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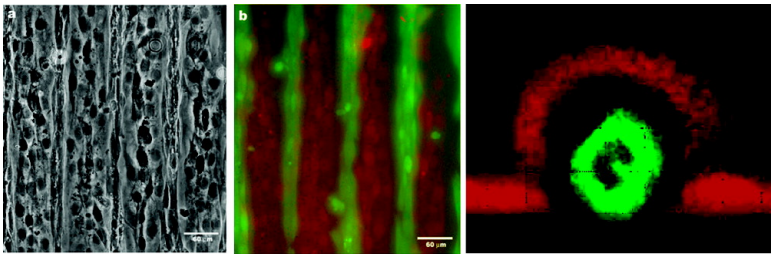

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Biocompatible Micropatterning of Two Different Cell Types

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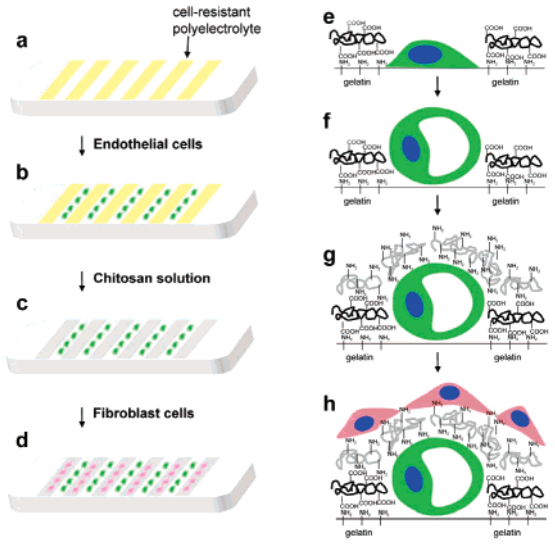
The past decade has brought notable advances in culturing cells on bioactive degradable scaffolds to guide their assembly into functional tissues. However, the single most important feature of most living tissue, complexity and cooperative function resulting from the specific spatial organization of multiple cell types, has remained largely unaddressed. Replicating in vitro this complexity and function is not possible using traditional co-culture techniques wherein multiple cell types are randomly seeded among themselves. Here, we demonstrate a new method based on polyelectrolyte assembly for controlling the arrangement of multiple cell types with subcellular resolution on biomaterials. The efficacy of this technique is demonstrated by organizing two different cell types, 3T3 fibroblasts and endothelial cells, on chitosan. The significance of this technique and its potential to guide the formation of blood vessels that deliver oxygen and nutrients, thereby eliminating constraints on the size of tissues that can be engineered in vitro, are demonstrated by co-cultures of 3T3 fibroblasts with line patterns of endothelial cells induced to form capillary tube-like structures having a central lumen.

Coordinated communication and heterotypic cell interactions are central to the function of many tissues, for example, hepatocyte function is enhanced when hepatocytes are co-cultured with supporting fibroblasts or endothelial cells,[1] blood vessels form only when endothelial cells and smooth muscle cells interact properly,[2] and nerve systems function only with proper neuron−glia cell interactions.[3] Controlling the extent of these heterotypic cell interactions, through precise arrangement of different cell types relative to each other, is key to significant advances in the development of functional engineered tissues.

The spatial distribution of individual cell types can now be routinely controlled through soft-lithography-based micropatterning techniques.[4,5] Controlling the spatial organization of multiple cell types, however, remains a challenge. Bhatia et al.[1] have elegantly patterned hepatocytes and fibroblasts using photolithography and manipulation of serum content of cell culture media. However, the success of this approach is dependent on the relative adhesiveness of the two cell types toward the substrate. Chiu et al.[6] have developed a versatile three-dimensional microfluidic approach to direct the flow and patterning of different cell types. Although this technique can be applied to pattern multiple cells into complex structures, the microfluidics limit pattern size, and the channel structure precludes heterotypic cell interactions. Yousaf et al.[7] have developed electroactive substrates to pattern two fibroblast populations by electrochemical switching of the substrate adhesiveness. Although indispensable for understanding heterotypic cell interactions, electrically conductive substrates may be unsuitable for tissue engineering applications. Here, we present a new approach for organizing two different cell types on biodegradable chitosan and gelatin substrates through multilayer assembly of cell-resistant and cell-adhesive polyelectrolytes.

