PROGRESS REPORT

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Synthetic Biomaterials to Rival Nature's Complexity—a Path Forward with Combinatorics, High-Throughput Discovery, and High-Content Analysis

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Cells in tissue receive a host of soluble and insoluble signals in a context-dependent fashion, where integration of these cues through a complex network of signal transduction cascades will define a particular outcome. Biomaterials scientists and engineers are tasked with designing materials that can at least partially recreate this complex signaling milieu towards new materials for biomedical applications. In this progress report, recent advances in high throughput techniques and high content imaging approaches that are facilitating the discovery of efficacious biomaterials are described. From microarrays of synthetic polymers, peptides and full-length proteins, to designer cell culture systems that present multiple biophysical and biochemical cues in tandem, it is discussed how the integration of combinatorics with high content imaging and analysis is essential to extracting biologically meaningful information from large scale cellular screens to inform the design of next generation biomaterials.

1. Introduction

The concept that cell behavior in vivo is dictated by interactions within a "niche" has motivated the design and creation of synthetic materials to mimic the behavior of this cellular environment. Although the niche is most commonly associated with the specific and highly regulated microenvironments that allow stem cells to maintain homeostasis,[1–3] recent studies in cancer have highlighted a role for specific niches in regulating pathological phenotypes.[4–6] In actuality niches exist for all cell types, and uncovering the constituents and mechanisms of how they function is critical for designing new biomaterials and therapeutics.

Stem cell niche environments include multiple signals that can drive the cells to proliferate, self-renew, or differentiate. Often these signals are conserved, and cross-talk between signaling pathways serve to complicate the identification of the

roles of specific pathways.[7] The natural niche is fluid and dynamic, and one of the ways cells can "feel" their environment is through traction forces exerted by the cell with the surrounding matrix. These forces lead to changes in cell shape and intracellular cytoskeletal tension, and ultimately can influence gene expression.[8] While the precise mechanics involved in how biological signals are induced by material properties is not yet fully understood, at the core is the link between the cell cytoskeleton and the underlying substrate material.[9] In their natural states, cells interact mostly with the extracellular matrix (ECM), and understanding this interaction between cells and the components of the ECM is the key to unlocking the complex signaling cascades that controls cell and tissue form and function.

During development, cells are exposed to a complex milieu of biophysical and biochemical cues which collectively constitute the cellular microenvironment or niche. Ultimately these signals, which include cell–cell interactions and physical–chemical interactions between extracellular matrix and soluble factors, are able to organize single cells into the complex and diverse tissues necessary to sustain life. A major challenge in studying these cell–material interactions lies in the enormous complexity of the components. For instance, a single ECM protein may have multiple binding domains and configurations, each buried or exposed depending on the local biophysical and biochemical environment.[10] Furthermore, the ECM is composed of hundreds of large complex proteins. These various proteins not only serve as independent signal transducers, but can also harbor and establish chemotactic gradients of growth factors[11] as well as provide "cross-talk" signaling between ECM protein and soluble growth factors.[12] ECM proteins can be divided into major classes:

Structural proteins, such as collagens and elastins: collagens are fibrillar chains of polypeptides (over 20 types are known to be present in the human body) that serve as the primary structural support in the human body.[13] Elastins are the other major structural protein in the ECM, and along with fibrillin, is responsible for the flexibility of many tissues.[14,15]

Multidomain adhesive glycoproteins such as fibronectin, vitronectin, and laminin, which bind to many other ECM components such as proteins, growth factors, signal receptors, and adhesion molecules.[14,15] One of the most well studied, fibronectin (FN),

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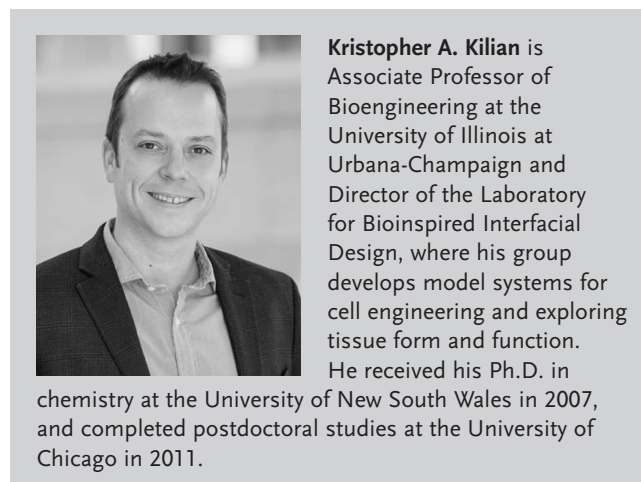E-mail: kakilian@illinois.edu

is known to contain binding sites in its FNIII domain for the RGD amino acid sequence.[16]

Glycosaminoglycans (GAGs) such as hyaluronan, and proteoglycans (PGs), which can often contain GAG side chains (e.g., heparan sulphate proteoglycans (HSPGs)) serve to bind almost all the structural proteins in the ECM[15] as well as also acting as potential reservoirs for growth factors and cytokines.[17]

