Fabrication of Free-standing Casein Microstructures with Bioimprinted Cellular Surface Features

A. Hashemi[1], I. Mutreja[1], M. A. Ali[2], M. M. Alkaisi[1], V. Nock[1]

[1] MacDiarmid Institute for Advanced Materials and Nanotechnology, Electrical and Computer Engineering, University of Canterbury, Christchurch, New Zealand

[2] Department of Applied Sciences, University of Otago, Dunedin, New Zealand

Micro-scale devices made of casein, the main protein of cow's skimmed milk are gaining increasing interest for use as biodegradable, stand-alone orthopaedic implants[1] and tissue engineering substrates[2]. While a strong mechanical microstructure and no toxicity make casein a suitable material for implantation, acute inflammatory reactions of the surrounding tissue remain a problem[1]. To influence and potentially ease reaction of the surrounding tissue[3], we propose the incorporation of biomimetic tissue-like surface features onto the cross-linked protein surface. In this paper we demonstrate for the first time the transfer of positive and negative cellular bioimprints[4-6], with combined micro- and nanoscale resolution onto biodegradable protein devices.

To achieve this we developed a two-step fabrication procedure (see Fig. 1). The first step involves the replication of desired shapes, such as biological material, using polydimethylsiloxane (PDMS) replica casting to produce a negative mold. This is followed by a second cast of the initial mold to produce a positive PDMS mold. Liquid casein solutions of various compositions are then cast onto both types of molds to produce free-standing protein structures with positive or negative high-resolution surface features. In this paper the patterning of casein will be characterized using regular lithography-defined shapes, as shown in Fig. 2. We will also discuss protein cross-linking, bioimprint replication (see Fig. 3) and the effect of the imprinted surface features when used in culture with secondary cells.

References:

1) W. Wang, et al., *Macromol. Biosc.*, 2011, 11, 914-926.
2) B. Dhandayuthapani, et al., *Int. J. Pol. Sci.*, 2011, 2011.
3) W. Y. Tong, et al., *Biomaterials*, 2012, 33(31), 7686-7698.
4) J. J. Muys, et al., *J. Nanobiotechnol.*, 2006, 4(1), 1.
5) L. M. Murray, et al., *J. Vac. Sci. Technol. B*, 2012, 30, 06F902-6.
6) L. M. Murray, et al., *J. Nanobiotech.*, 2014, 12(1), 289

