

High-Throughput and Combinatorial Technologies for Tissue Engineering Applications

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As the field of tissue engineering progresses, new technology is essential to accelerate the identification of potentially translatable approaches for the repair of tissues damaged due to disease or trauma. The development of high-throughput and combinatorial technologies is helping to speed up research that is applicable to all aspects of the tissue engineering paradigm. This diverse technology can be used for both the rapid synthesis of polymers and their characterization with respect to local and bulk properties in a high-throughput fashion. The interactions of cells with many diverse materials in both two- and three-dimensions can be assessed rapidly through the use of microarrays and rapid outcome measures and with microfluidic devices for investigation of soluble factor and material combinations. Finally, small molecule screening of large libraries is being used to identify molecules that exhibit control over cell behavior, including stem cell differentiation. All of these approaches are aimed to move beyond traditional iterative methods to identify unique materials and molecules that would not have been identified otherwise. Although much of this work is only applicable for *in vitro* studies, future methods may translate into rapid screening of these approaches *in vivo*.

Introduction

OVER THE PAST FEW DECADES, scientists and engineers have sought to develop combinations of cells, scaffolds, and signaling molecules as the ideal microenvironments for application in tissue engineering.¹⁻³ The important environmental factors vary with the tissue of interest, as well as the type of cells recruited or delivered in the approach. Through this research, numerous scaffolds have been developed from a wide range of materials and incorporating numerous molecules (e.g., growth factors) to enhance matrix production and accumulation.² This has advanced our understanding of the importance of many cues toward optimal tissue engineering approaches, yet the field of tissue engineering has proceeded in a relatively iterative fashion with the use of tedious low-throughput studies. Due to the nature of the field, the input parameters to a tissue engineering approach are nearly endless and include the material selected, material properties, cell choice, culture environments, and soluble cues.

With this in mind, the complexity of cell signaling with the environment due to the multitude of factors involved may have slowed down tissue engineering development, due to the difficulty in isolating effects to one parameter. For example, it is necessary to examine not only the bulk material properties such as mechanics and degradation, but also how scaffolds interact with cells through toxicity studies, as well as their ability to regulate cellular behavior such as adhesion,

proliferation, or even stem cell differentiation.^{3,4} Adding to the complexity, proliferation and differentiation are largely controlled by cell-cell and cell-extracellular matrix (ECM) interactions, soluble factor stimuli, and the physical structure of the microenvironment itself.^{3,4} Additionally, intrinsic cellular factors such as small molecules and RNA also play an important role in cell signaling and differentiation.³

Traditional methods of assessing these various factors have involved iterative approaches that require a tremendous amount of time and effort. Recently, the development and use of high-throughput and combinatorial techniques in tissue engineering have significantly enhanced this process through efficient and productive platforms. For example, polymers may be synthesized through combinatorial processes, which accelerate the often tedious synthesis and purification schemes that may be necessary for development of nontoxic and biodegradable polymers.⁵ Upon synthesis, the identification of optimal properties from large numbers of materials may also be performed through newly developed rapid assessment techniques.⁵ This continues with the assessment of optimal cell-material interactions in a rapid fashion, often through the development of microdevices or use of printing technology.⁶ For example, radically polymerized polymers with various functionalities can be synthesized in nanoliter volumes and plated in combinations onto a microarray to analyze the influence of the biomaterial on cell growth and differentiation.¹ These techniques also include platforms such as

microfluidics, which can analyze combinations of biomaterial gradient streams in a dynamic and temporally changing three-dimensional microenvironment, and high-throughput screens to characterize the role of small molecules on cellular behavior through the screening of large libraries of soluble factors.^{2,3} Notably, all of these areas may lead to the more rapid identification of optimal tissue engineering components and their unique combinations.

While these techniques also have applications in gene therapy and drug candidacy analysis, this review will focus specifically on applications in tissue engineering for injury and degenerative disease treatments.⁴ Regenerative medicine techniques are being developed for a wide range of treatments for conditions such as cardiac ischemia, liver disease, and spinal cord injury, and combinatorial and high-throughput approaches are accelerating the process for many tissue types.⁶ As this field progresses, existing and new high-throughput techniques will be crucial in efficiently and rapidly developing ideal synergies between microenvironments and signaling factors for engineered tissues to be successful in clinical applications. This review will present the current landscape of high-throughput and combinatorial techniques as they apply to tissue engineering applications, the experimental and clinical benefits of these techniques, and the future directions of this rapidly growing field. Specifically, the focus will be on (1) combinatorial polymer synthesis, (2) tools for the rapid assessment of material properties, (3) high-throughput assessment of cell–material interactions, (4) microfluidic devices for the rapid development of engineered environments, and (5) high-throughput screening (HTS) for identification of small molecules that influence cellular behavior.

