



# Collagen scaffolds for tissue engineering and angiogenesis

Asma Yahyouché<sup>1</sup>, Dr Jan Czernuszka<sup>1</sup> and Dr James Clover<sup>2</sup>

<sup>1</sup>Materials Department, University of Oxford, Parks road, Oxford, OX1 3PH.

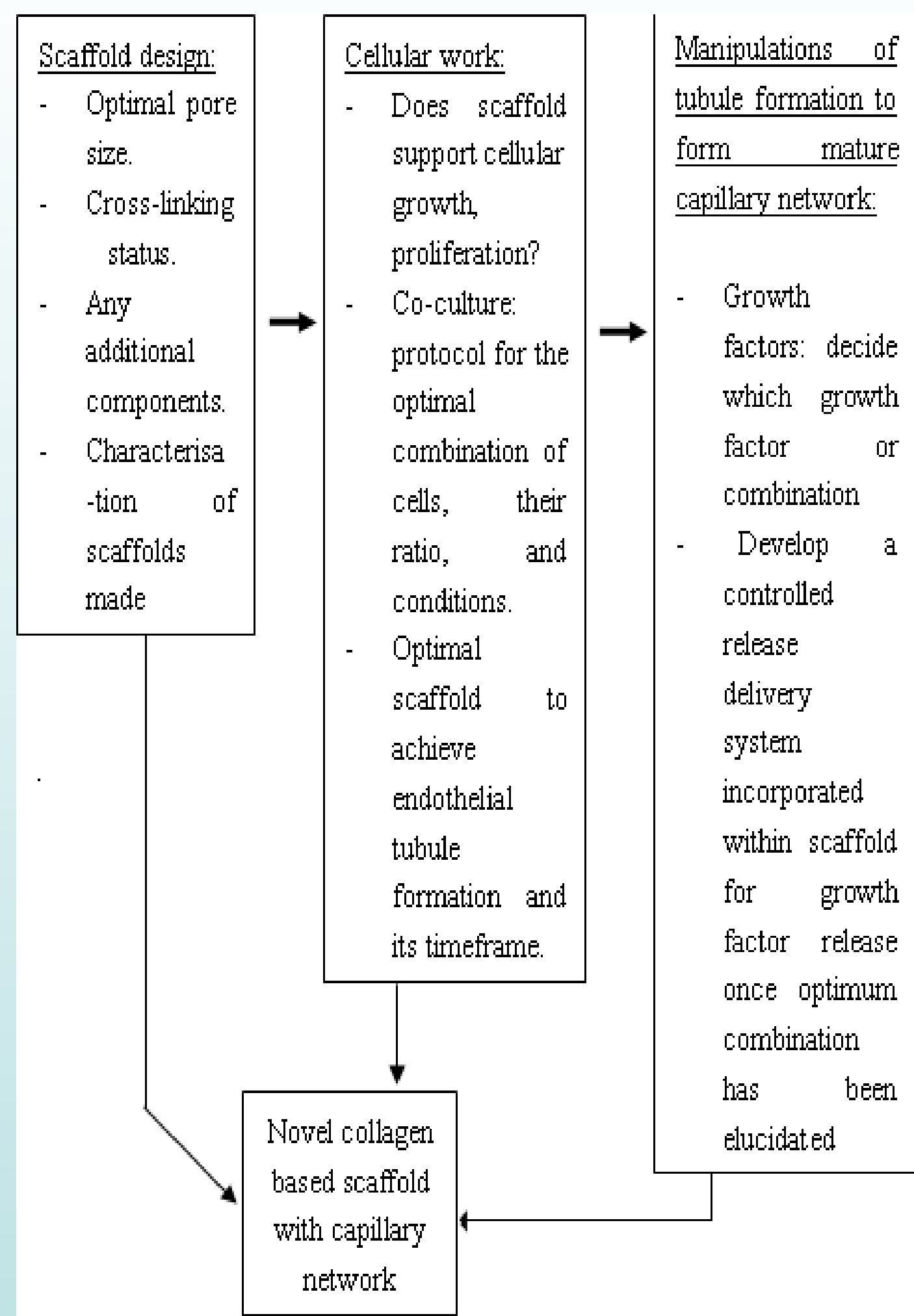
<sup>2</sup>Nuffield Department of Surgery John Radcliffe Hospital, Oxford, OX3 9DU