Cellular interaction with the various ECM proteins occurs primarily through integrins. Integrins are a family of heterodimeric glycoproteins consisting of α and β subunits. These units are non-covalently linked transmembrane proteins with large extracellular domains. 18 α and 8 β subunits have been discovered, though only 24 different α-β combinations are known.[18] Integrins play a key role in cellular adhesion as well as regulating cytoskeletal organization and transmembrane signal transduction.[19] When specific integrin receptors bind to ECM molecules, a change in the cytoplasmic domain of the receptor occurs.[20] These changes are associated with integrin clustering and focal adhesion formation.[21] Focal adhesion formation is mediated by a variety of proteins including talin, vinculin, and paxilin[15,21,22] Integrin binding modulates the actin cytoskeleton, which can in turn mediate major signal transduction pathways, leading to changes in cell shape, motility, proliferation, and differentiation (**Figure 1**).[22,23]

ECM can also regulate cellular function through the binding of growth factors by charged GAG side chains. For example, HSPGs have been shown to augment fibroblast growth factor (FGF)/fibroblast growth factor receptor (FGFR) binding and promote FGFR dimerization.[24] Using surface plasmon

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resonance to measure binding constants of FGF to the FGF receptor in the presence and absence of heparin, Ibrahimi et al. concluded that HSPGs both influences the thermodynamics of receptor/growth factor interactions by stabilizing the ternary complexes required for binding, and also improves the kinetics by concentrating growth factor and limiting the diffusion of soluble factors.[24]

Integrins can thus activate cell signaling pathways independently, but often they act synergistically with other growth factor receptors.[12] The ability of heparin to bind to many different classes of proteins ranging from growth factors and cytokines,
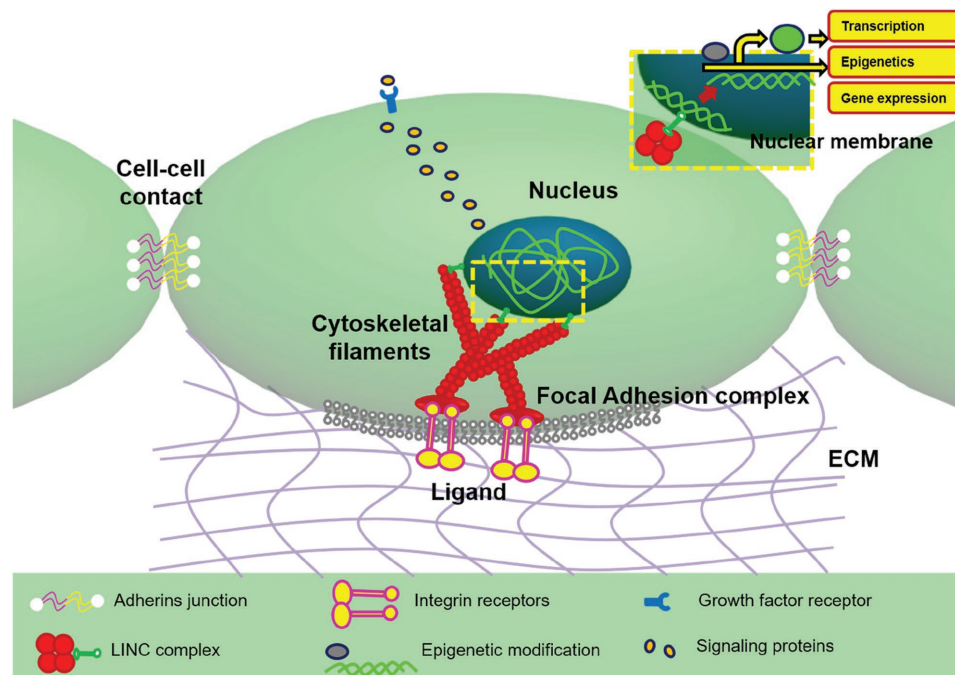

Figure 1. Signaling from the extracellular environment can be felt by the cell through a variety of mechanisms. Cell-cell signaling is mediated through adherin junctions. Mechanotransductive cues can occur through binding of integrin receptors to ligands on ECM molecules. This in turn forms focal adhesion complexes containing specialized proteins which undergo conformational changes to elicit further signaling downstream. At the nuclear membrane, the linker of nucleoskeleton and cytoskeleton (LINC) complex transmits the mechanical forces carried by the cytoskeleton to the chromatin. External forces can thus be directly translated within the nucleus in the form of changes to transcription factor binding, epigenetic changes, and ultimately changes in gene expression.

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to metabolic enzymes and other structural proteins[15] suggests the importance of proteoglycans in signal regulation as well. These interactions between ECM proteins and growth factors thus promote distinct cellular niches that can regulate cell fate and behaviour.[20]

In this progress report we discuss recent advances in the design and development of high throughput approaches to discover natural and synthetic biomaterials, and use these materials systems for diverse applications. From microarray platforms that present multiple biochemical cues to adherent cells, to protein and polymer arraying strategies, these tools have facilitated the unraveling of matrix composition-cell function relationships towards new biomaterials that guide cell behavior and functional outcomes. Furthermore, the development of high throughput robotics and high content imaging systems has paved the way to new biomaterials-driven assays and screens that include multivariate cues.

