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A New Reinforced Fibrin Collagen Glycosaminoglycan Material to Resist Tissue Contraction in Heart Valves

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INTRODUCTION

- Heart valve (HV) prostheses for paediatric patients have well documented shortcomings, predominantly related to the need for multiple surgeries as the child grows.
- A tissue engineering approach may provide an effective alternative by allowing the implanted valve to grow and remodel with the patient.
- Fibrin has been shown to be an excellent scaffold for HV tissue engineering studies, however, fibrin based constructs have shown an inability to maintain an appropriate seal upon closure, as a result of cell-mediated contraction of the HV leaflets [1,2].
- We are currently investigating how a collagen glycosaminoglycan (GAG) scaffold, infiltrated with fibrin, can provide a fully natural scaffold, which has sufficient stiffness to resist the contractile forces of cells acting upon it and thus resist this cell mediated contraction.
- Collagen and GAGs are ideal natural materials to support fibrin in this application, as collagen is the major extracellular component of the native HV, while GAGs provide necessary fatigue resistance against the repeated shearing between the different layers of the native heart valve.

AIMS

- To develop and characterise a cross-linked, multicomponent scaffold of collagen, GAG, and fibrin (CGF) in a HV shape.
- To assess vascular smooth muscle cell (VSMC) distribution, and response within this CGF scaffold.
- To investigate the cell-mediated contraction of this CGF scaffold when containing VSMCs.

METHODS

- A HV shaped collagen-GAG (CG) scaffold was fabricated through freeze drying a CG slurry in a custom built mould (see Figure 1). Parameters were optimised to produce a CG scaffold with a homogenous pore size structure.

