Fabrication of Artificial Surface-bound Morphogen Gradient for Controlling Progenitor Cell Fate

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Statement of Purpose: During embryogenesis, morphogen gradients organize cells and determine patterns of tissue organization as a result of concentration-dependent ligand binding (Gurdon JB and Bourillot PY. Nature. 2001;413:797-803). In order to understand these mechanisms at molecular level, a method is needed to generate stable and predictable concentrations of morphogenic molecules, which are highly potent and secreted in tiny amount (Ingham PW and McMahon AP. Genes Dev. 2001;15:3059-3087). Here, a method is described for fabricating controlled concentrations of a morphogen, sonic hedgehog (SHH), on polymeric substrate via immobilization of its specific affinity heparan sulfate (HS) carrier. The potential usage of SHH gradient surfaces to control tissue patterning is also demonstrated through the differential lineage commitment of embryonic chick limb bud progenitor cells on them.

Methods: Polyethylene terephthalate (PET) film was preirradiated with UV using a previously reported technique (Li B, et al. Biomaterials. 2005;26:1487-1495). Selective exposure was achieved by moving substrate on a stage against the photomask. The substrate was then put in 10 wt% acrylic acid (AA) and exposed to UV irradiation for 10 min. The substrate was then incubated with 4 mM biotin hydrazide, 100 mM 1-Ethyl-3- (3-dimethylaminopropyl)-carbodiimide hydrochloride, and 120 mM N-hydroxysuccinimide for 24 h, followed by incubation with 0.1 mg/ml streptavidin for 30 min. It was then treated with biotinylated HS for 1 h. Finally the HS-derivatized PET substrate was supplemented with recombinant myristoylated N-terminal fragment of SHH (SHH-N, R & D Systems) at 10 ng/ml for 1 h.

Results: A multi-step surface immobilization process was performed to fabricate SHH-bound polymer substrates (Figure 1A). Poly(acrylic acid) (PAA)-grafted PET substrates were prepared by UV preirradiation (preUV) followed by graft polymerization of AA. Biotin hydrazide (BH) was subsequently reacted via carbodiimide coupling with the exposed –COOH moieties of PAA. Streptavidin (SAV) was affinity-bound to the grafted biotin and it was then used to attach biotinylated HS. The resulting anchored HS immobilized SHH morphogen molecules, providing a functionally-active substrate of signaling molecule.

Controlling the surface density of –COOH by preUV time allowed for immobilizing specific surface concentrations of SHH. When the substrate was moved underneath a photomask, it remained in a given position for a specific amount of time. Translating a window across the substrate at variable speed generated a gradient whose steepness and profile may be varied (Figure 1B-D). To further verify that the surface density of HS could be decided by preUV time, ESCA analyses were performed with the substrates at various stages of reaction. The element ratio N/C, representing the richness of surface-bound HS, increased with prolongation of preUV time (Figure 2). All these indicated that HS immobilization was controlled by the PAAc grafting density, which in turn was determined by preUV exposure.

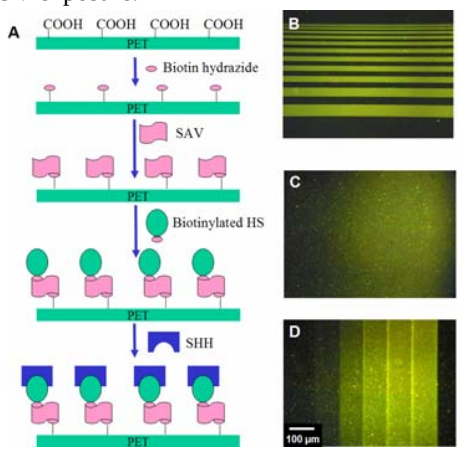

Figure 1. (A) Schematic of the procedure for immobilizing SHH on polymer surface. (B-D) Pattern or gradients of surface-immobilized fluorescein-labeled HS.

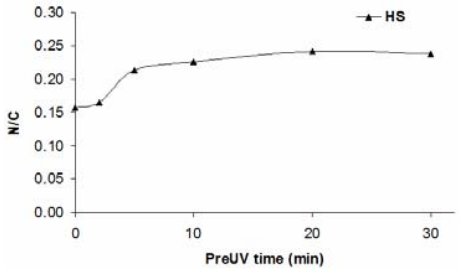

Figure 2. Relationship of the density of surface-bound HS and UV preirradiation time.

Next SHH was affinity-bound to HS surfaces using the known mechanism (Perrimon N and Bernfielf M. Nature. 2000;404:725-728). The amount of surface-bound SHH was too little to be directly measured therefore the cellular responses on SHH-bound substrates were tested instead. In preliminary cell culture trials with naïve limb bud precursor cells on a stepped surface gradient of SHH, cells plated on the strips above 5 min of preUV went from a precursor mesenchymal shape to rounding up and producing long, neuritic-like cytoplasmic extensions. On strip of 5 min exposure, an elongated bipolar phenotype became evident. Such changes were further confirmed by immunoblotting and semi-quantitative PCR for marker of specific lineages, implying the possibility that SHH alone can trigger important feedback loops needed for contextual lineage development on a 2-D surface.

Conclusions: A facile approach has been developed for creation of stable and predictable morphogen gradients on polymer substrate. Limb bud precursor cells exposed to these gradients substrates showed differentiation in relation to the amount of SHH-associated HS. This study established a means of directing precursor cells into various lineages using gradients of morphogen created on artificial bioinformational platforms.