2. Microarray Platforms for Studying Cell–Material Interactions

In order to analyze such a complicated system, many groups have developed high-throughput screening platforms in which individual components of the ECM can be investigated in parallel. These microarray screening platforms are typically conducted on a 2D substrate (most commonly 25 mm × 75 mm glass slides) and can contain hundreds to thousands of unique spots, each representative of a synthetic microenvironment. This strategy allows simultaneous analysis and parallelization of large combinations of biomolecules common to the ECM, allowing investigators to parallel some of the complexities of the in vivo niche.

Microarray technology provides the flexibility of choosing many different biomolecules for recreating a synthetic niche. However, in order to effectively model the ECM several design elements must be incorporated. They should be well defined in terms of ligand identity, presentation, and density,[25] and present an inert background to discourage nonspecific biomolecule adsorption. In this progress report we highlight important considerations in the preparation of microarrays as well as key advances and insights gleaned from various array platforms.

2.1. Substrate and Printing Method

Most microarray techniques employ traditional glass slides as the substrate and then further modify the surface to suit a specific chemistry (e.g., glass surface for silane-based chemistry or gold surface for thiol-based chemistry, etc.). Traditional glass slides provide flexibility as many downstream processes such as cell culture and imaging support standard 25 mm × 75 mm dimensions.

The most common printing methods are contact printing, inkjet printing, and photolithography (**Figure 2**). In contact printing, typically a robotic handler system dips a small metallic pin into a solution, then deposits nanoliters of solution onto a surface by making contact with the substrate. This method is preferred for its ease of transfer as well as the small amounts of solvents required. Inkjet printing is a non-contact method that allows for multiple drops of solution to be deposited at the same location. Monomers and initiators can thus be mixed this way to form polymer or hydrogel microarrays.[26] Photolithography approaches involve irradiating a substrate with high energy beams (typically UV light) through a photomask to initiate polymerization in discreet spots. Similarly, an elastomeric stamp containing set patterns can be fabricated using soft lithography and this stamp can be subsequently used to generate a patterned surface. Many reviews have been written on the topic of methods for generating microarrays and their applications;[27–30] here we highlight recent progress in leveraging microarray technologies to emulating the complexity of the niche in physiological and pathological contexts.

The typical microarray workflow consists of three main steps (**Figure 3**a): the monomers to be investigated are chosen and combined. These are then deposited onto a substrate (typically a glass slide), and treated to prevent non-specific adhesion to non-deposited regions. Cells are seeded onto the array, allowed to adhere to the biomaterial interface, and cultured for a specific timepoint that corresponds to a bioactivity of interest (e.g. migration, proliferation, differentiation, etc.). Finally, after a pre-determined time point, the microarray is imaged with a high-content imaging system and a readout is analyzed. The remainder of this section will focus on the specific biomolecules that are typically deposited in these microarray platforms and their applications in studying cell-ligand interactions and how these interactions affect downstream cellular processes.
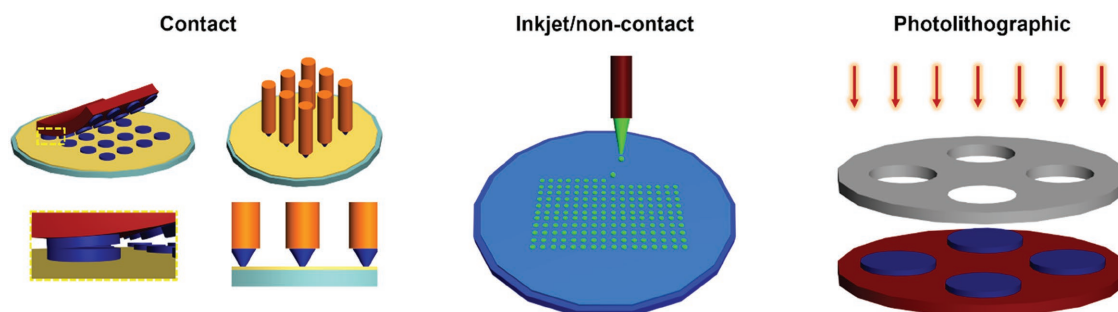