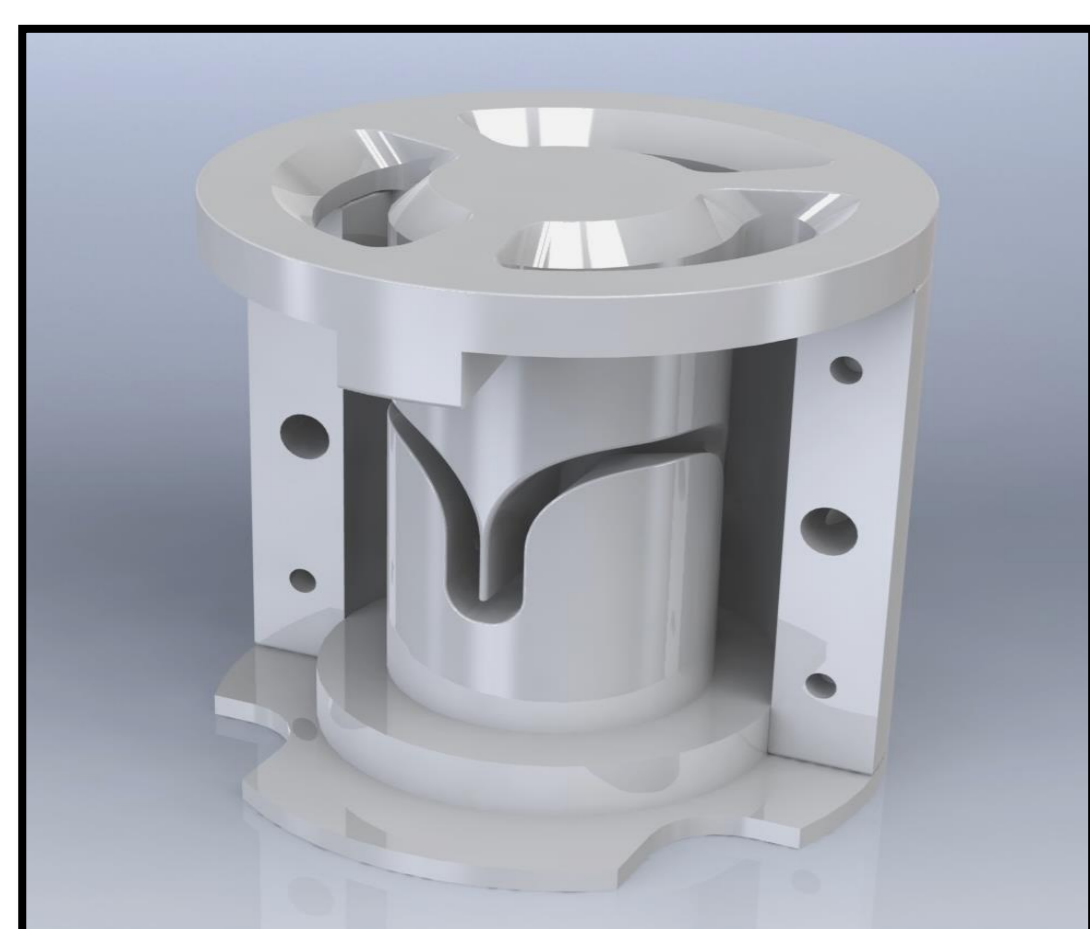


Figure 1. We have previously developed a mould for the construction of tri-leaflet HV conduits [1, 3, 4]. This mould was modified for producing freeze-dried CG scaffolds.

- Once freeze dried, scaffolds were crosslinked physically by dehydrothermal (DHT) treatment at 105°C for 24 hours and cross-linked chemically using 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDAC) in the presence of N-hydroxysuccinimide (NHS) solution, which stiffens the scaffold while maintaining elasticity.
- Different concentrations of both collagen and GAG were assessed to find the most stable concentration of CG to work with. 0.75%Collagen together with 0.044%GAG was found to be the most stable once injected with fibrin (see Figure 2).

METHODS

- The infiltration of fibrin throughout the CG was assessed using Masson's Trichrome staining (see Figure 3).
- Mechanical properties of the scaffolds were tested using a Zwick/Roell Z050 machine (see Figure 4).
- Vascular smooth muscle cells (VSMCs) were successfully seeded into 5mm thick CG scaffold discs using an injection technique with fibrin itself as the carrier and cultured over 7 days (see Figure 5).
- The diameters of the discs were recorded and comparisons drawn to the fibrin control, which was seeded at the same seeding density (see Figure 6).
- VSMC proliferation was measured using a PicoGreen assay (see Figure 7) and live dead cell staining allowed cell viability to be assessed (see Figure 8).

RESULTS



Figure 2. This image shows a sample of the heart valve shaped CG material, post cross-linking and post infiltration with fibrin. The material stands up under its own weight and is easy to handle.

