Polymeric Substrates with Bioimprinted Micro- and Nanoscale Topography for Regulation of Chondrocyte Re-Differentiation

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Polymeric substrates have the ability to influence attachment, proliferation and gene expression of anchorage-dependent cells such as chondrocytes. Three-dimensional scaffolds provide a repair strategy for cartilage defects but require extensive in-vitro cell expansion[1]. Chondrocytes, spherical in-vivo, lose phenotype or re-differentiate to more fibroblastic shape during expansion in monolayers. Surface modification techniques which regulate re-differentiation of expanded human chondrocytes are thus of significant clinical importance. Bioimprint is a nanolithography technique capable of creating permanent surface modifications with micro- and nanoscale cellular features[2]. In this paper we demonstrate the use of this technique to fabricate polymeric substrates with imprinted chondrocyte replicas at various expansion stages.

The substrates were fabricated by cell patterning using polydimethylsiloxane (PDMS) stencils[3] and subsequent bioimprinting, as illustrated in Fig. 1. The stencils were placed on glass substrates and contained by macroscopic PDMS wells. Human nasal chondrocytes were cultured in the wells and bioimprinted at various expansion stages at 1 h, 6 h, 12 h and 24 h. Primary imprints were separated from the substrate, washed to remove cell material, and characterized using light- and atomic force microscopy (AFM, see Fig. 2b). Additionally, secondary positive replicas were formed by curing liquid PDMS pre-polymer on the initial imprints, as shown in Fig. 1(4b). These secondary replicas can then be imprinted back into other materials, yielding homogeneous substrates with biomimetic, expansion-stage specific topography equivalent to the original cells (see Fig. 3). This is demonstrated by the successful replication of early un-adhered spherical cells (1 h) vs. flat expanded fibroblast-like cells (24 h) into PDMS, and successively PS. We will further discuss the effect of these surfaces on chondrocytes in expansion and how the technique can be used to yield novel insights into general cell mechanics and tissue formation.

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[1] Woodfield, T. B. F., et al., Biomaterials, vol. 27, pp. 1043-1053, 2006.
[2] Nock, V., et al., J Vac Sci Tech B, vol. 28, pp. C6K17-C6K22, 2010.
[3] Folch, A., et al., J Biomed Mater Res, vol. 52(2), pp. 346-353, 2000.

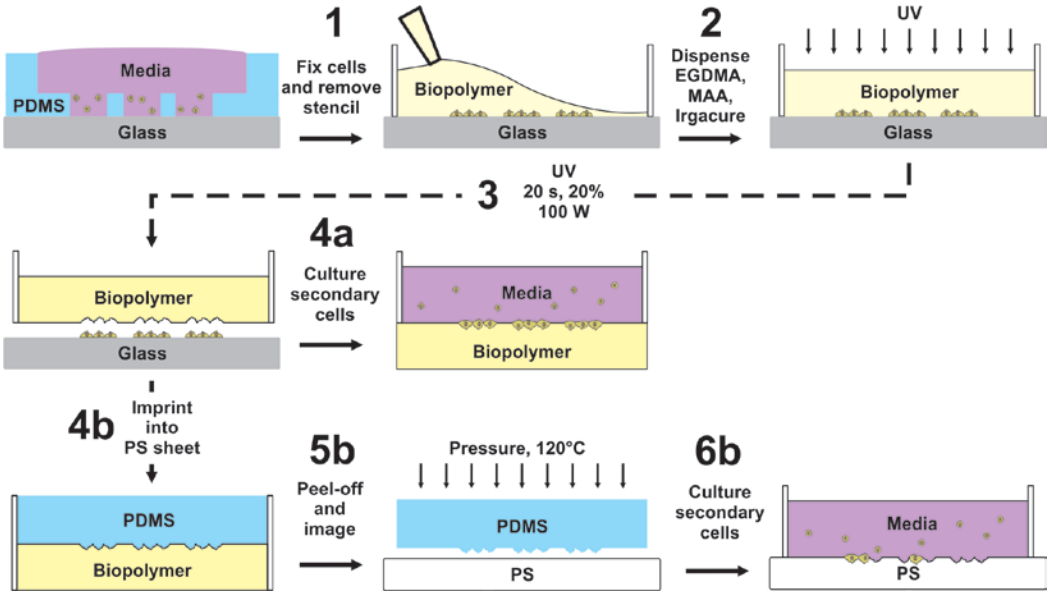

Figure 1: Schematic of the Bioimprint process.