Figure 2. Many techniques have been reported for printing microarrays. Contact based approaches are the most common and allow for precise control of printed features. Inkjet or non-contact printing approaches offer advantages of allowing multiple solutions to be deposited at each spot, allowing for greater chemical flexibility. Photolithographic approaches use aligned masks to generate the spotted array.
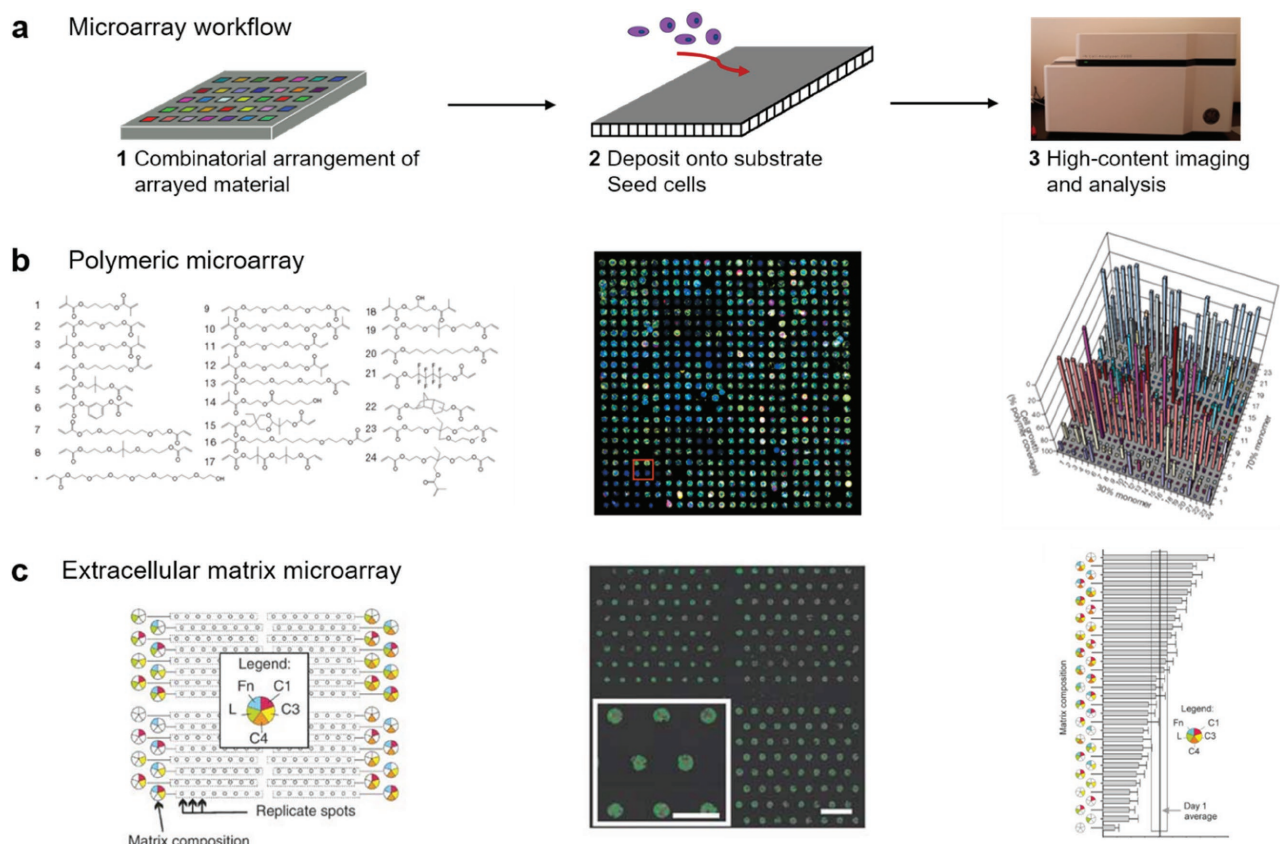
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Figure 3. A typical microarray workflow is shown (a). The material to be deposited is combined and deposited onto a substrate. Cells are seeded onto this array and allowed to culture for a number of days. High content imaging systems are used to quantify cell number, adhesion, or other readouts specific to the array. An early demonstration of a polymeric microarray deposited acrylate monomers onto pHEMA coated glass slides to investigate hESC growth and differentiation on various polymer spots (b). Reproduced with permission.[32] Copyright 2004, Nature Publishing Group. For extracellular matrix arrays, typically ECM proteins will be combined and spotted. Rat hepatocytes cultured on these combinations of ECM molecules can be stained to determine how different ECM proteins influence the hepatic phenotype (c). Reproduced with permission.[40] Copyright 2005, Nature Publishing Group.

2.2. Polymeric Microarrays

An early application demonstrating the efficacy of synthetic polymer arrays was described by Kohn et al., who prepared a combinatorial library of aromatic polyesters by reacting 14 diphenols and 8 diacid monomers to produce 112 distinct polymers. They used this polymer array to study fibroblast adhesion and proliferation and found that increased proliferation was correlated with surface hydrophobicity.[31] Later, a study by Anderson et al. used robotic handling technology to deposit pairwise combinations of 24 different acrylate containing monomers with a radical initiator onto a poly(hydroxyethyl methacrylate) (pHEMA) coated glass slide (Figure 3b). They investigated human embryonic stem cell (hESC) growth and differentiation on these various polymer spots.[32] Although such synthetic polymers are not "true" biomolecules in the sense that these are not naturally occurring within the body, almost all in vitro cell culture today is performed on similar polymers such as polystyrene. Such studies then are instructive in that they allow us to analyze proper culture conditions for specific cell types. The same group later used a similar strategy to screen for polymer substrates which could maintain clonal

growth and self-renewal of pluripotent stem cells,[33] where the pluripotency of hESCs was maintained for more than two months in culture. In this way, they demonstrated that a chemically defined polymer substrate had a similar propensity for ensuring clonal growth compared to mouse feeder cells, an important advancement for the eventual use of hESCs as therapeutics.