Combinatorial Polymer Synthesis

In the pharmaceutical industry, researchers have used high-throughput, combinatorial techniques including automation, miniaturization, and parallel synthesis to transform their approach to developing new small molecules and discovering drug candidates.⁷ These approaches are now being utilized in biomaterial development and in the field of tissue engineering due to the numerous advantages to accelerating material design and development. This includes the synthesis of polymers with a wide range of chemical components and bulk properties. Typically, polymer development has involved the use of tedious synthetic and purification steps, along with iterative design for material development since it is often difficult to predict the resulting polymer properties based on the polymer chemistry and structure. With advanced approaches where syntheses are performed more rapidly, many polymers can be developed quickly and then screened for the desired properties.

For over two decades, the pharmaceutical industry has developed and utilized methods in combinatorial synthesis and high-throughput analysis in its drug discovery efforts (originally reviewed by Gordon *et al.*⁸ in 1994). The goal was to create large, diverse combinatorial libraries and characterize their pharmaceutical effects through a range of techniques. The synthesis and screening of combinatorial libraries primarily used either tagged methods (e.g., phage technology, peptides-on-plasmids, and encoded combinatorial libraries) or untagged synthesis/analysis techniques (e.g., mixture

synthesis and portioning-mixing).⁹ These initial approaches have been expanded, and there has been extensive research and development in combinatorial synthesis and characterization in identifying lead compounds and therapeutically relevant combinations for pharmaceutical applications.^{10,11} Beyond drug development, similar techniques have been applied to gene therapy efforts over the years to identify unique polymers that would be most effective in nonviral gene therapy approaches on a range of length scales.^{12–15} One approach has been the combinatorial synthesis of degradable cationic polymers [e.g., poly(β -amino ester)s (PBAEs)] and the analysis of structure/function relationships, something that would not be possible on this scale without combinatorial synthesis procedures.^{12–14} While there are numerous applications that would benefit from this technology, this section will focus on polymer development applicable to the field of tissue engineering.

Some of the early combinatorial polymer libraries were created for subsequent high-throughput analysis of material properties specific to an application of interest (e.g., engineered bone and artificial medical implants). One such library, developed by Kohn and coworkers,¹⁶ combined tyrosine-derived diphenols and diacids in permutationally designed monomer systems to produce alternating A-B-type copolymers (A—contains reactive group for pendent chain attachments; B—allows for systematic variations in polymer backbone structure). Combining these copolymers, the system allowed for the production and analysis of a library of 112 polyarylates with small structural differences and some similar properties, but also with slight differences in polymer free volume, bulkiness, flexibility, hydrophobicity, and cellular response that affected their potential utility in medical implant applications.¹⁶ Another study investigated more traditional polymer combinations of poly(D,L-lactide) and poly(ϵ -caprolactone) and their resulting influence on osteoblast activity.¹⁷ This study employed composition spread and temperature gradients (and specifically heat-induced phase separation) to synthesize and screen hundreds of combinatorial polymers with varying characteristics and thereby demonstrated differences in osteoblast response.

Since these early models, researchers have developed many additional methods of parallel, automated, and combinatorial polymer synthesis, including polycondensation, radical polymerization (free-radical polymerization and controlled radical polymerization), ring-opening polymerization, polyolefins (mostly for applications in the chemical industry), and supramolecular polymerization.⁵ For example, Gravert *et al.*¹⁸ utilized free-radical polymerizations to construct block copolymers, which were subsequently twice split and used as macroinitiators to polymerize other sets of monomers. The final polymers displayed variable solubility profiles and functionality and demonstrated that this process may be used for a variety of high-throughput applications. Numerous other synthesis methods in controlled radical polymerization and ring-opening polymerization have been effective as well.^{5,19–21} Additionally, the development of synthesis methods for supramolecular polymers, which include both covalent and noncovalent bonds (as opposed to only covalent bonds), has introduced another variety of polymers with potential biomedical applications.⁵ This research included the first method for fully automated production of supramolecular coordination polymers developed by Schmatloch *et al.*,²²

which helped realize the potential for developing many new supramolecular polymers.⁵