E-mail: asma.yahyouché@materials.ox.ac.uk

## Introduction:

Tissue engineering as a multidisciplinary science primarily aimed at meeting the need to replace organs and tissues lost due to diseases or trauma. Though it has moved a long way since it was first defined in 1987, it is still in its early stages[1]. One of the bottleneck problems to engineer unlimited tissue size is the vascularisation[2]. The development of an inherent capillary bed *in vitro* is crucial for ensuring the viability and functionality of tissue-engineered tissues, as is the ability to connect with the host vasculature once implanted. The current approach in vascularising bioengineered tissue is the integration of various parameters observed in angiogenesis process *in vivo* with the biological *in vitro* tissue engineering research. Thus, the combination of highly porous interconnected scaffolds made of synthetic or ECM components with/without vascular cells in presence/ absence of growth factors have been investigated. However, in such a combinatorial approach, many parameters need to be optimised before achieving a viable vascular analogue *in vitro* maintained for tissue engineering. Unless these difficulties of creating an intrinsic circulation can be overcome, tissue engineering will be restricted to thin structures. Hence there is a strong need for developing strategies that can promote angiogenesis in 3-D scaffold constructs of biodegradable polymeric scaffolds. This particular need forms the basis of the proposed project. A cellularized tissue engineered scaffold could have a host of clinical applications. There would be a particular advantage to plastic and reconstructive surgeons. Applications could include body resurfacing for injuries caused by burns and trauma.

## Outline of the project:



## Basis of the project:

*In vivo*, most cells do not survive more than a few hundred micrometres from the nearest capillary, due to diffusion limitations [3]. Capillaries and the vascular systems are required to supply essential nutrients including oxygen, remove waste products and provide a biochemical communication highway [4]. *In vitro* culturing of cells provides such nutrient through standard culture media by diffusion of ions into the cell membrane. However as they start to migrate within the scaffold and produce extracellular matrix, diffusion is limited to a critical distance. Angiogenesis is the sprouting of blood vessel which is a principal mechanism of blood vessel formation. Endothelial lining of blood capillaries consists of single layer of flattened cells whose basal surface interacts with extracellular matrix. Since blood capillaries *in vivo* entirely surrounded by extracellular matrix containing collagen as a major component, collagen could be material of choice for the scaffold [5]. Endothelial cells are the key regulator of this process, by forming endothelial tubules. However, these neovessels are dependant on growth factor concentration. The neovessel becomes independent of these growth factors once a smooth muscle or pericyte coat has been achieved. Growth factors play a vital role in angiogenesis. They influence the activation, proliferation and maturation of endothelial cells. Consequently, co-culture of endothelial and smooth muscle cells is vital if stable vessels are to be produced. This project aims to produce a collagen based scaffold with an inherent capillary network.

## Preliminary results:

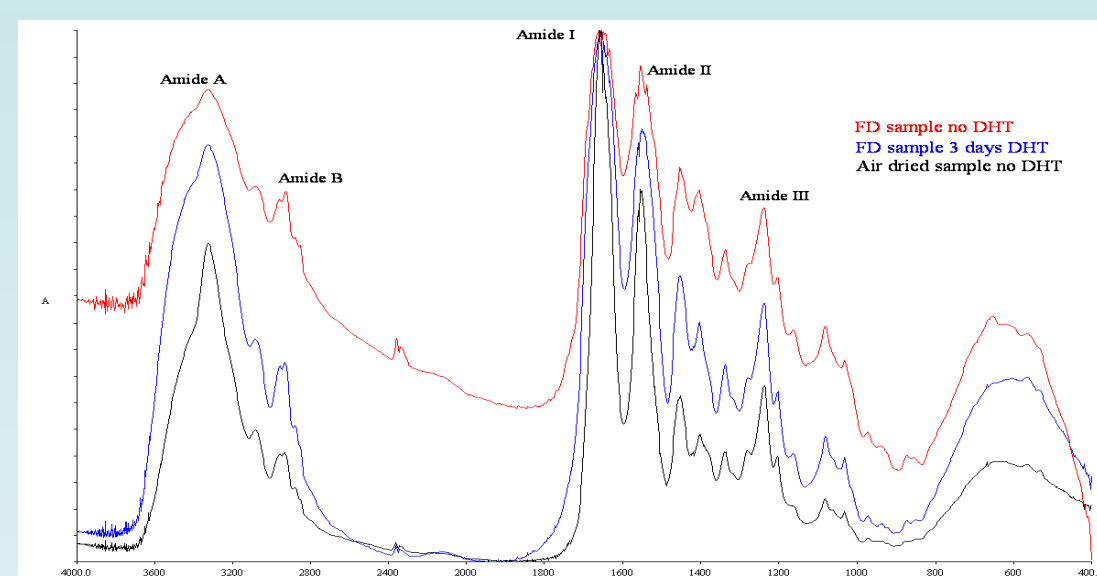
A bovine collagen type 1 (Devro) scaffolds were fabricated using freeze drying (FD) and cross linked by DHT (Figure 1). Scaffolds were characterised using DSC (Table 1), FTIR (Graph 1) and SEM (Figure 2 & 3). Enzymatic degradation studies were carried out using type I bacterial collagenase and final weight analysis giving the percentage of collagen lost from a sample over the period of 1hr (n=3, St.Dev. plotted as error bars in graph 3). Microchannels were introduced in some scaffolds in attempt to increase mass transfer of nutrients (Figure 1). *In vitro* seeding of scaffolds was done using endothelial and smooth muscle cells. Live-dead assay was used to assess the cytotoxicity of scaffolds.



