as in **Figure 2**. Moreover, the pore size influences the meso-scale tissue surface. Absorber porosity and pore size can be adjusted independently to control the resulting tissue characteristics.

Figure 3
Non-invasive thickness measurement *in situ* and real-time
The Lein Applied Diagnostics CTS1 uses the principles of confocal microscopy to measure tissue thickness. It is used routinely to measure RAFT tissue thickness for RAFT R&D, QC and QA purposes, and in special circumstances used for real-time measurements *in situ* during tissue formation.

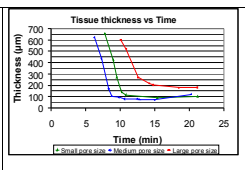

Figure 4
Monitoring of tissue formation in real-time
Exemplar real-time thickness measurement tracings taken during tissue formation with gels being absorbed by fibre absorbers of either relatively large, medium or small pore size as in **Figure 2**. For a larger pore size, capillarity is reduced, and endpoint thickness becomes relatively thick as a consequence.

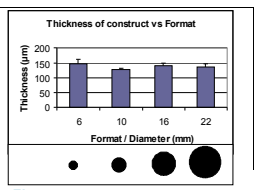

Figure 5
Formats for tissue production of variable tissue size and number
RAFT absorbers were produced in different diameters, keeping other parameters to scale, and used to produce tissues of variable diameter and number per batch. From a starting gel height of 6500 µm, the absorbers produced tissues with mean thicknesses all within the 128-147 µm range (sample size varied between n=6 and n=140), confirming suitability of process scaling.

Bibliography
Brown, R.A., Wiseman, M., Chuo, C.B., Cheema, U., Nazhat, S.N. (2005) Ultrarapid engineering of biomimetic materials and tissue: Fabrication of nano- and microstructures by plastic compression. *Advanced Functional Materials*, 15, pp. 1762-1770.

Acknowledgements
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