In 2006, the first combinatorial library of degradable photocrosslinked biomaterials was synthesized by Anderson *et al.*,²³ who built on a previously developed library of potential gene carriers.¹³ Specifically, a combinatorial approach was used to synthesize a library of 120 acrylate-terminated PBAE macromers that could be formed into networks using a photoinitiated polymerization. The synthesis did not involve any purification steps and used commercially available reagents, which accelerated the synthesis process. This rapid process produced polymers that varied greatly in their property characteristics, including degradation behavior (e.g., mass loss) and mechanical properties (e.g., elastic modulus).²³ The general synthesis procedure and an example of the diverse mass loss between polymers are shown in Figure 1. Brey *et al.*^{24,25} also found that properties such as molecular weight and macromer branching significantly influence the resulting PBAE network and bulk properties. With the appropriate screening process, photocrosslinked polymer scaffolds could be selected to meet design criteria, including optimal degradation profiles and mechanical properties for specific engineered tissue functions.

Combinatorial syntheses can also be performed on the nanoliter-scale by mixing reactive macromers and spotting onto a surface.^{1,26,27} This was performed with 24 poly-

mers in various combinations and ratios to produce 3456 individual spots on a single array with the goal of analyzing cell–biomaterial interactions.²⁷ This platform relied on the use of a photoinitiated radical polymerization compatible with the technology. A robotic handling system was modified to handle the unique challenges of polymerizing diverse monomers, including the viscous nature of some acrylate monomers and the inhibition of radical polymerization by oxygen.^{1,27} Therefore, array synthesis was performed in an atmosphere of humid argon with oxygen present at <0.1% and used a long-wave UV lamp to quickly polymerize the monomers.^{1,27} One of the benefits of this technology is that it is relatively universal to radically polymerized materials, a group of materials that is gaining interest in tissue engineering.²⁸ For example, natural polymers such as hyaluronic acid (HA) can be modified to be photopolymerizable and utilized in tissue engineering applications.^{28,29–33} Schmidt and coworkers³² constructed a variety of photopolymerized, crosslinked glycidyl methacrylate-HA hydrogels with a corresponding range of degradation rates, swelling, mesh size, and other properties and demonstrated their potential use in wound-healing applications by implanting them subcutaneously in rats. Subsequently, in 2005, Burdick *et al.*³⁴ analyzed the use of photopolymerizable HA as a scaffold for both 3T3-fibroblasts and auricular swine chondrocytes for macromers with a range of molecular weights and formed into gels at different concentrations. With the range of polymers available, these approaches may help in identification of unique materials as well as their combinations (i.e., copolymers) that would be tedious to characterize individually. Overall, the development of a large variety of advanced polymer libraries, both synthetic and natural, has laid the framework for the identification of highly effective microenvironments for tissue engineering applications.

These synthetic techniques can also be used with a variety of preparation techniques to further their combinatorial possibilities. These preparation methods include flow-coating devices that produce thin films with gradients in thickness and temperature-gradient thin film platforms that provide composition/thickness, composition/temperature, or thickness/temperature two-dimensional libraries, and methods using photopolymerization to create a gradient polymer film.^{5,35–37} A more recent setup employed the use of a microextruder with two feeder heads designed by Potyrailo *et al.*³⁸ that was capable of producing polymers with a variety of step or gradient polymer combinations for subsequent analysis. Gradients provide the ultimate combinatorial synthesis method because polymer development extends beyond discrete formulations, providing even more compositions, as long as characterization techniques are available for assessment. All of these synthesis and preparation methods have contributed to the development of combinatorial polymer libraries with unique properties and functionalities, which can then be analyzed in high throughput to identify ideal polymeric combinations for applications such as tissue engineering. Polymer synthesis in three-dimensional environments is also of interest for tissue engineering applications. For example, scaffolds were constructed from varying compositions of two biodegradable tyrosine-derived polycarbonates, resulting in three-dimensional combinatorial libraries on 96-well plates.³⁹ This three-dimensional design should provide a variety of microenvironments that are more similar to *in vivo*