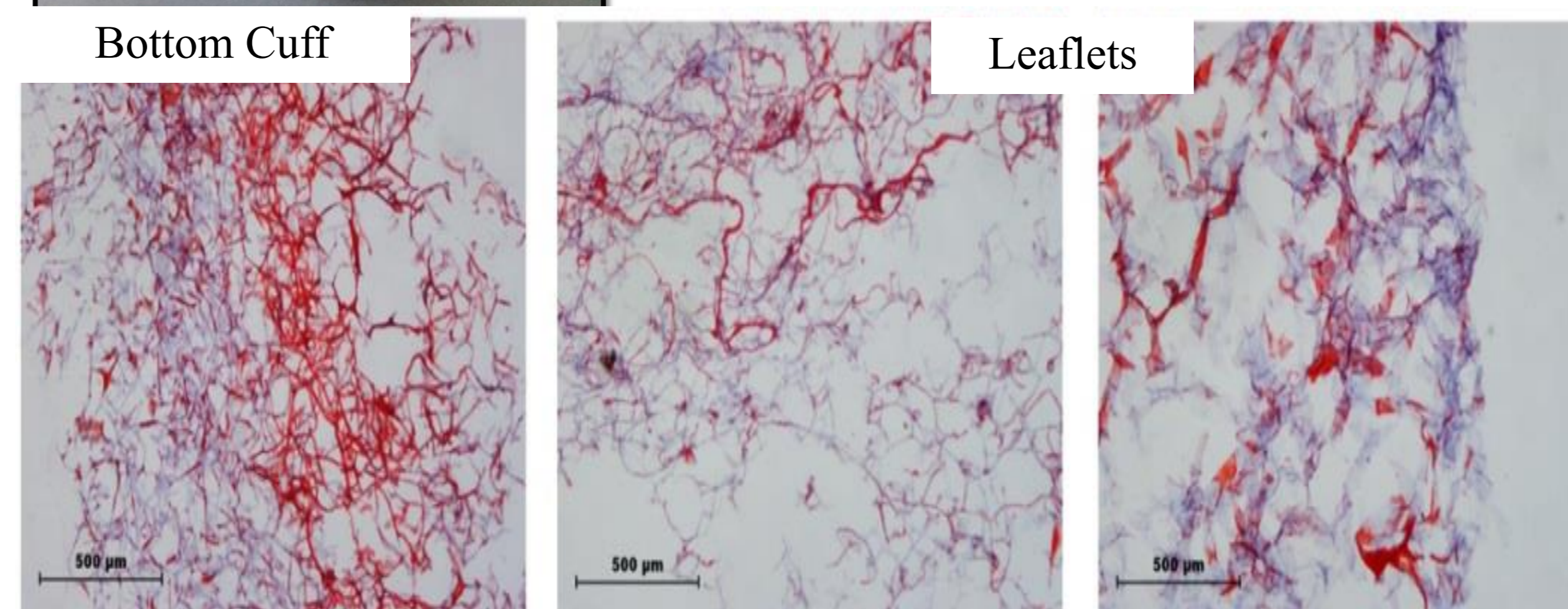


Figure 3. Masson's Trichrome staining shows fibrin (red) has infiltrated through all sections of the collagen-GAG (blue) heart valve shaped scaffold.

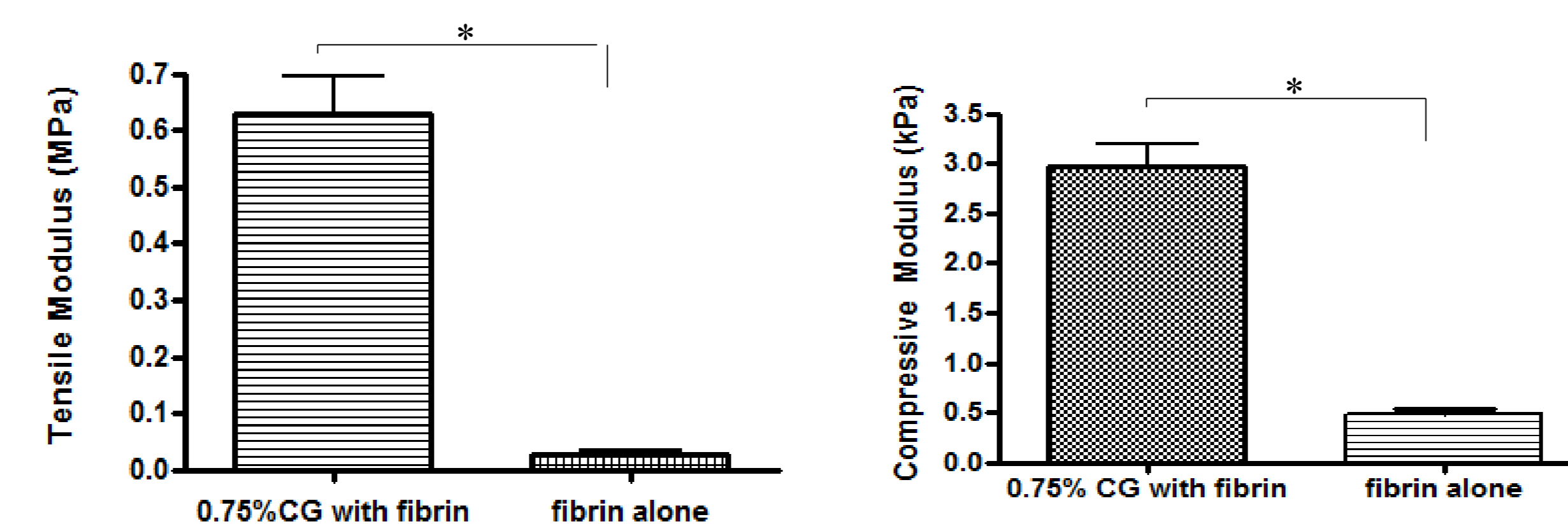


Figure 4. Tensile testing and compressive testing shows when fibrin gels are supported with a 0.75%Collagen 0.044%GAG matrix, their mechanical properties change significantly.