Scheme 1 [a]



[a] (a−d) Strategy for patterning two different cell types on chitosan or gelatin films. (b) Micropatterns of cell-resistant anionic polyelectrolyte on biomaterial surfaces confine the attachment of the first cell type. (c) Adsorption of cell-adhesive cationic chitosan on cell-resistant polyelectrolyte renders these regions cell-adhesive, allowing for the attachment of a second cell type (d). (e−h) Strategy for assembling tube-like vessels of endothelial cells within a second cell type. Cell nuclei are colored blue.

The principal challenge in micropatterning multiple cell types lies in the development of a noncytotoxic procedure for converting background cell-resistant regions that define the arrangement of the first cell type into cell-adhesive regions to allow for the attachment of another cell type. The technique we developed to accomplish this transformation is shown in Scheme 1a−d. First, with a chitosan substrate, a cell-resistant anionic copolymer of oligoethyleneglycol methacrylate and methacrylic acid, poly-(OEGMA-co-MA), is microcontact printed as a series of 60 μm lines.[8] Monolayers of the first cell type (human vascular endothelial cells) naturally attach and proliferate only within the 20 μm lines of bare chitosan separating the 60 μm wide lines of cell-resistant polyelectrolyte. The substrate is subsequently immersed into a medium containing cationic chitosan that electrostatically binds onto the 60 μm wide lines of cell-resistant polyelectrolyte, rendering these regions adhesive to a second type of cells (3T3 fibroblasts). Optical phase contrast (Figure 1a) and fluorescence (Figure 1b) microscopy demonstrate that endothelial and fibroblast cells can be sequentially patterned with micrometer accuracy, leaving no gaps between the complementary line patterns of the two cell types. Cross-sectional confocal imaging (Figure 1c) reveals that the fibroblast cells are offset vertically by ∼0.25 μm, which can be adjusted by varying the concentration of the poly(OEGMA-co-MA).

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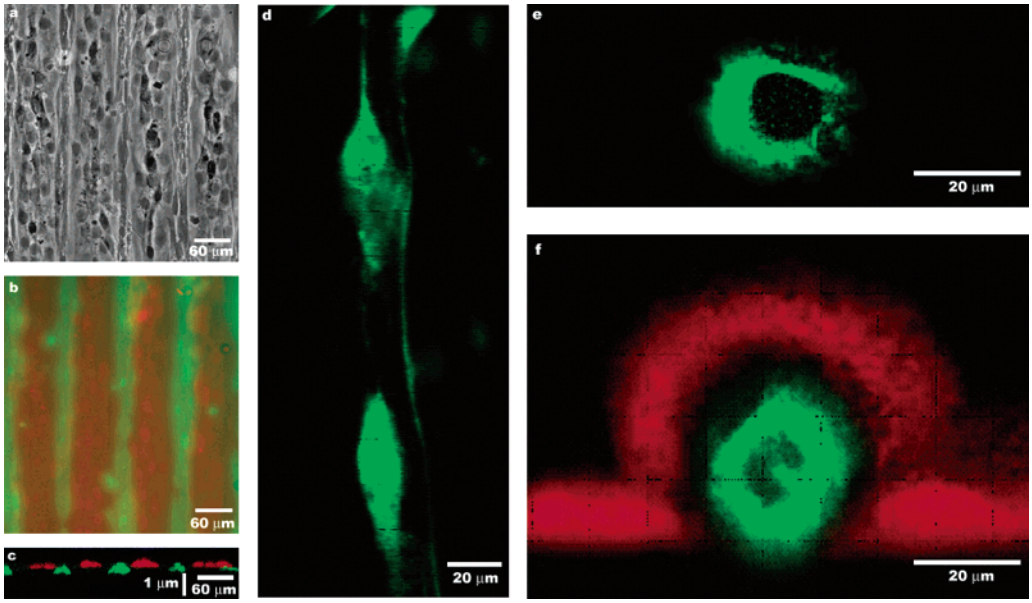

