Engineering Dynamic Biointerfaces

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Abstract

Recent advances in dynamic biointerfaces enable spatiotemporal control over cell position and migration after attachment using substrates that employ chemical, optical, thermal, or electrical triggers. This review focuses on flexible and accessible methods for the fabrication of cellular arrays or co cultures for fundamental studies of cell biology or regenerative medicine.

Introduction

The biological function of most tissues results from the specific arrangement of different cell types. For example, hepatocytes and hepatic stellate cells interspersed with endothelial cells form permeable sinusoids that facilitate transport to and from the bloodstream. This fine alternating arrangement of multiple cell types arises from time-varying spatial heterogeneities of the extracellular matrix. While the details of how multicellular tissue patterns form is currently under intense investigation, it is clear that geometric control of heterotypic cell-cell and cell-substrate interactions is key to engineering functional tissues and addressing fundamental questions in developmental cell biology. Three-dimensional bioprinting of biocompatible materials and cells creates several tissue types;[1] however, the sensitivity of living cells to shear generated by narrow channels in the printing nozzles sets opposing constraints on printing speed and resolution. In this review, we focus on methods for patterning different cell types with single-cell precision (Figure 1) and directing the migration of individual cells for use with cell-sheet engineering[2] or bioprinting for the efficient generation of cellular ensembles with single-cell precision.

Engineering Cell-Surface Interactions

Cells respond to substrate heterogeneities by altering aspects of their behavior, including spreading,[3] cell-cycle progression[4], apoptosis, polarity,[5] and migration.[6] Generally, hydrophobic, charged, and electron-accepting groups on surfaces promote protein adsorption and subsequent cell attachment, while poly(ethylene glycol) and zwitterionic materials inhibit attachment of protein and cells. Rather than passive ECM adsorption, surface-bound peptides with high integrin binding affinity such as Arg-Gly-Asp (RGD) attach directly to cell adhesion receptors.

Whitesides[7] pioneered control over cell shape and spreading area using microcontact printed poly(ethylene glycol)-terminated thiol inks on gold substrates(Fig 1A). Poly(ethylene glycol) (PEG) resists adsorption of extracellular matrix proteins and thus prevents cell deposition. Other PEG-based methods for cell organization include ultraviolet photolithography of PEG diacrylates,[8] microcontact printing of cell-resistant polyelectrolytes,[9] photochemically effected click reactions,[10] sequential removal of Parafilm,[11] and culturing in aqueous two-phase systems.[12] While these methods direct adhesion of a cell population, they do not readily allow temporal influence over cell adhesion.

Temporal control of cell adhesion

Dynamic chemical[13-17], optical, thermal[18] or electrical[19-21] cues modulate cell-adhesion characteristics of substrates. Mimicking the *in-vitro* behavior of heterotypic cellular contacts requires micrometer-scale control over cell attachment and spreading, imposing limitations on the technique. For example, while poly(N-isopropylacrylamide) permits reversible attachment and detachment of cells as a function of temperature,[18] establishing steady and sharp temperature differences to control adhesion characteristics over cellular length scales remains a challenge.

Switchable substrate can be formed by binding a biocompatible ligand with affinity for cells opposite that of the original surface. An early and straightforward technique employs electrostatic chitosan adsorption onto microcontact-printed patterns of cell-resistant copolymers, converting the printed polymer to cell-adhesive.[13] Switching cell-adhesion characteristics facilitates sequential co-patterning of different cell types or dynamic control over cell spreading.[22] Likewise, avidin-biotin bioconjugation yields protein and cell patterns from a surface appended with biotin,[14] and organoclick chemistry switches a ligand-bearing surface. Strained alkynes spontaneously couple with an azide, without a cytotoxic copper catalyst; addition of cyclooctyne RGD to cells cultured on an azide-bearing surface enables dynamic control of cell spreading.[15] A substrate-bound boronic acid clicks with a diol-conjugated RGD, producing a cell-adhesive surface. However, unlike the Huisgen click reaction and poly(cation) adsorption (Fig. 1C₁), boronate esters readily interconvert when exposed to

vicinal diols. Addition of sugar to the culture media results in surface loss of RGD and recovery of cell resistance.[16, 17, 23]