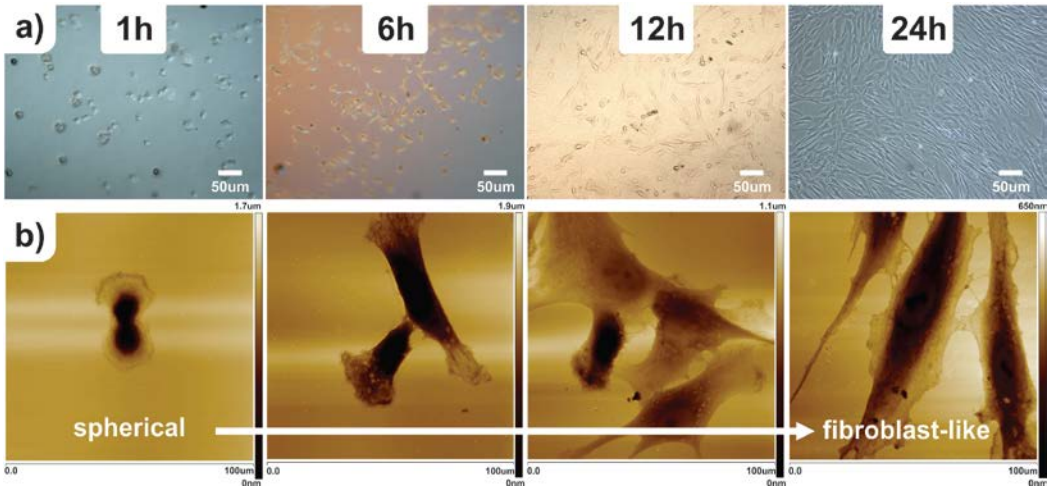

Figure 2: a) Chondrocyte bioimprints at different stages. b) Typical AFM scans.

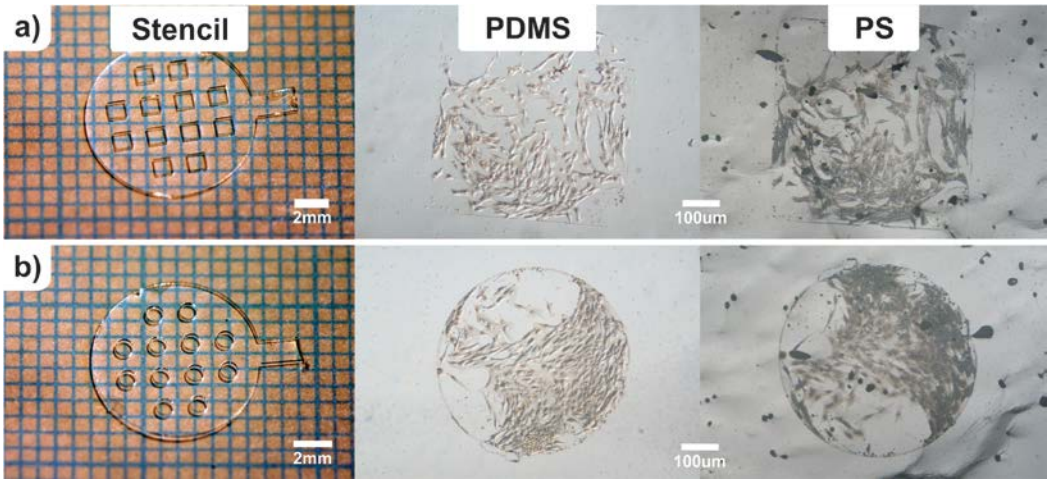

Figure 3: a) Square and b) round chondrocyte micropatterns replicated into PS.