Figure 5. This image shows a CGF scaffold that was seeded with VSMCs at a seeding density of 3,000 cells/mm³. This image was taken after 7 days in static unrestrained culture. The gel is 12mm in diameter. The CG dry scaffold was 11mm diameter prior to infiltration with fibrin containing VSMCs at day 0.

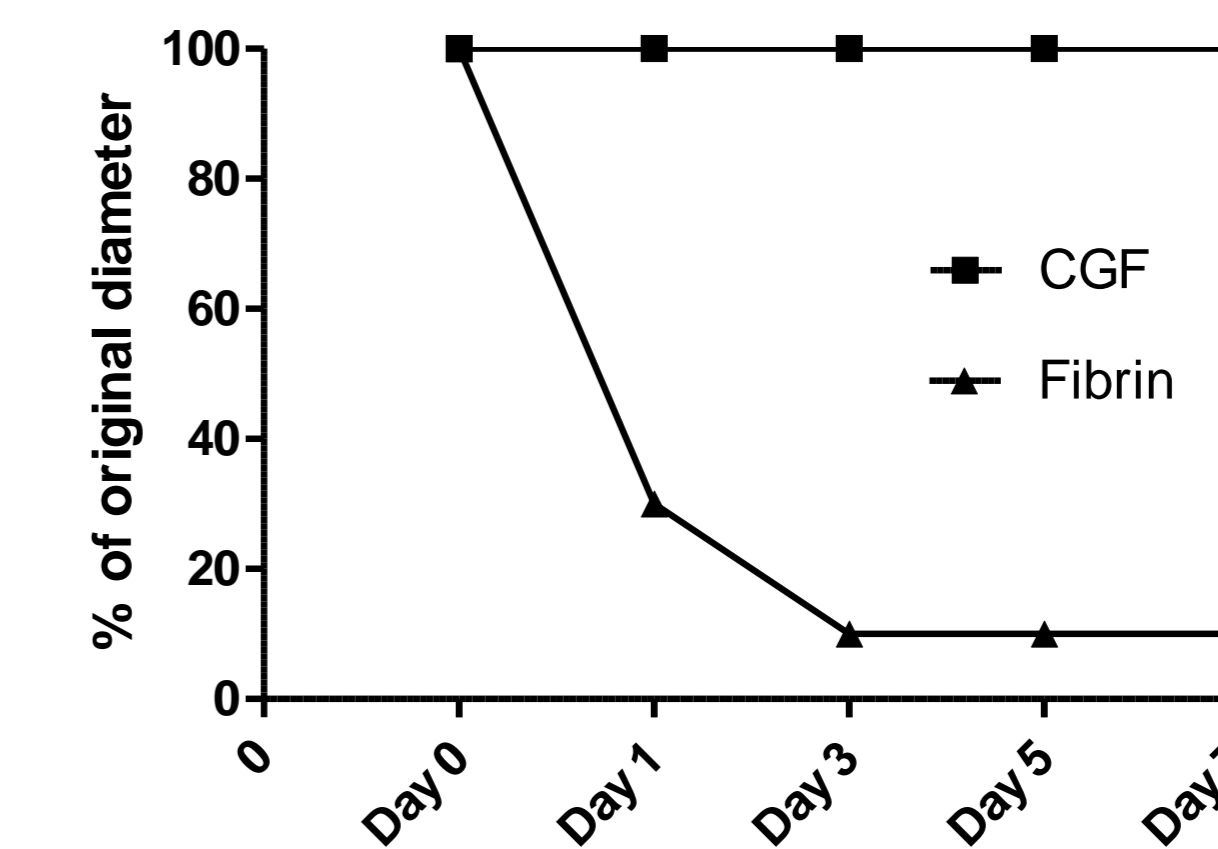


Figure 6. Negligible contraction over 7 days was observed in the CGF scaffold seeded with VSMCs. In comparison, fibrin-only scaffolds with the same seeding density had contracted to approximately 90% of their original diameter after 3 days (n≥9).