Light offers a convenient trigger for dynamic alteration of substrate cell-adhesion characteristics, since photolithography intrinsically offers sub-cellular resolution. Photosensitive linkers most often exploit the synthetic accessibility and high quantum efficiency of the nitrobenzyl ester functionality. While cleavage occurs at near-UV ($\lambda = 365$ nm) wavelengths, cells tolerate the small dose required. Attachment of a photosensitive nitrobenzyl group to RGD eliminates its cell-adhesive behavior. Coupling nitrobenzyl-RGD to glass[24] or gold[25] creates an initially cell-resistant surface. Spatially-defined photolysis releases the nitrobenzyl cage, revealing RGD to promote cell attachment (Fig. 1D₁); uncaging nitrobenzyl RGD with transdermal light exposure regulates cell adhesion *in-vivo*. [26]

Linking RGD through a nitrobenzyl group triggers release of adhered cells into solution upon illumination through detachment of adhesive peptides from the surface.[27] Conversely, coupling a cell-resistant group to a surface through a nitrobenzyl linker yields a platform that becomes cell adhesive upon exposure to light to, for example, externally manipulate cell spreading. Preexisting surface chemistry ordinarily dictates the synthetic technique for photosensitive poly(ethylene glycol) attachment. (Fig. 1D₂) PEG-nitrobenzyl succinimidyl esters, PEG-nitrobenzyl azides and PEG-nitrobenzyl thiols functionalize amino, propargyl, and gold[28] surfaces, respectively. Photoconvertable cell-resistant polymers offer a substrate-independent alternative, as they do not require covalent surface modification.

A more general approach relies on initially cell-resistant photocleavable polymers. Printable on a variety of surfaces, these photo-switchable polymers enable light-controlled cell-adhesion without requiring covalent surface modification. Copolymerization of a nitrobenzyl alcohol methacrylate with poly(oligoethyleneglycol-methacrylate) yields a cell-resistant polymer, but the caged methacrylic acid limits aqueous solubility. Cleavage of this hydrophobic group causes the polymer to dissolve into solution, uncovering an underlying cell-adhesive substrate. Spin coating this polymer on a cell-adhesive substrate creates a cell-resistant surface that reverts upon illumination. Microscope projection lithography of the spin-coated polymer enables micrometer-scale control on the location, shape, and area of attached cells.[29-31] Integration of microcontact printing with photosensitive polymeric inks yields co-cultures without intervening photolithography. (Fig. 1D₃) A copolymer comprising poly(lactic acid) coupled to PEG through a nitrobenzyl group initially acts as a cell resist by virtue of the hydrophilic block. Illumination releases the cell-resistant portion of the polymer into solution, while the cell-adhesive block remains adhered to the surface. Co-cultures result from printing the ink with a micropatterned stamp, seeding the first cell type, flood illumination, and seeding the second cell type. The geometry of the printed stamp alone governs the configuration of the

heterotypic cellular array.[32] In these cases, photocleavage of the polymer renders the conversion between cell-adhesive and cell-resistant states irreversible.

Reversible switching of cell adhesion employs surface-bound functionality whose composition remains invariant, but undergoes a change in geometry or polarity with an external influence (Fig. 1D₄). Spiropyrans photointerconvert between uncharged and zwitterionic forms. UV-irradiation of spiropyran-modified surfaces triggers conversion to the resistant zwitterionic form, effecting cell release[33] while darkness regenerates the nonpolar form. Exposure to UV light isomerizes azobenzene from the thermodynamically stable *E* isomer to the *Z* configuration. Linking RGD to a cell-resistant surface through an azobenzene linker facilitates reversible cell adhesion by photoisomerization. The native *Z* form exposes the cell-adhesive peptide at the surface. Illumination to the *E* form conceals the peptide behind cell-resistant groups, causing release of adhering cells.^[34]

