Surface modification and patterning of proteins on photocrosslinkable glycosaminoglycans

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Natural polymers such as polysaccharides might have advantages for biological samples and cell-based devices because they are natural components of the *in vivo* microenvironment [1]. A method combining natural photocrosslinkable polymers such as hyaluronic acid (HA) with micromolding approaches was presented and characterized in order to fabricate hydrogels able to immobilize cells.

Recently, photolithography and soft lithography have been used to encapsulate live cells within microscale polymeric hydrogels (i.e. microgels) anchored onto two-dimensional (2D) surfaces. This offers great potential for diagnostics and cell screening applications. Yeh et al. [2] presented a micromolding approach to generate cell-encapsulating 3D hydrogels of controlled shapes and sizes in the form of harvestable free units. Cells were suspended in a hydrogel precursor solution containing photoinitiator, deposited onto hydrophilic poly(dimethylsiloxane) (PDMS) patterns, crosslinked under UV radiation, and retrieved upon hydration. Two common photocrosslinkable hydrogel materials, methacrylated hyaluronic acid (MeHA) and poly(ethylene glycol) diacrylate (PEGDA), were tested using this technique, yielding shape controlled microgels with homogeneous cell distribution at various viable cell densities.

In this work, two glycosaminoglycans (GAGs), MeHA and chondrotin sulfate methacrylate, were synthesized and microstructured combining soft lithography and UV crosslinking, initiated by addition of an ultraviolet photoinitiator, Irgacure 369 (kindly supplied by Ciba). Solutions of these GAGs were deposited on silicon substrates treated with 3-(trimethoxysilyl) propyl methacrylate [1]. PDMS stamps were moulded using silicon masters previously fabricated by conventional UV-Lithography and deep ultraviolet (DUV) optical lithography (λ = 248nm) and etched by a SF_6/C_4F_8 and $Cl_2/HBr/O_2$ gases combination, respectively. The PDMS stamp microstructures were transferred to the GAGs substrates using an EVG620 mask-aligner curing with UV light (Figure 1).

We have also studied the localized anchorage of proteins (for a subsequent study with human mesenchymal stem cells) over these materials by microcontact printing of poly(OEGMA-co-MA) [3], amino-PEG and PLL-g_PEG, so that areas that avoid the adhesion of proteins can be created, giving rise to well-defined patterns (Figure 2).

References

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[2] Yeh J., Ling Y., Karp J.M., Gantz J., Chandawarkar A., Eng G., Blumling III J., Langer R. and Khademhosseini A. Biomaterials **27** (2006) 5391.
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Figures

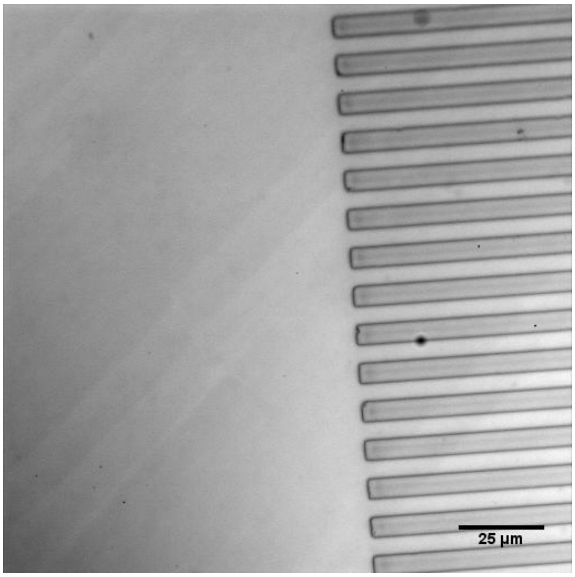

Figure 1. Methacrylated hyaluronic acid microstructures. Period = 11.3µm

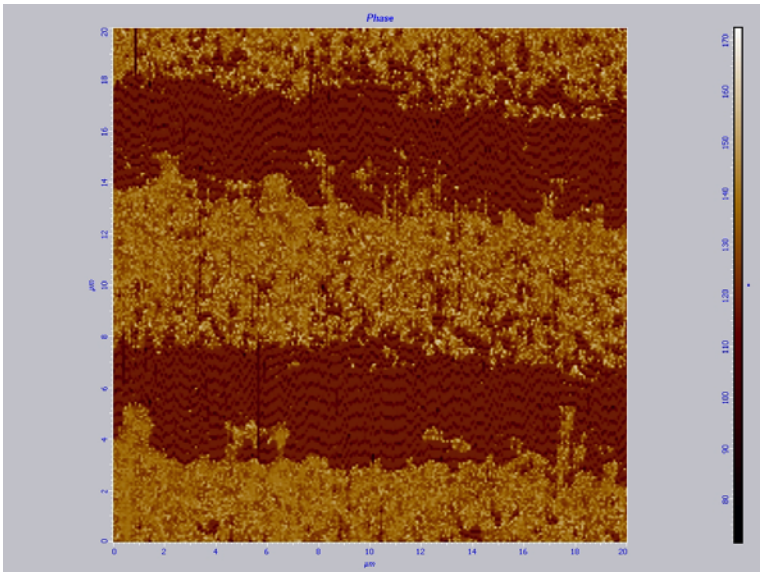

Figure 2. AFM - change of phase image showing microcontact printed P(OEGMA-co-MA) over a chondroitin sulfate methacrylate substrate. Period ~10µm