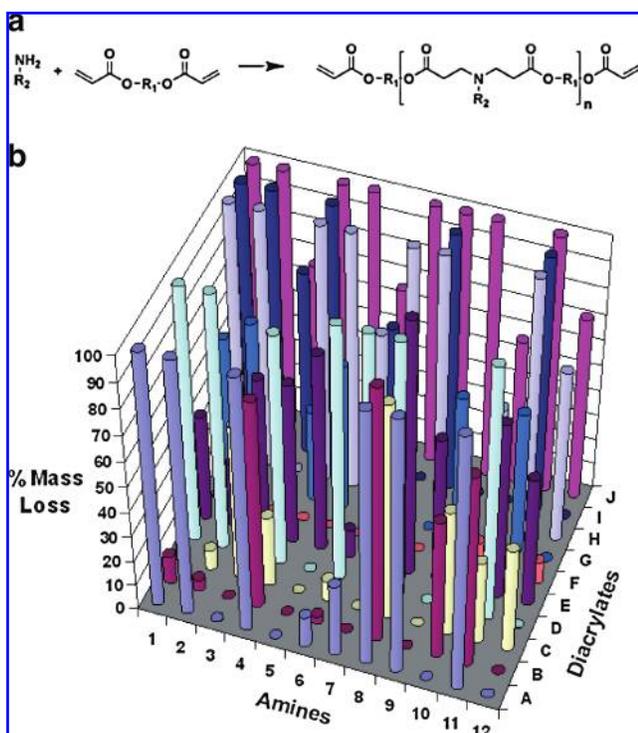


FIG. 1. Combinatorial polymer synthesis. (a) Schematic of synthesis scheme for fabrication of poly(β -amino ester)s (PBAEs) from primary amines and diacrylates, where the versatility is found in the selection of reagents for synthesis (i.e., R₁ and R₂). (b) Representative mass loss for polymers formed from a group of 120 PBAE macromers after 32 days. The PBAE library exhibits a range of degradation and mechanical properties for diverse applicability in tissue engineering applications. Color images available online at www.liebertonline.com/ten.

conditions and should thereby enhance the development of ideal polymer scaffolds for tissue engineering applications.

As identified by Kohn⁴⁰ in 2004 and further described by Kohn *et al.*⁴¹ in 2007, one important issue to consider as combinatorial molecule synthesis moves from drug discovery applications to material science and tissue engineering applications is the need for reproducibility in polymer synthesis (e.g., molecular weight and polydispersities). With small molecule development, the chemical structure is the essential driving factor; however, there are a variety of factors that are significant when considering polymer design, including the polymer molecular weight, the molecular weight distribution of polymers within the sample, the presence of trace impurities, and the mechanism of fabrication, which all play a role in resulting material properties.^{40,41} Maintaining uniformity in polymer design and accelerating characterization of polymers both on the molecular and bulk levels may be some of the limitations with this approach. One solution to this issue is the use of the parallel synthesis of polymers (i.e., using individual reaction vessels to synthesize many individual polymers simultaneously and separately).^{40,41} The next step requires the characterization of the synthesized polymers with respect to their formed properties, including surface protein adsorption, rate of degradation, cytotoxicity, level of biocompatibility *in vivo*, and cellular response.^{40,41}

Tools for the Rapid Assessment of Material Properties

The previous section indicates that the synthesis of a large number of polymers rapidly is possible; however, without ample techniques to characterize the properties of these materials, their rapid development may not be advantageous. One of the earliest methods of rapidly assessing material properties was demonstrated by Kohn and coworkers⁴² in 1998. This was aimed at assessing the combinatorial polymer library consisting of 112 polyarylates that was described in the previous section. In addition to creating many distinct polymers, this work enabled the researchers to determine relationships between polymer structure and glass transition temperature (T_g), hydrophobicity, and mechanical properties. These properties were determined by systematic analysis using gel permeation chromatography (for molecular weight), differential scanning calorimetry (for T_g), thermogravimetric analysis (for decomposition temperature), and goniometry and sessile drop method (for air–water contact angle). Some of their conclusions included that oxygen substitution in the backbone is effective in increasing polymer T_g and in decreasing hydrophobicity (for substitution in the backbone and/or pendent chain) and that additional methylene groups in the backbone and/or pendent chain can significantly diminish polymer strength and stiffness. Thus, these approaches can also be used to develop polymer structure/property correlations for future material development.