Figure 1: scaffold with and without channels.

Collagen samples	Temperature denaturation T <sub>d</sub> (°C)
Control Air dried 1% no DHT	61.06±0.4
Collagen scaffold FD no DHT	63.3±0.39
Collagen scaffold FD 3d DHT	55.47±0.3

Table 1: Effect of different processing on thermal properties of collagen



Graph 1: FTIR spectra of air dried and freeze dried collagen samples +/- DHT.

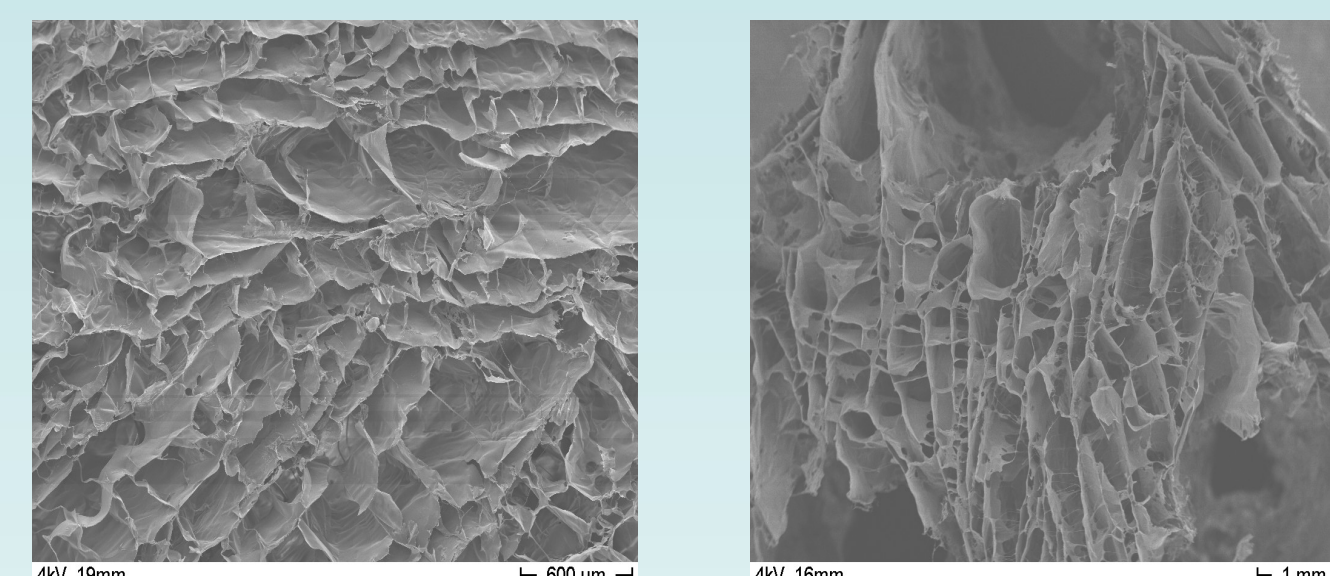
