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## **Engineering of Nano-Microscale Lamellae in a Model Collagen Based Scaffold**

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Collagen is the major structural component of most tissues and is commonly used in the form of a three-dimensional structural support lattice for cells in tissue-engineered constructs. Plastic compression (PC) is a new technique developed by our group for ultra-rapid tissue and biomaterial fabrication. A characteristic of the PC fabrication process is that gel fluid is expelled through one or more surfaces. This led to the hypothesis that fibrillar collagen would compact at high density at those surfaces, with potential for controlled nano-microfabrication of 'tissue lamellae'. The hypothesis was tested with polarised light and transmission electron microscopy.

Rectangular slab acellular type I collagen gels were prepared and compressed (standard PC protocol) to 7% of the original gel wet-mass. Control collagen gels were allowed to pre-compact for 3 hours under their own weight in a humid chamber to achieve maximal substrate stability (with 57% loss of fluid by weight). Gels were fixed for routine transmission electron microscopy. Image analysis showed a pronounced 'layered' effect where fibril density was 1.5 fold greater at the edge compared with the gel core ( $p < 0.005$ ). The effect of PC on collagen fibril diameter was also investigated. Polarised light microscopy of the compressed collagen constructs (stained with Picosirius Red) identified birefringent aligned collagenous 'lamellae' on the surface of the construct.

It is concluded that PC treatment of collagen constructs leads to the predictable and controlled formation of lamella structures, including alignment which may be useful in biomimetic materials and nanofabrication of tissues.

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