Several other groups have employed a similar polymer-based screening approach to identify synthetic surfaces that can enhance cell binding and culture compared to traditional tissue culture plastic.[34] Zhang et al. reported on a class of thermoresponsive hydrogels that allow long term hESC culture in a xeno-free environment,[35] Hay et al. describe a urethane and acrylate-based microarray for screening polymers that support the attachment of hESC-derived hepatocytes as well as long-term ability to retain a hepatic phenotype,[36] and Brafman et al. identified several polymers that could support the renewal of human pluripotent stem cells.[37] Together these reports highlight the utility of an array-based platform for identifying material conditions that support ESC cell culture and maintenance of the pluripotent phenotype. A commonality to each of these polymer-based systems is the ability of the surface coating to

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promote pluripotent colony growth through combined integrin mediated adhesion and robust cell-cell contact. However, a central effector that governs the long-term pluripotent stem cell growth on these polymer surfaces remains to be revealed. One potential factor that may be involved in supporting ESC culture across these different materials could be specific bioactivities associated with physisorption of ECM proteins in the media or secreted by the cells to the chemical groups at the polymer interface.[33] While there are clear advantages to a synthetic coating in cell culture for industrial and clinical settings, many investigators continue to focus on natural ECM protein-based materials, and combinatorial approaches have the potential to reveal unique tissue-mimetic protein formulations for cell culture.

2.3. Extracellular Matrix Protein (ECM) Microarrays

Compared to the relatively inexpensive monomers of polymer arrays, ECM proteins are generally expensive and are mostly derived from animals, thus they provide a greater risk of containing contaminants. However, microarray technology offsets much of the cost associated with using proteins, as typically only small volumes of liquids (nL to μL scale reactions) are required. Studying cell-protein interactions also holds great potential as ECM proteins generally form the backbone of the cellular niche and are capable of initiating cellular signaling cascades on their own or in combination with other signaling molecules.[38] In 2000, MacBeath and Schreiber reported on a method to spot proteins onto chemically modified glass slides using a traditional DNA microarray spotter.[39] They demonstrated that a contact printing approach dispensing nanoliter volumes of protein samples was capable of creating high resolution arrays for studying specific protein–protein interactions in a high-throughput format. Since then, many other investigators have used a similar approach to create ECM protein arrays for a variety of applications. Flaim et al. examined 32 different combinations of 5 common extracellular matrix molecules and used a traditional DNA microarray spotter to print these proteins onto an acrylamide-coated glass slide (Figure 3c). They cultured rat hepatocytes on this ECM array and found that the hepatocytes adhered differentially on the various combinations. They also cultured mouse embryonic stem cells on the ECM array and reported that protein combinations containing collagen IV helped promote hepatocyte function, while collagen I and fibronectin appeared to help differentiate ES cells into a more hepatic phenotype.[40]

Similar studies followed, using combinatorial protein arrays and factorial analysis to discover preferential protein conditions for other biological functions. Kuschel et al. used an array containing 14 different ECM proteins to demonstrate that protein arrays could be used to generate adhesion profiles for different cell types.[41] Soen et al. mixed extracellular matrix proteins with growth factors and adhesion molecules to create a synthetic molecular microenvironment array. They examined the effects of these various protein combinations on neural differentiation of human neural precursor cells and found that the presence of Wnt and Notch co-stimulation favored maintenance of a multipotent state whereas the presence of bone morphogenetic protein 4 induced differentiation of the precursor cells.[42] Other groups also reported high throughput platforms using mixtures of ECM proteins and growth factors. Nakajima et al. observed growth factor synergies with ECM matrices on neural stem cells and their ability to undergo neuronal or glial specification,[43] Brafman et al. investigated the role of hepatic stellate cell phenotype,[44] Jones et al. screened hepatocytes for anti-fibrotic and anti-apoptotic effects,[45] and Huang et al. examined murine embryonic stem cells and their ability to remain pluripotent on combinatorial arrays featuring ECM proteins and growth factor morphogens.[46] As array technology becomes more accessible with commercially available tools,[47] scientists are able to expand screens to include more proteins. While proteins are powerful investigative tools, the use of cheaper xeno-free bio-ligands are attractive for future applications of next-generation biomaterials.

2.4. Biomimetic Peptide Microarrays

Proteins present several limitations for studying precise ligand–receptor interactions. Adsorbed layers of proteins are heterogeneous, and adsorption depends mostly on the choice of substrate, making control of protein distribution and orientation immensely challenging.[48] Thus, model systems (such as self-assembled monolayers of alkanethiolates on gold) that present short bioactive ligands, have been proposed as important mechanistic tools for investigating such ligand-receptor interactions. In many ways short peptides are advantageous over full length proteins in that they are easy to synthesize and purify, and can be covalently conjugated to a complementary surface chemistry in a well-defined manner.[49] This is especially amenable to industrial scale-up and reproducible modification of synthetic materials for potential applications in tissue engineering and clinical devices. In addition, cell spreading only requires a minimal surface concentration of peptide ($\approx$1 fmol cm^{-2}).[50] Studies on model surfaces have demonstrated the utility of peptide sequences to promote cell spreading,[51] cell mobility,[52] proliferation,[53] and differentiation[54] on peptide-modified biomimetic surfaces. Just as cross-talk between ECM proteins and growth factors can affect signaling transduction pathways, so too can the interaction of peptides. In order to elucidate the effects of these peptide ligands on cell behavior, investigators have employed high-throughput approaches to screening and analysis.