Azobenzene linkers enable photomanipulation of RGD orientation relative to a static cell-resistant background. However, the thermal reversion from the resistant state requires on the order of hours. Effecting this transformation electrostatically with microelectrode arrays enables rapid and reversible electrostatic control over cell adhesion (Fig. 1E). RGD attached to an amino-bearing, thiol-terminated linker interspersed with PEG thiol co-anchored on a gold surface yields a substrate for electrical cell manipulation. At zero applied potential, RGD extends beyond the static PEG chains, promoting cell attachment. Application of a negative charge draws the RGD tether toward the substrate by way of the positively charged amine groups, exposing the underlying hydrophilic cell resistant substrate.[35] Rather than electrically manipulating RGD, termination of PEG with a charged group controls cell adhesion by changing the orientation of the cell resist. A PEG chain capped with sulfonate folds toward a positively charged surface, while PEG-trimethylammonium folds toward a negatively charged surface. Patterning both positively- and negatively-charged groups together with RGD furnishes a substrate where the sign of the applied potential dictates the particular region that becomes cell adhesive.[20]

Directed migration and 3D bioprinting

Regenerative medicine's need for assembling different cell types over large areas with single-cell resolution motivates the development of dynamic biointerfaces. In addition to the switchable dynamic substrates discussed above, a promising hybrid approach involves bioprinting cell clusters at low-resolution as reservoirs, with 2D or 3D polymer micropatterns, co-printed at high resolution, that direct cell migration from the reservoir to their final positions. Cells respond to changes in either the two-dimensional geometry of a patterned culture surface (Microarray Amplification of Natural Direction Persistence-MANDIP) or the three-dimensional topology of a scaffold (Topographic Amplification of Natural Direction

Persistence-TANDIP). 2D MANDIP and 3D TANDIP patterns require no gradients and thus, have no intrinsic limitations on the distance they can direct cells. Figure 2 demonstrates the directional migration of fibroblasts cells guided by the three-dimensional T-shaped junctions imprinted in gelatin. This technique overcomes the shear limitation of microscale cell printing. [36, 37] Spontaneous creation of a cellular co-culture based on guiding directional migration of different cell types based on MANDIP (Figure 3) relies solely on the natural migratory response of cells to asymmetric micropatterns [38].

Conclusions

Recent studies demonstrate the utility of dynamic interfaces for engineering cell–material interactions. Spatial and temporal control over different cell types allows both scaffold preparation for engineering tissues and investigation of fundamental biological questions. Among the various external stimuli, light has the best spatial and temporal control. The sensitivity of cells to shear limits the resolution and speed of cell inkjet printing. The use of 2D or 3D printed cell-directing patterns in conjunction with low-resolution bioprinting of cell clusters offers a promising hybrid approach for the efficient assembly of functional tissues requiring precise arrangement of different cell types.

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enable reversible control of cell adhesion. E) A conducting surface controls cell adhesion by attracting or repelling an electrostatically charged tether.

Figure 2: Uniform width three-dimensional microchannels with overhanging T-junctions can induce polarization of 3T3 fibroblasts and human umbilical vein endothelial cells by expanding the cell front at each turn. Topographical amplification of natural directional persistence (TANDIP) microchannels with T-junctions preferentially direct A) 3T3 fibroblast and B) Human umbilical vein endothelial cells (HUVEC) to the right (time in hours). The angle at the T-junctions modulates the flux of cellular traffic. Staining shows F-actin (green), the nucleus (blue), and D) the Golgi apparatus (red). Scale bars in A, B: 100 μm . Scale bars in C, D: 30 μm . Reprinted from Ref. [37] with permission.

Figure 3: MANDIP pattern for guiding the self-assembly of two different cell types. Using the MANDIP design in the left, two different cell types, 3T3 fibroblasts and human microvascular endothelial cells (HMVEC), were guided into a patterned co-culture. The parallel zigzag arrays originating from the left seeding area consist of teardrops with the blunt ends pointed to the right, while the parallel zigzag islands originating from the right seeding area had the blunt end of the teardrops pointed to the left. Cell A 3T3 fibroblasts (green) and Cell B HMVEC (red) migrate in opposite directions from the left and right reservoir. (A) Time-lapse images (in hours) show the movement of human microvascular endothelial cells along the MANDIP pattern. (B & C) Self-assembled co-culture of 3T3 fibroblasts and HMVEC visualized by fluorescence and phase contrast microscopy after 7 days of seeding. Scale bars: 100 μm . Reprinted from Ref. [38] with permission.

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