

## Single Unconfined Compression of Cellular Dense Collagen Scaffolds for Cartilage and Bone Tissue Engineering

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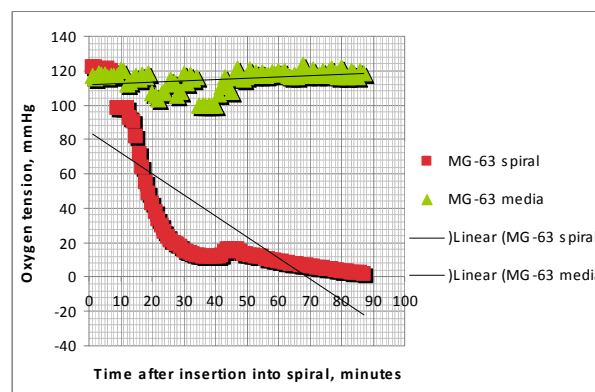
**INTRODUCTION:** Cell seeded collagen matrix scaffolds have been extensively evaluated recently as potential systems for *de-novo* tissue regeneration and repair for a variety of tissue types. While collagen gels are biologically excellent as starting point scaffold materials, their use is limited by the lack of cohesive structure and inherently weak mechanical properties due to a high liquid content (>99%). An ingenious method of combining unconfined plastic compression (PC) with capillary action has shown that these scaffolds can be rapidly processed into tissue like structures, which can be immediately implanted into the host<sup>[1]</sup>. It has been shown that the rapid increase in fibrillar collagen density dramatically enhanced the mechanical properties of such scaffolds thus potentially eliminating the need for long term cellular action. This simple project investigated the effect of single unconfined compression on cartilage-cell seeded collagen matrices in terms of cell viability, proliferation and oxygen consumption.

**METHODS:** *Scaffold preparation:* Neutralized collagen gels (2ml) were prepared in rectangular moulds (10mmx30mm). Cellular constructs were produced by single compression (SC). Unconfined compressive load of 1.5 KPa for 5 min was initially applied to produce collagen sheets, which were rolled to give cylinder scaffolds of ~2 mm diameter. Collagen content using SC and DC was measured gravimetrically after freeze drying. MG63 bone cells and ATDC-5 cartilage cells were incorporated between neutralization and setting/multiple compaction at a pre-compression density of  $5 \times 10^5$  cell/ml. Acellular scaffolds were used as controls.

*Assessment of Cell Viability:* Samples were fixed in formalin at days 1, 5 and 10 and wax embedded. 5µm histology sections were obtained and labeled with DAB conjugated TUNEL based cell apoptosis detection kit. A minimum of 1000 cells were counted in random light microscope fields (x20 objective), evaluated for TUNEL reactivity and morphological signs of cell necrosis, and a cell death index was obtained. *Proliferation:* at days 1, 3, 7 and 10 samples were subjected to Alamar Blue proliferation assay – a non-destructive assay that may be used on the same samples over time.

*Oxygen Monitoring:* Fibre optic oxygen probes (Oxford Optronix, Oxford, UK) were inserted into the centre of SC constructs and oxygen levels in the centre of cell-seeded constructs were measured over 5 days.

**RESULTS:** COL-I protein content averaged 12%, and for SC processed scaffolds. Despite the evidence of cell death (approx. 13 % of the total cell count) after 24 h in culture, no significant increase in the number of dead cells occurred as a function of time in the SC processed scaffolds for either cell type. Cell proliferation within the collagen sheets was confirmed for both cell types, and reached levels expected for the numbers of cells seeded within the scaffolds after 10 days in vitro. Oxygen levels in the core of such constructs were significantly low, showing depletion of oxygen by MG63 cells (*Fig. 1*).



**CONCLUSION:** SC collagen scaffolds maintained integrity and considerable MG63 and ADTC cell viability up to 10 days in culture and should be therefore further investigated as potential scaffolds for hard-tissue engineering. We have established a method for the monitoring of oxygen in the model PC collagen cultures and shown that the high density PC collagen material allows good perfusion of oxygen.

**REFERENCE:** [1] RA Brown, M Wiseman, C-B Chuo, U Cheema, SN Nazhat. *Advanced Functional Materials* 15, (2005) 1762.