Kiessling and co-workers demonstrated that self-assembled monolayers of peptide-conjugated alkane thiols could be facilely arrayed onto gold surfaces to generate well-ordered and chemically defined spots (**Figure 4**a).[55] They used this array platform to screen for laminin-derived peptides that could maintain hESC pluripotency.[56] The "biopanning and rapid analysis of selective interactive ligands" (BRASIL) technique[57] was employed to discover novel peptides that would bind to embryonal carcinoma cells and support the growth of human embryonic stem cells.[58] Recently, they have also used the peptide array technology to identify heparin-binding peptides that can support long term culture of multiple pluripotent cell lines.[59] Notably, these peptide reagents were shown to be
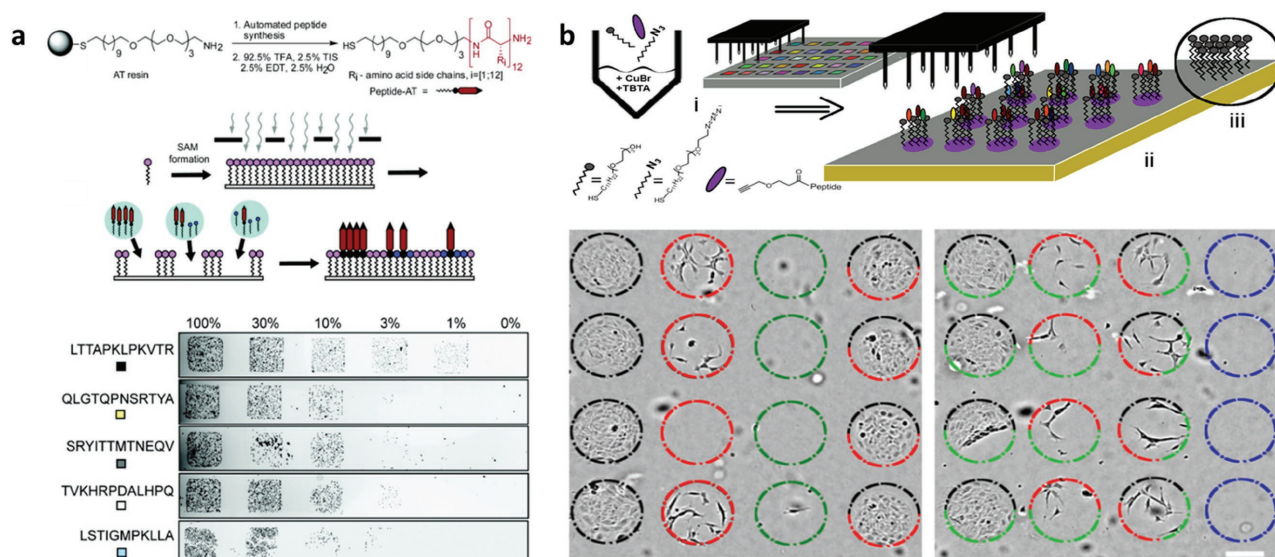
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Figure 4. a) Peptide microarrays generated by synthesizing peptides directly onto an alkanethiolate resin. A photomask is used to selectively remove regions of a background self-assembled monolayer, and the purified peptide-alkanethiolates can then be spotted onto the exposed regions. This strategy allows for screening of specific sequences that can support adhesion and growth of pluripotent cells. Reproduced with permission.[58] Copyright 2010, American Chemical Society. b) Conjugation of peptides to an alkanethiolate within 384-well plates, followed by spotting the resulting solution onto gold coverslips. 78 combinations of biomimetic peptides were screened to investigate combinations that could promote osteogenesis in mesenchymal stem cells from adipose tissue. Reproduced with permission.[63] Copyright 2014, Royal Society of Chemistry.

amenable to translation to cell culture plasticware for ease of use in the laboratory and clinical settings.

Other groups have also demonstrated the utility of peptide arrays for ligand discovery and development. Yousaf and colleagues used an electrochemical surface chemistry to explore the role of ligand composition and density on mesenchymal stem cell differentiation in an array format.[60] They were able to quantitatively assess the concentration of ligand at the surface using cyclic voltammetry, and observed that higher densities of ligand promoted higher adipogenic differentiation. Koepsel et al. combined BMP-binding peptides, proteoglycan binding peptides, and integrin binding peptides and examined the effects of ligand binding on human mesenchymal stem cell alkaline phosphatase production.[61] They noted that when the RGD binding peptide was absent, the proteoglycan peptide and BMP peptide seemed to have no significant effect on alkaline phosphatase production. This unexpected result highlights the importance of synergistic ligand interactions as well as the need to control signaling contexts (i.e. substrate elasticity, ligand density, etc.). Lin et al. arrayed peptides targeting integrins and TGFβ receptors and showed murine mammary epithelial cells only displayed markers of epithelial to mesenchymal transition (EMT) on combinations of peptides known to stimulate EMT.[62] Recently our lab reported on a technique that uses "click" chemistry to conjugate alkyne-terminated peptides onto azide-terminated alkanethiolates, and then spotted the resulting species onto gold-coated coverslips.[63] We first demonstrated that this technique was robust enough to support and differentiate between two different cell types (mouse embryonic fibroblasts and adipose derived stem cells) for over one week in culture without degradation of the array spots. We then used this array platform to study the effects of a panel of twelve biomimetic peptides on the tumor initiating properties of B16F0

murine melanoma cells (Figure 4b).[64] We found that a specific peptide combination enhanced the expression of several melanoma cancer stem cell markers, as well as the migration and invasion characteristics of the melanoma cells. By investigating the signaling pathways associated with that specific peptide combination, we demonstrate that this strategy of using microarrays to screen for cell-material binding properties can serve more functional purposes beyond identifying surfaces to support cell adhesion.