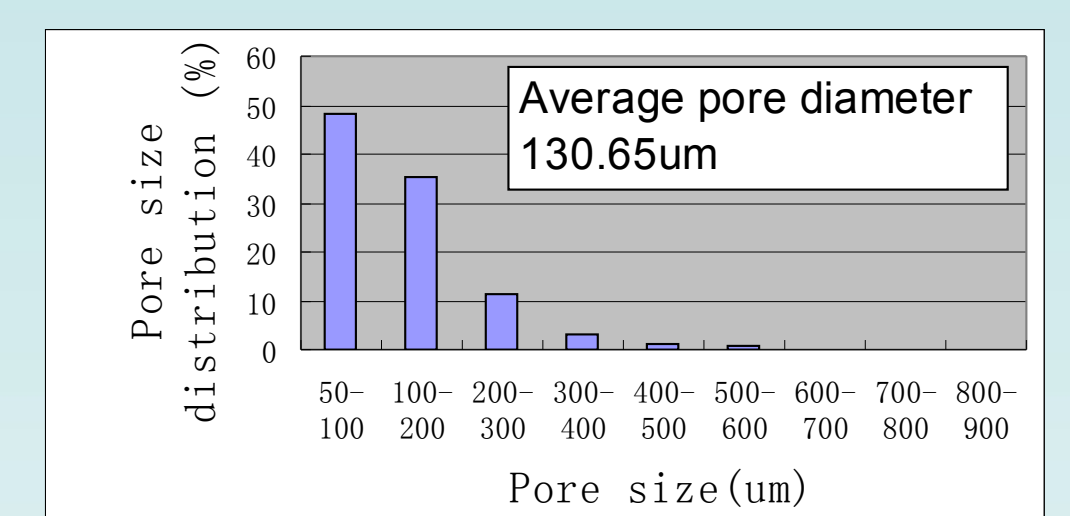
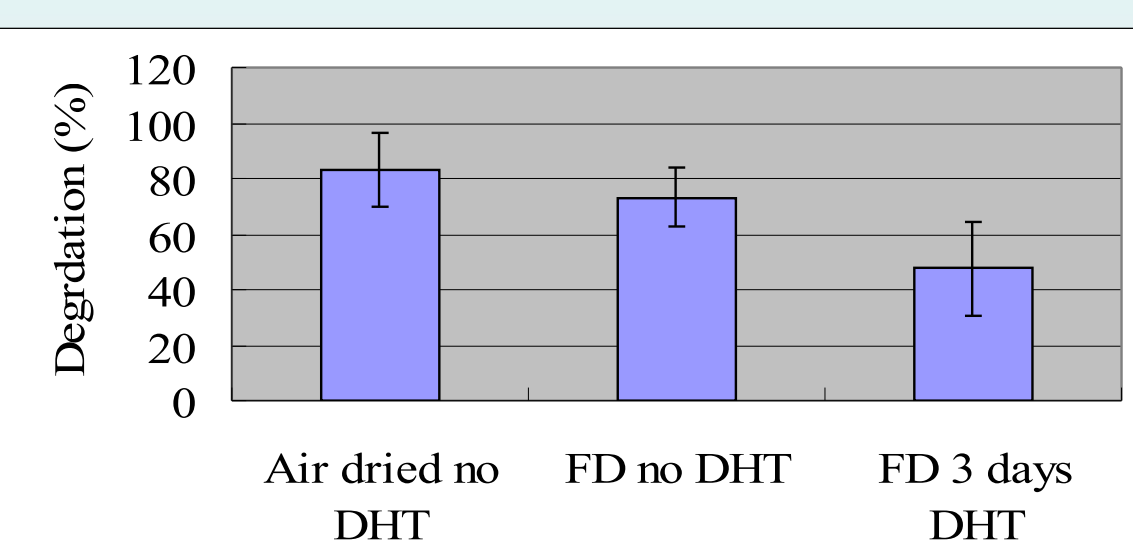


Figure 2: SEM cross section images of: left) 1% collagen scaffold FD, and right) collagen scaffold FD with 3channels



Graph 2: pore size distribution and average pore diameter of 1% FD collagen scaffold



Graph 3: Enzymatic degradation of collagen samples using 50 units of bacterial collagenase at 37C for one hour.