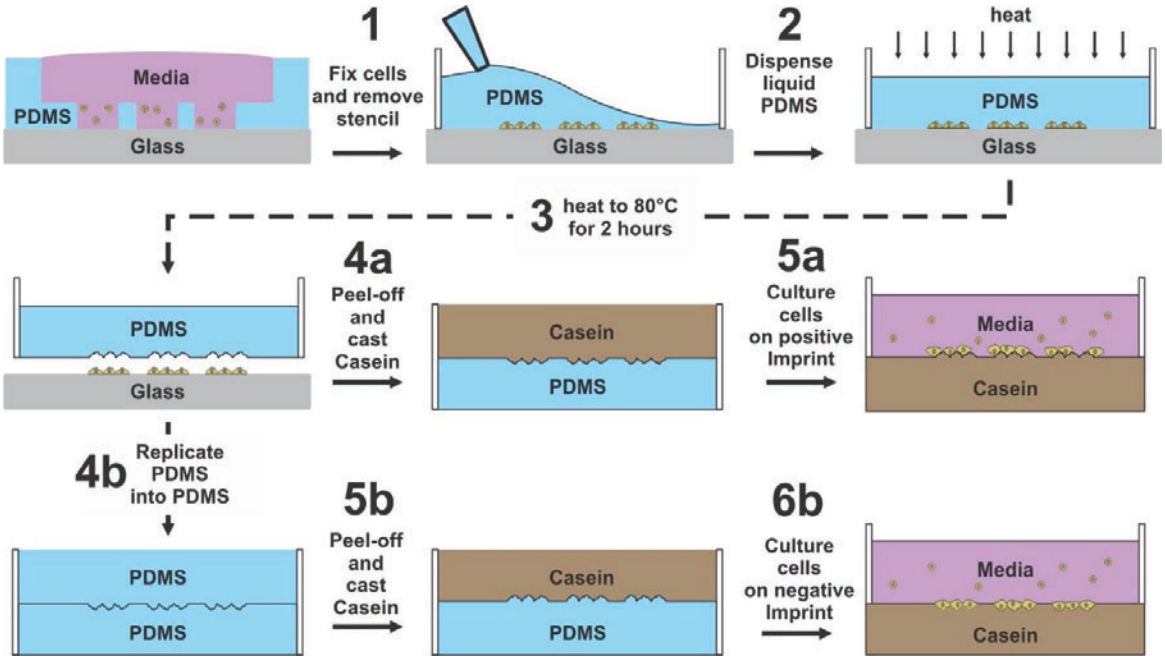

Figure 1: Schematic of the replication of 3D bioimprint patterns onto freestanding casein structures with 4a) negative PDMS mold and 4b) positive PDMS mold to yield positive (5a) and negative (6b)) casein imprints, respectively.

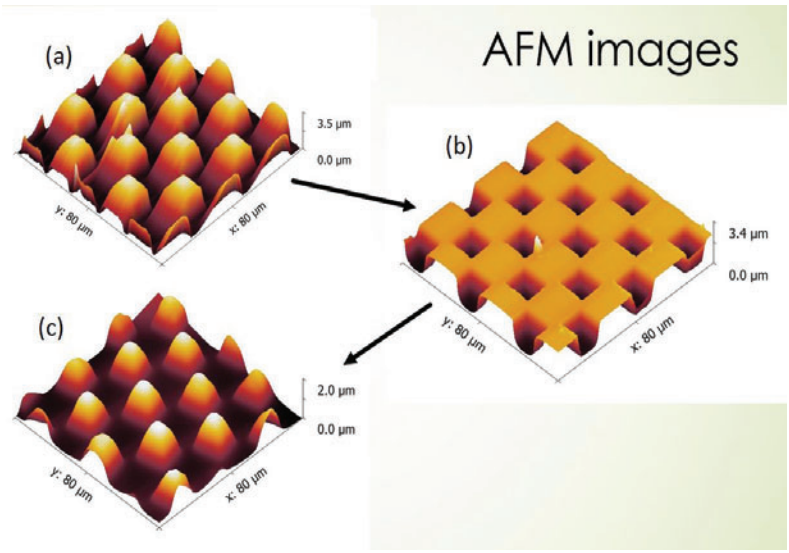

Figure 2: Atomic force microscopy (AFM) images of photo-lithographically defined micron-scale patterns used to characterize feature transfer from photoresist (a) via PDMS (b) into casein (c).

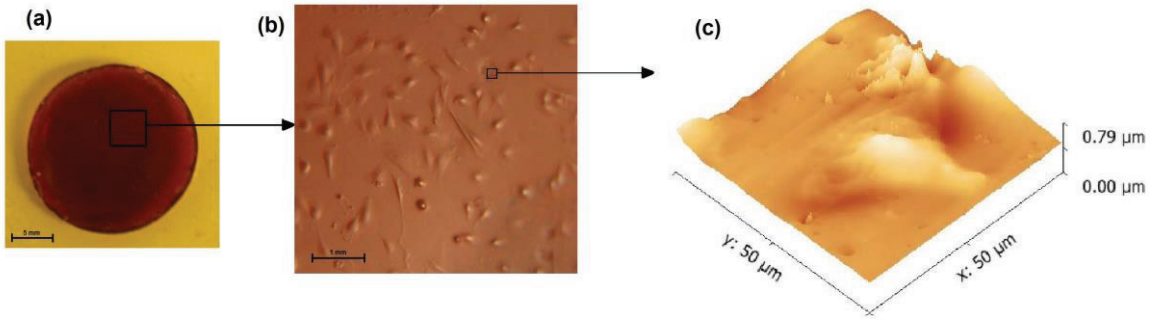

Figure 3: a) A cross-linked casein film, b) optical image of positive bioimprints on the casein film, and c) atomic force microscopy (AFM) image of the positive imprint of a single cell on the casein surface.