2.5. Combinatorial Arrays Modulating Physical and Chemical Cues

The array systems described thus far are able to provide insight on biochemical interactions between cells and the underlying substrata. As our understanding of these cell–material interactions grow and as the tools to investigate these phenomena become more widely available, researchers are able to adopt increasingly advanced platforms to probe both chemical and physical cues in parallel. For instance, ECM elasticity has been known to be a key determinant for downstream cellular signal transduction cascades.[65] Classical experiments by Engler et al. using mesenchymal stem cells demonstrated that differentiation can be determined solely by the compliance of the substrate, with cells favoring a stiffness that matches the modulus of their natural in vivo niche.[66] Next-generation array platforms have now been reported to allow tunability of this additional parameter. Recently, the Lutolf laboratory developed a method using robotic spotting on soft hydrogels that could simulate complex microenvironment niches by not only altering the combinations of proteins spotted, but also the modular stiffness of the underlying substrate.[67] By examining

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both biochemical and mechanical cues in parallel, they showed that concurrent pathways could be activated for adipogenesis of mesenchymal stem cells; biochemical cues from the proteins appear to be more dominant on lower rigidity substrates and become less dominant as mechanical cues override the biochemical cues at higher rigidity.[68] Yet despite these advances in array-based techniques, it remains an impracticality to screen every combination of cell–ligand interaction. As arrays become more complex and include more parameters, it also becomes increasingly difficult to deconvolute the role of each effector. In this regard, studies to elucidate the fundamental signaling pathways downstream of cell-material binding remain a challenge. However, with rational experimental design and a focus on physiologically relevant systems and biologically meaningful read-outs, microarray technology can provide a powerful tool in the design of new biomaterials and therapeutics.

3. New Applications of Microarray Technology and Future Outlook

Microarray applications have largely focused on identifying new substrates to support adhesion and proliferation of various cell types. Although adhesion is perhaps the most basic readout to evaluate for microarray platforms, it remains an important parameter and there exists no uniform method for quantifying adhesion. The classic method for quantifying adhesion involves counting the number of cells within each spot,[69] or measuring the confluency of each spotted region.[56,63] However, these methods to profile "adhesion" provide only a basic understanding into what is actually occuring at the cell–material interface. For example, a recent investigation by Moghe et al. demonstrated that simply by characterizing variations in cell shape and cytoskeletal organization of human mesenchymal stem cells, long term lineage differentiation could be predicted.[70] This study highlights the importance of developing novel analyses to incorporate into existing screening platforms. Although many microarray platforms exist, these techniques should function as more than just basic characterization tools. Next generation microarray technology should be able to combine multiple functionalities and allow the identification of specific downstream signaling that occurs at the biomaterials interface. The incorporation of additional tunability to the traditional microarray format by introducing 3D elements,[71,72] cell–cell contacts using co-cultures within the microarray,[73] micro and nano topography[74,75] and inclusion of inorganic materials[76] will improve control over the context in which cues are presented and received, thus providing more meaningful biological read-outs.

3.1. Microarrays Presenting Multivariate Cues

As introduced in section 2.5, tissues within the body contain mechanical properties spanning orders of magnitudes.[77] Although ECM elasticity has become one of the most studied factors, many of the techniques used to perform these studies are low-throughput, requiring gels or polymers to be cast individually. Currently, most microarray platforms are still slide-based, and offer limited flexibility for tuning substrate elasticity. The study by Lutolf et al. reported a method in which ECM proteins were deposited onto silicon pillars, which were then compressed into a hydrogel layer.[67] Doing so allowed combinatorial spotting of proteins within hydrogel wells of moduli 1–50 kPa and revealed optimal microenvironments for MSC and NSC culture. Similarly, Murphy et al. demonstrated a technique involving differential wetting of alkanethiolate SAMs to pattern arrays of hydrogels with different stiffenss and adhesion ligand concentration. Both of these approaches and others will enable the precise tailoring of biophysical and biochemical cues that cells are exposed to in 2D and 3D environments.

In addition to matrix mechanics, biological materials in vivo are often structured at the micro and nanoscale,[78] which can influence the presentation of adhesion and other signaling molecules. To explore the role of topographical cues, de Boer et al. demonstrated a topographical biomaterials library to select microstructured interfaces that guide cellular processes.[75,79] Using this approach, unique surface topographies can be identified that promote a desired cell state. These and other techniques that allow multi-functional tuning of chemical as well as physical parameters will prove instructive in understanding complex biophysical processes and diseases. Although traditional microarrays are useful for the study of ligand interaction, substrates that can approximate the mechanical properties of soft tissues have been shown to be more predictive of in vivo outcomes.