Cell number based on DNA fluorescence

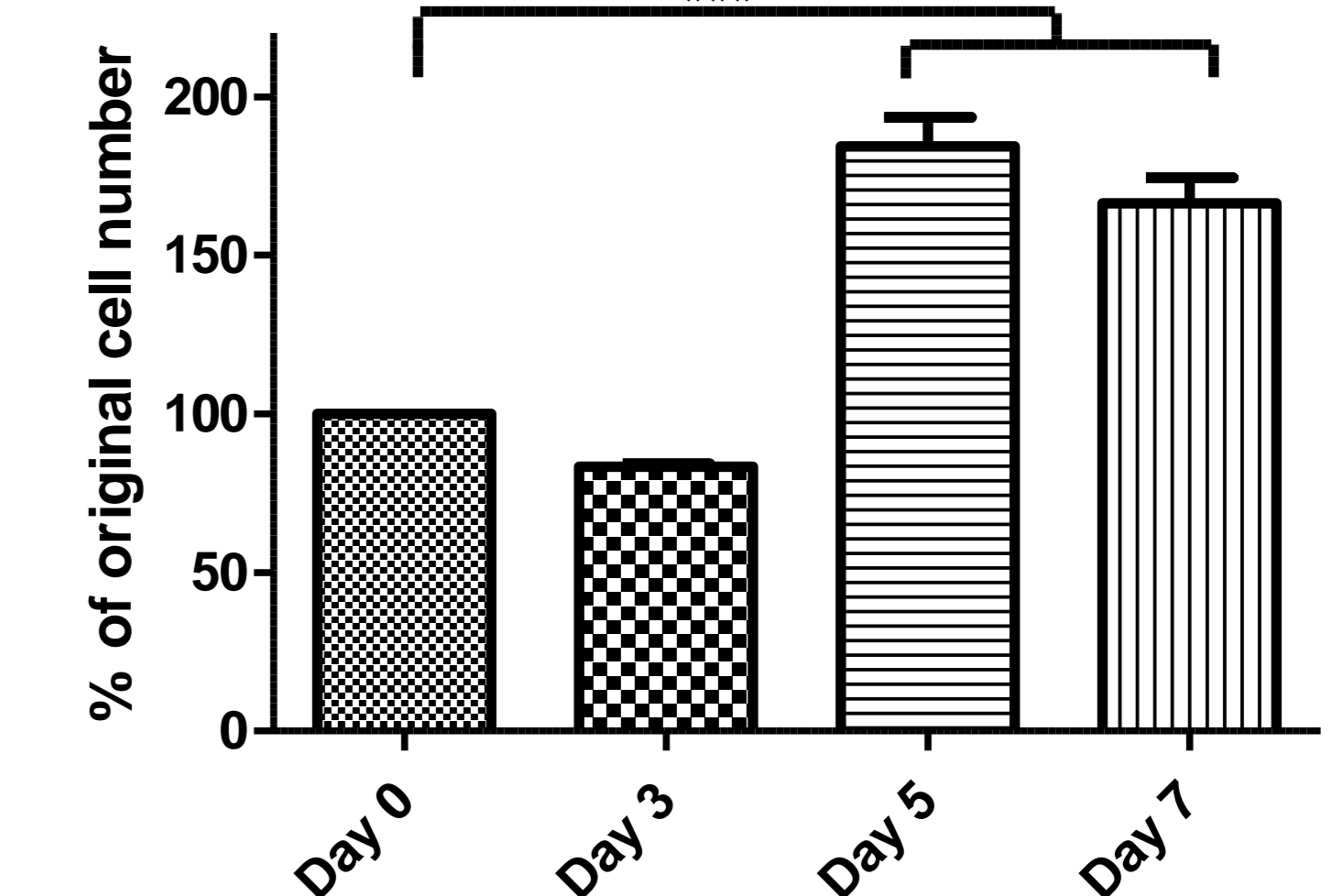


Figure 7. When compared to Day 0, there was a significant increase in dsDNA at both Day 5 and Day 7 time points (n=3, p<0.05). This demonstrates the ability of cells to proliferate within the CGF scaffolds.