Figure 1. (a−c) Micropatterning of human microvascular endothelial cells and 3T3 fibroblast cells prelabeled with Cell Tracker Green and Orange, respectively. (a) Phase contrast image of endothelial and fibroblast cells patterned within 20 and 60 μm wide lines, respectively. (b) Fluorescence image of the two cell types shown in (a). (c) Confocal image of the cross section corresponding to (b). (d−f) Endothelial capillary-like tubes. Horizontal (d) and vertical (e) confocal image cross sections of human microvascular endothelial cells cultured on a 20 μm line show a central cavity extending along cells. Bulges are due to the cell nuclei (colored blue in Scheme 1e−h). (f) Confocal image of the vertical cross section of tube-like structure formed by endothelial cells within a second cell type, fibroblasts.

To demonstrate how this approach can be applied in tissue engineering, we apply it to the assembly of structured co-cultures of fibroblasts with endothelial cells that have been induced to form capillary tube-like structures.[9] Fibroblasts were used here as a model, and other cells can be co-cultured using this approach. Unlike larger diameter blood vessels, implantation of capillaries assembled in vitro is difficult because of their small size and fragility.[10]

In this procedure, shown schematically in Scheme 1e−h, endothelial cells attach within 20 μm lines of bare gelatin separated by 60 μm wide lines of cell-resistant poly(OEGMA-co-MA) (Scheme 1e). After 5 days of proliferation, the micropatterned endothelial cells form capillary tube-like structures (Scheme 1f). Confocal images of horizontal and vertical cross sections (Figure 1d,e) confirm the existence of a central lumen, which appears as a dark central space extending over multiple cell lengths. Bulges in the confocal cross sections are due to the cell nucleus, which is colored blue in the schematic (Scheme 1e−h). The substrate with its assembled capillary is subsequently immersed into a solution of water-soluble chitosan that binds onto the separating gaps of cell-resistant polyelectrolyte and the capillary itself (Scheme 1g), rendering these surfaces adhesive to a second cell type, fibroblasts (Scheme 1h). Confocal imaging of the vertical cross section (Figure 1f) confirms that the fibroblast cells fully cover both the capillary and previously cell-resistant regions of poly(OEGMA-co-MA). Although capillary tube-like structures have been formed on patterned gold substrates,[9] this is the first demonstration of capillaries patterned on a biomaterial in the presence of a second cell type. Many questions and challenges remain to be addressed before assemblies of tissue-specific cell types with embedded capillaries can be implanted in vivo; for example, how do the mechanical properties of these capillaries compare with those in vivo? Will embedded capillaries integrate with existing vascular structure in vivo? Do mechanical forces due to blood flow influence capillary architecture?

In comparison to other techniques for patterning two different cell types, the polyelectrolyte assembly approach reported here is noncytotoxic and allows arbitrary geometric patterns to be formed on biocompatible biodegradable substrates, such as chitosan and gelatin, without the need for electroactive substrates or external fields. However, the biocompatibility of poly(OEGMA-co-MA) remains to be tested, and synthesis of cell-resistant polyanions with enhanced biodegradability is now in progress. Nonetheless, the approach presented here is highly scalable, and the extension to three-dimensional tissue structures can be accomplished by stacking layers of two-dimensional patterns.[11] The organization of multiple cell types on biomaterials is an important first step toward the bottom-up assembly of cells to replicate tissue complexity and function.

Supporting Information Available: Experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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