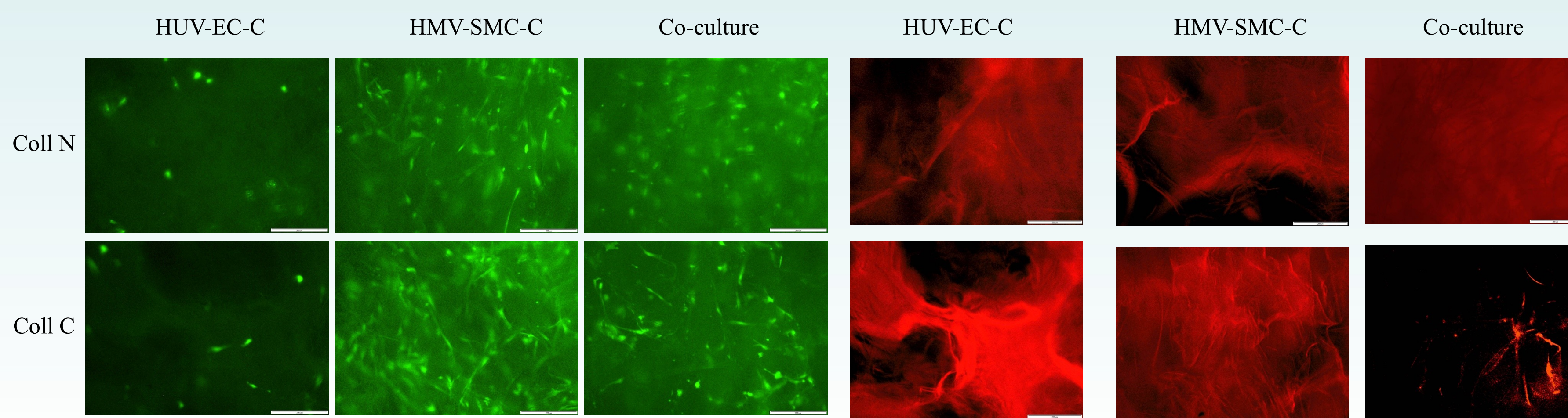


Figure 3: Live/Dead staining of cells after 7 days seeding on scaffolds. CollN: collagen scaffold without channels. CollC: collagen scaffold with channels. HUV-EC-C: endothelial cells. HMV-SMC-C: smooth muscle cells. Co-culture: smooth muscle cells and endothelial cells seeded together on scaffold. Green dots: live cells. Red dots: dead cells

## Conclusions:

- The air dried sample T<sub>d</sub> falls within the range of collagen denaturation which is 60 to 70°C. It is clear from table 1 that making collagen scaffolds using freeze drying and cross-linked with DHT had a negative effect on thermal stability of hydrated collagen.
- All FTIR spectrums showed the characteristic peaks of collagen (Amide A, B, I, II and III). The presence of these bands in IR spectrum confirms that the primary structure of collagen has not been modified. All structural changes that occurred during processing of collagen affected a higher level of collagen structure. The processing slightly affected the tertiary structure of collagen due to some shifts in main peaks.
- The average pore size of 1% collagen scaffold was approximately 131µm with the majority of pores falling within the range of 50-200µm. The channels could be easily viewed and the structure in between still porous and interconnected.
- Cross linking collagen scaffolds with DHT improves their resistance to degradation.
- Collagen scaffolds used are not toxic to both types of cells since hardly any dead cells are detected by live/dead assay.

## Glossary:

- ECM:** extracellular matrix.
- Scaffold:** 3D polymer structures to provide a 3D network for cells to attach to and on which to proliferate that can be implanted into a tissue defect site.
- FTIR:** Fourier Transform infrared analysis is a method of determining the chemical bonds present in a material and their state of vibration (bending, stretching, and contracting).
- DSC:** Differential scanning calorimetry measures the amount of energy (absorbed or released heat) required to heat or cool a specimen at a controlled rate, keeping the specimen at nearly zero temperature difference from an inert reference material.
- Cell culture:** cell expansion *in vitro* to generate a large mass of cells. cells are placed at 37°C and 5% CO<sub>2</sub>. The medium contains a variety of components : salts, amino acids and sugars as well as low level of hormones and growth factors required for each particular type of cells.
- FD:** Dissolved polymer and water are emulsified and freeze-dried to remove water and solvent
- DHT:** dehydrothermal treatment, physical cross linking induced by simply heating dry collagen under vacuum to temperature normally around 100°C.

**References:** 1) Liu, C.Z., et al. J Biomed Mater Res B Appl Biomater, 2008, 85(2): p. 519-28. 2) Rose, F.R., Q. Hou, and R.O. Oreffo, J Pharm Pharmacol, 2004, 56(4): p. 415-27. 3) Sachlos, E., et al., Biomaterials, 2003, 24(8): p. 1487-97. 4) Frerich, B., et al. Int J Oral Maxillofac Surg, 2001, 30(5): p. 414-20. 5) Montesano, R., L. Orci, and P. Vassalli J Cell Biol, 1983, 97(5 Pt 1): p. 1648-52.