3.2. Translation to Biomimetic 3D Systems

An additional application of the microarray platform is the identification of clinically relevant ligands for incorporation into next generation 3D hydrogels. In contrast to 2D culture, 3D culture can more accurately mimic the architecture of natural matrices.[80–82] The need for 3D culture systems is especially apparent in the field of drug screening. For instance, 3D model systems for therapeutic development have shown pronounced differences in cell behavior when compared to 2D models.[83,84] Solid tumors are composed of a complex and dynamic macromolecular architecture where signaling between multiple cell types and the surrounding matrix plays a decisive role in progression. Towards understanding the complex network of signals underlying oncogenesis, it is desirable to develop biomimetic in vitro models that faithfully recapitulate the tumor microenvironment. However, the majority of available model systems are not ammenable to accurate representation of the dynamic in vivo microenvironment. Recently we demonsrated a technique to fabricate high-throughput co-cultured alginate fibers in a single step, presenting a ECM adhesion peptides to facilitate macrophages and tumor cell adhesion. Using this platform we investigated breast adenocarcinoma cells and macrophage interactons, and demonstrated a pharmacological inhibitor screen for disruption of a macrophage-tumor cell paracrine loop (**Figure 5**).[85] Future studies could readily incorporate ligands identified from microarray screening within these complex 3D architectures. The use of small bioactive ligands allows easy scale-up and translation to a wide range of biomaterials. Further research into identifying ligands that can mimic the in vivo tumor microenvironment will only improve
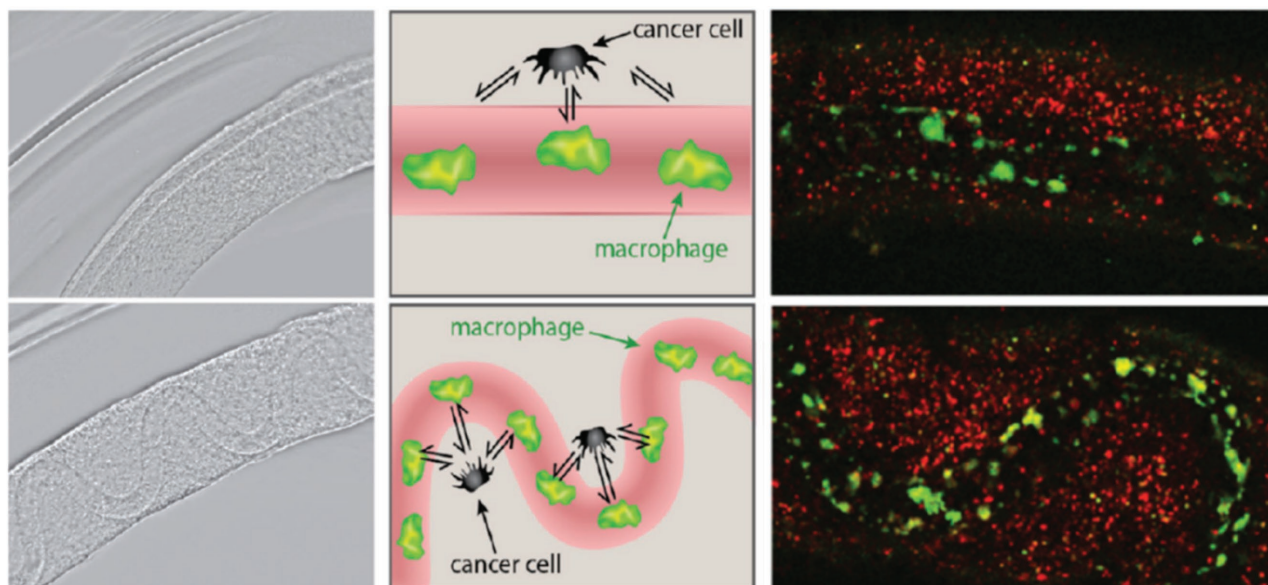
Figure 5. Straight and patterned hollow alginate structures can be extruded using a simple microfluidic technique (left). These alginate backbones are peptide-conjugated to allow for cell attachment and culture. Such a structure can be used to model the interaction between cancer cells and stromal cells (middle). By labeling the individual cells, migration is tracked within the 3D microenvironment. One application of this technique is for drug screening (right). Green = macrophages, Red = cancer cells. Reproduced with permission.[85]

the biological and physiological relevance of next-generation in vitro tissue models.[86]

4. Conclusions

Nature has optimized the properties of biomaterials through combinatorial processes to facilitate a broad range of functional cellular activities. Efforts to recreate the complex multivariate presentation of signals inherant to natural materials has been fueled by advances in biomaterials science and engineering, high throughput techniques, computational tools, and high content imaging and analysis. Better control at the cell–biomaterial interface will enable improved read-out of biologically meaningful activities towards deciphering 'materials structure-cell function' relationships. From the design of implant coatings and tissue engineering scaffolds, to new cell-based assays in clinical and pharmaceutical settings, the emergence of high throughput/high content approaches has expanded the scope in which materials can be assayed with cellular systems.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

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