In early 2004, Meier *et al.*⁴³ provided a comprehensive overview of the current development in HTS and assessment of material properties, which built on a previous review published in 2003.⁵ For the high-speed characterization of standard polymer molecular weights, recent high-throughput techniques have focused on using methods such as gel permeation chromatography in combination with high-speed columns, parallelization, and/or flow-injection analysis.^{43,44} Parallelization utilizes more machines to run test samples, but

is obviously limited by lab space and costs. New, higher speed columns allow for rapid size exclusion separations to occur in 2–6 min rather than 30 min to 3 h.⁴⁴ Additionally, researchers have utilized matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) techniques for absolute determinations of molecular weight, molecular-weight distribution, and end group analysis of macromolecules (which can be optimized using multiple-layer spotting sample preparation techniques, online monitoring of reactions, and/or ink-jet printing).^{45–47} Next, optical screening methods have been increasingly improved for high-throughput property and chemical composition assessment purposes.⁴³ These methods have been enhanced by combining them with imaging and commercial reader technologies for absorbance, fluorescence, and infrared (and near infrared) spectroscopy.⁴³

Infrared and near-infrared spectroscopy-based techniques, including attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR), are effective tools for measuring monomer/polymer compositions and polymerizations,^{48–50} while fluorescence spectroscopy approaches have been used to determine properties such as molecular weight, amount of branching, and catalyst selectivity.⁵¹ Lastly, numerous methods have been developed to address the area of screening for morphology and physical properties, particularly for polymer film analysis.^{52–57} These methods include using relationships between various physical properties and the preceding optical data, as well as the evaluation of thin films to determine adhesion, crystallization, or dewetting property characteristics.^{52,53,55–57} Fast differential scanning calorimetry analyses have been considerably accelerated with automated large sample array differential scanning calorimetry. This field is still developing and should continue to improve the speed of property characterization while maintaining equal or even better accuracy of data compared to traditional methods.⁴³

In combination with the above-mentioned synthetic nanoliter-spotting techniques, Tweedie *et al.*²⁶ synthesized a library of over 1700 photopolymerizable acrylate-based biomaterials using automated array synthesis and characterized the resulting mechanical properties with nanoindentation. Nanoindentation is a method of measuring nanometer-scale displacement with respect to load while depressing the material's surface with a rigid indenter. These methods were found to be quick and accurate in determining numerous mechanical response characteristics and could potentially be applied to other sets of combinatorial/crosslinked polymers.²⁶ This example presents a situation where a technology was modified to accelerate material property characterization with a new, rapid synthesis process. An additional method for measuring mechanical properties of thin films was strain-induced elastomer buckling instability for mechanical measurements.⁵⁸ This method utilizes the light scattering produced by the buckling of two mismatched polymers, the glassy thin film of interest and a softer silicone sheet.

The rapid characterization of polymer properties and cellular responses will be vital for the continuing development of tissue engineering research. As Kohn *et al.*⁴¹ described, the chemical composition of polymeric scaffolds significantly affects its mechanical/cell interaction properties and, therefore, will often determine the applicability of a potential engineered tissue scaffold. Kohn *et al.*⁴¹ cite recent studies that demonstrate how slight differences in polymer compo-

sition and structure [poly(L-lactic acid) vs. poly(DTE carbonate) and poly(DTE carbonate) vs. poly(DTB carbonate), respectively] can significantly affect cell response and long-term tissue growth, including differences in bone resorption, inflammatory response, degradation, tissue in-growth, and foreign body response.⁵⁹ The considerable effects of minor polymeric differences support the need to develop and utilize high-throughput methods that rapidly and systemically characterize many polymer compositions. In the future, this may increasingly depend on computational programming as we will discuss in the subsequent section on assessing cell-material interactions.⁴¹ Additionally, x - y spatially resolved libraries, in which polymer composition varies across the x and y coordinates, respectively, have also been utilized to assess a wide range of polymer mixtures using some of the analytical methods previously described (i.e., optical microscopy).^{36,41,60} This type of method should also play an important role in the future development of rapid material characterization.

