Introducing stable microgrooves in to fluid-leaving surface of plastic compressed collagen by embossing.

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INTRODUCTION: Micropatterning of polymers is a technique used in tissue engineering to create additional features on the surface usually to manipulate cell behaviour. However, methods used for fabrication of such features on the surface of synthetic polymers are not always suitable for natural polymers. Here we describe a method of embossing developed for plastic compressed collagen constructs. Plastic compression (PC) of collagen is a technique that allows cell-independent fabrication of dense, tissue-like collagen constructs without compromising viability of resident cells (1). Method of PC creates potential anisotropy of the opposite surfaces – stiffer fluid leaving (closest to the blotting elements) and more elastic non-fluid leaving surface. We hypothesized that embossing into these surfaces will give more stable features on the fluid-leaving surface (FLS) as opposed to non fluid-leaving surface (NFLS). Objectives were to develop methods of embossing onto both surfaces, assess these features and to determine stability of the embossed pattern over time with and without cells. Slow-dissolving phosphate-based glass fibers were used as the embossing template in this study as fiber diameter and spacing is easily controlled.

METHODS: Collagen type I (acid-soluble, rat-tail, FirstLink, UK) was neutralized and set prior to plastic compression. Glass fibers (diameter 35-40 µm, average spacing 70 µm) were pressed into the collagen gel during standard PC, into the FLS or NFLS (Fig1) for pattern embossing.

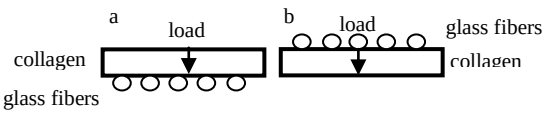

Fig1. Schematic representation of the PC embossing on to the FLS (a) and NFLS (b). Arrow – direction of fluid flow.

Constructs were fixed and features analyzed using SEM and routine wax histology. Stability of the grooves embossed on to FLS was then tested by culturing acellular and cellular (seeded with human corneal fibroblasts, 5x10 ^{5} per construct) constructs in the standard culture medium (DMEM) for two weeks. Grooves dimensions were measured at days 1, 7 and 14 using SEM and wax histology. Data are presented as mean ±SD. Statistical analysis was carried out using non-parametric unpaired t-test (GraphPad Prism 3.0 software, USA); value of $p < 0.05$ was considered significant.

RESULTS: Embossing of the glass fibers on to PC collagen resulted in deeper, more pronounced grooves on the FLS as opposed to the NFLS (Fig.2a, b). Measured depth of the indentations was almost 3 fold greater in the FLS (23±3.8 µm) than NFLS (8.2±2.2 µm).

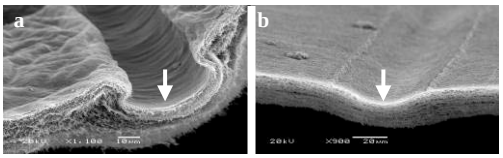

Fig. 2. SEM images of features, embossed in to the PC collagen (arrows). a- FLS, b-NFLS

Importantly, the depth of grooves embossed on to FLS did not change significantly over two weeks culture period in either cellular or acellular constructs (Fig.3a, b).

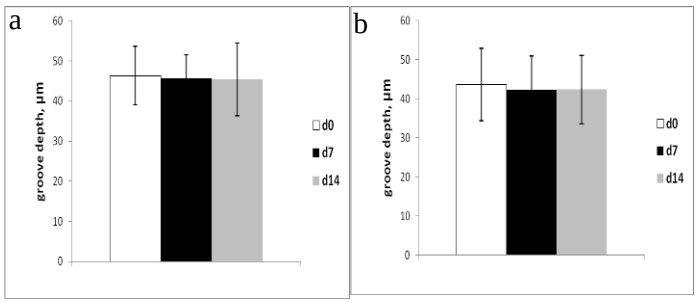

Fig.3 Average depths of the grooves embossed on to FLS of a – acellular PC collagen constructs b - seeded with corneal fibroblasts. Measured at days 1, 7 and 14 in culture.

DISCUSSION & CONCLUSIONS: Embossing of the predictable microfeatures is more effective on to the FLS of the PC collagen and features are stable for at least 2 weeks culture regardless of cell activity.

REFERENCES: [1]Brown R.A. et al. (2005) *Adv. Funct. Mater*. **15(11).** 1762-1770

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