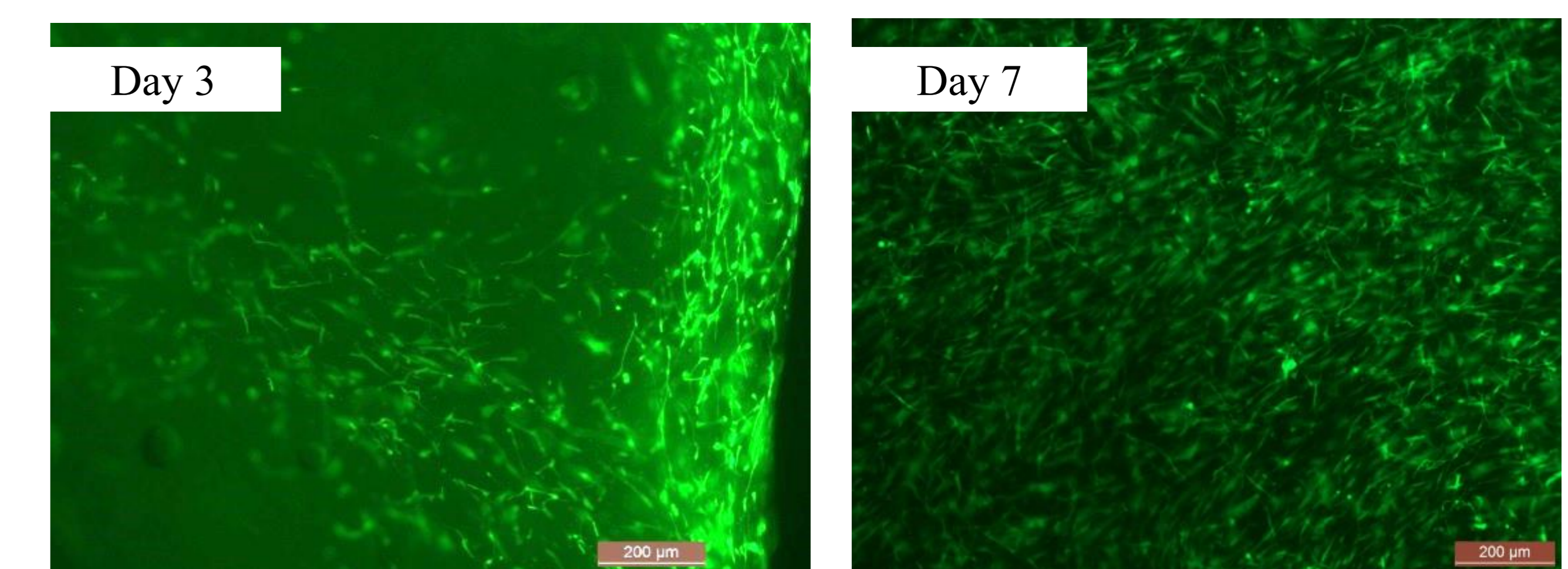


Figure 8. Live/dead staining of VSMCs showing live cells (green) and dead cells (red) at Day 3 and Day 7 time points (original magnification: 100X). At Day 3, excellent viability is seen with some areas of unpopulated scaffold remaining. Day 7 shows confluent populations of viable cells, supporting the observed increase in proliferation as demonstrated in Figure 7.

CONCLUSIONS

A crosslinked, multicomponent scaffold of collagen, GAG and fibrin has been characterised for heart valve applications. Fibrin gels reinforced with a 0.75% collagen, 0.044% GAG scaffolds can resist VSMC induced contraction significantly more than fibrin-only gels, while allowing cell proliferation and maintaining excellent cell viability. This improvement in structural integrity may facilitate the use of fibrin based materials for heart valve tissue engineering.

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