As important as the different methods for measuring outcomes is the use of statistical methods to minimize the number of repeats,^{61,62} design experiments to isolate critical variables,⁶³⁻⁶⁸ and mine large amounts of data for optimal conditions. This is important for all topics covered in this review. For example, by using high-quality assays with sufficient separation between positive and negative controls, the calculation of the Z-factor greater than 0.5⁶² allows chemical hits to be determined with high confidence without using replicates. Likewise, a formalized "Design of Experiments," using full and fractional factorial designs, allows for the variation of several parameters at once to determine optimal conditions.^{66,68} Traditional methods of single parameter variation for optimization rapidly increases the amount of experimentation as more variables are introduced, while fractional factorial designs can greatly reduce the studies needed to determine critical inputs. Methods for using informatics to mine the large amounts of data produced using combinatorial and HTS are crucial to gain the most insight into processing capabilities. While biochemical assays often are looking for the discovery of hits, for combinatorial library screening it is important to try and tease out relations between properties with the chemical structures and processing parameters. For these types of studies, cluster analysis can be used to link patterns of material properties with their associated processing parameters, whereas principal component analysis reduces data into descriptive vectors to more rapidly describe structure property relationships.⁶⁹

The availability and quality of high-throughput assays for material characterization are commercially relatively limited, leading to inefficient iterative approaches for analysis. Recently, there has been significant progress in characterizing various biomaterial assays and developing reliable and advanced high-throughput assay systems. Additionally, the use of computational systems to artificially determine cell-biomaterial interactions based on known properties has gained traction, but remains limited due to the complexity of these interactions.⁴⁰ These algorithmic systems have the potential to exponentially increase efficiency in developing and analyzing combinatorial virtual libraries by bypassing the actual, physical production of thousands of polymeric combinations. It is clear that there is still room for the development of new techniques to rapidly characterize material properties

from large libraries of polymers. Beyond bulk properties and toward tissue engineering applications, it is also necessary to rapidly characterize how cells interact with a range of materials.

High-Throughput Assessment of Cell-Material Interactions

Using the vast array of combinatorial polymer libraries and many of the tools for material property analysis, the next step in developing the ideal microenvironment for engineered tissue is the high-throughput assessment of cell-material interactions. This ideal microenvironment will often seek to mimic the natural environment of particular cell types or even improve on this environment by cultivating growth and proliferation.⁴ Particularly in the field of tissue engineering, the extracellular environment including surface properties such as roughness and hydrophobicity and specific cell-surface interactions maintains a large influence on cellular attachment, spreading, overall growth, and differentiation in the case of stem cells.¹ The use of high-throughput systems to improve the speed and breadth of characterizing and understanding these effects on cellular responses would significantly accelerate the process of developing effective engineered tissue constructs that could be utilized in medical applications.

The development of systems for analyzing cell-material interactions in bulk began in the late 1990s, shortly after the production of the first large combinatorial polymer libraries. In addition to developing a combinatorial polymer library and demonstrating rapid polymer property characterization, Kohn and coworkers⁴² also performed one of the earliest high-throughput analyses of cell-material interactions in 1998. This analysis studied the interaction between 42 polyarylate polymers (a subset of the 112 polymer library) and fibroblast cells, focusing on proliferation using an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. In general, these investigators found a linear correlation between decreased proliferation and increased surface hydrophobicity of materials derived from non-oxygen-containing diacids. The analysis also showed that all polymers derived from oxygen-containing diacids acted as good substrates for fibroblast proliferation. Along with these findings and others, this study illustrated the ability to determine important material interactions with only small amounts of polymer material.

