Bottom-up Modification of Biomaterial Surface for Control of Cell Adhesion and Alignment

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Statement of Purpose: Selective cell adhesion and cell alignment play an important role in a number of biomedical applications including tissue engineering and regenerative medicine.[1] Surface modification[2] combined with microfabrication and micropatterning[3] techniques is widely utilized to spatially control and modulate cell behavior. Here we report a simple method of chemical modification of glass surface to introduce hyaluronic acid (HA), which directs the specific adhesion and aligned orientation of cells on the substrate without using any top-down patterning approaches.

Methods:

Glass surface modification and characterization. Glass slides were silanized to create an amine-modified surface. HA was covalently attached to the amines using carbodiimide chemistry (EDC/NHS) via its carboxylic acid groups. The silanized glass slide was oriented in the vertical direction and dipped into a solution of HA with EDC/NHS. Each step of glass surface modification was characterized and verified using XPS analysis.

Cell culture and adhesion on modified glass surfaces. Modified and unmodified glass was placed in cell culture dishes. T98 human glioma cells and normal human dermal fibroblasts (NHDFs) were cultured on different glass surfaces (untreated glass, amino-silanized glass, and HA-modified glass) in complete medium with serum or in serum-free medium for up to one week and imaged.

Results and Discussion:

XPS analysis. XPS results of glass, amino-silanized glass, and HA-modified glass showed the expected characteristic peaks of binding energy, verifying the success of each surface modification step.

Cell adhesion to modified glass substrates. T98 cells and NHDFs adhered and spread to all types of glass substrate when cultured with complete medium containing serum.

Cell orientation and alignment on HA modified glass substrates in serum and serum-free conditions. T98 cells appeared to be elongated and aligned in the direction and orientation of HA modification (Fig. 1). NHDF attachment, alignment, orientation, and morphology did not appear to change when cultured on the different substrates (A, E). In serum condition, T98 cells attached, aligned, and oriented on HA-modified glass surface (F), and appeared elongated compared to cells cultured on untreated glass (B). In serum-free condition, T98 cells attached HA-modified surfaces (G and H), but the alignment and orientation of the cells occurred at a slower pace (G and H compared to F). When cultured on untreated glass without HA, the cells appeared rounded (C and D compared to B, G, and H).

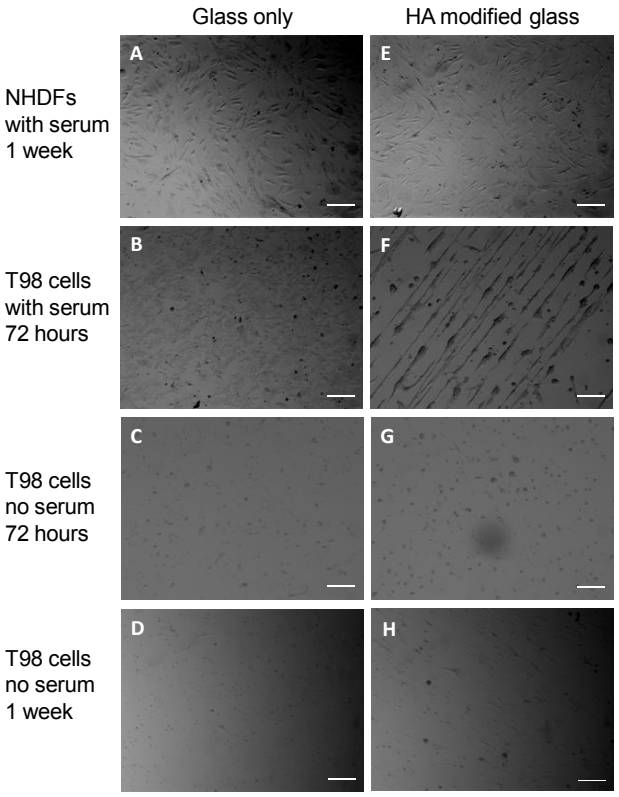

Figure 1. NHDFs and T98 cells cultured on untreated glass (panels A, B, C, D) or HA-modified glass surfaces (panels E, F, G, H) in serum (A, B, E, F) or serum-free condition (C, D, G, H) for 72 hours and 1 week. Scale bar = 250 μm

Conclusions: Modification of non-specific cell-binding surfaces with selective/specific cell-binding biopolymers may provide control of cell-substrate interactions. Our method of chemically modifying glass surfaces with HA results in adhesion and oriented alignment of cells without using any top-down printing or lithographic approaches. The observed cell alignment is presumably due to the alignment and self-assembly of HA polymer chains chemically attached to the glass surface. We consider the use of T98 cells and NHDFs as proof-of-principle cells, and aim to investigate the potential use of therapeutically relevant cells and controlling their adhesion and orientation by modulating the biomaterial substrate.

References:

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3. Khademhosseini A. *et al*. Biomed. Microdevices. 2007; 9:149-157.