Since these early pioneering analyses, high-throughput assessment has seen significant growth in the volume of analyses, the breadth of cells and polymers studied, and the variety of cell responses that have been characterized. In one study, over 1700 combinatorial nanoliter-scale polymer spots of various (meth)acrylates were introduced to a single slide, thereby providing a high-throughput platform for analyzing cell attachment, cell spreading, and cell-type-specific growth.^{1,7} This work examined the effects of these polymers on human embryonic stem cells (hESCs) and C2C12 cells (a mouse myoblast progenitor cell line), including cell growth, adhesion, and proliferation.¹ Additionally, positive matches for cell attachment and spreading were also tested for differentiation into cytokeratin-positive cells. A representative polymer array with cells attached is shown in Figure 2. The process of successfully identifying cell-material interactions

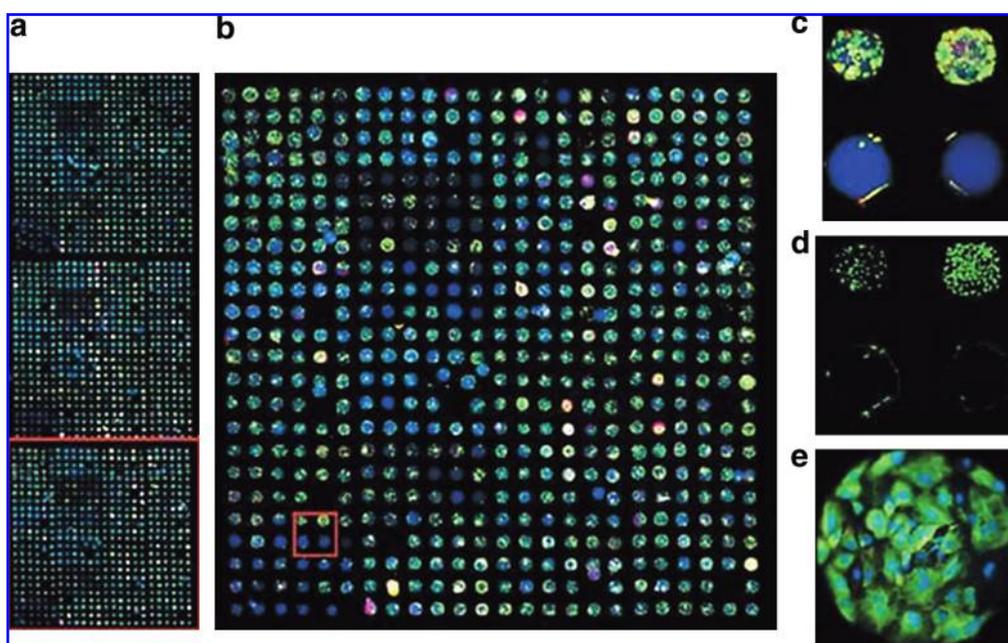
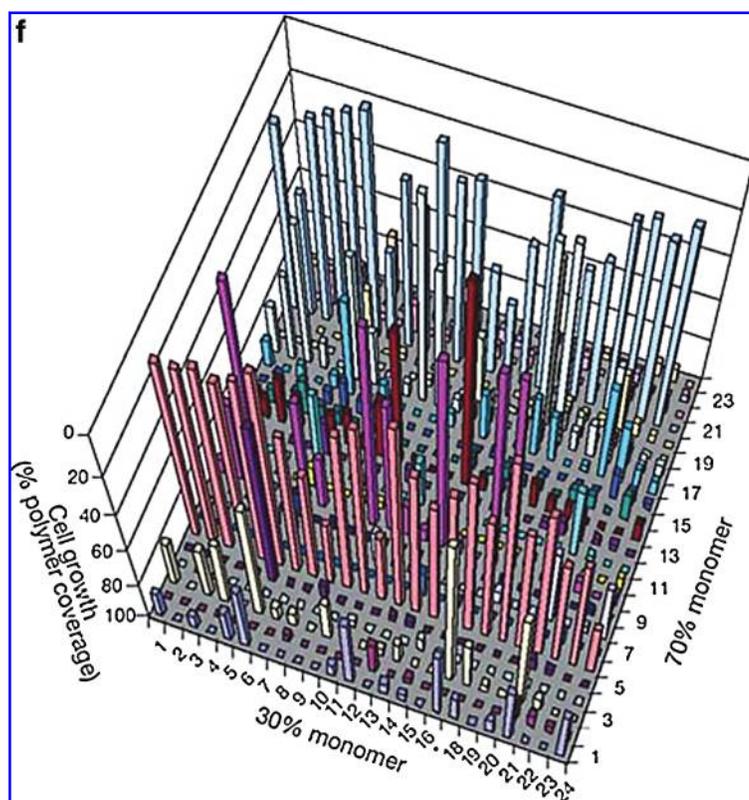


FIG. 2. Polymer arrays to assess cell–material interactions. (a–c) Examples of fluorescent images of human embryonic stem cells (hESCs) cultured on polymer arrays in the presence of retinoic acid for 6 days and then stained for cytokeratin 7 (green) and vimentin (red) (a–c, e) or nuclei (green) in (d). (f) Quantification of cell coverage as a function of polymer composition after 6 days. These techniques are useful to identify unexpected relationships between material chemistry/properties and cellular interactions, including stem cell differentiation. Reprinted with permission from Anderson *et al.*¹ Color images available online at www.liebertonline.com/ten.



and the ease of producing and characterizing cytokeratin-positive cells in particular could be utilized in developing engineered epithelia tissue. C2C12 cells showed attachment and growth on almost all material combinations. This differentiation between the two cell types could also be effectively used in advanced scaffolding for engineered tissue applications. Lastly, it was found that cell growth and proliferation is

dependent on the presence of retinoic acid for some specific polymer combinations, which could potentially be employed to control cell behavior in tissue engineering. This study relied on fluorescent techniques that could be quantified with image analyses. The importance of this work is that it assessed many formulations, including material/soluble factor combinations, rapidly and that it went beyond identifying correlations

and focused on the identification of random combinations by utilizing the benefits of high-throughput studies.

This technology was further expanded to encompass arrays consisting of 3456 individual combinations of biodegradable polymers, and the interactions of human mesenchymal stem cells (hMSCs) were assessed with regard to properties such as attachment and spreading.²⁷ This platform maintained the added benefit of being able to synthesize and characterize polymeric materials using conventional methods instead of producing nanoliter-scale materials on the array. Many of the combinations were found to foster attachment and growth, including poly(lactic-co-glycolic acid) (PLGA) combined with L-lactide. These characterizations display the potential of designing effective biomaterials to enhance stem cell development, including neural stem cell growth and perhaps differentiation through engineered, combinatorial microenvironments. Looking forward, Anderson *et al.*²⁷ identified the screening of biomaterial interactions with other microenvironment components such as proteins as a potential application for this specific high-throughput microarray platform.

As researchers have continued to study cell–biomaterial interactions, these analyses have become increasingly complex and multifunctional. For instance, Neuss *et al.*⁷⁰ developed a grid-based system for the screening of various combinations of 7 different stem cell lines (including pluripotent embryonic and multipotent adult stem cells) and 19 polymeric materials. Through this research, five factors (morphology, vitality, cytotoxicity, apoptosis, and proliferation) were characterized using multiplex assays for each of 140 combinations. From this standardized, parallel analysis, they were able to identify combinations of stem cells and polymer scaffolds, including human dental pulp stem cells on poly-DL-lactic acid and human preadipocytes on texin, that support cell adhesion and proliferation and prevent apoptosis and necrosis. This type of diverse combinatorial assessment of numerous cell/material parameters should be extremely useful in quickly characterizing cell and scaffolding combinations for specific tissue engineering functions.

As the field has progressed, high-throughput methods have increasingly taken advantage of fluorescence-based analysis of cellular responses. For instance, investigators have recently developed a high-throughput method for testing the growth, adhesion, and morphology responses of mouse connective tissue fibroblast cells (L929) on a set of 214 polyurethane-based polymers.⁷¹ In this study, a probe composed of nonfluorescent 5-chloromethylfluorescein diacetate was utilized to enter the cell and then display fluorescence when it was cleaved by intracellular esterases. Combining image capture and screening analysis with the appearance of fluorescence enabled the team to rapidly quantify the growth, adhesion, and morphology of the fibroblasts. In addition to identifying clear trends relating cellular adhesion and polymer structure, the researchers also emphasized that this methodology could be scaled up to 1024 individual experiments per slide, allowing very high-volume and quick analyses with only small quantities of materials.⁷¹ Numerous other studies have also utilized fluorescence-based analyses of cell behavior^{27,64,72,73} or protein–material interactions,⁷⁴ as these techniques are easily applied and effective. Modern transfection techniques have allowed researchers to transfect cell lines with genes for fluorescent proteins that are activated when the

reporters of genes of interest are activated.^{3,75} This allows for passive observation of genetic activation of cell differentiation.

Another trend is the progression toward complete micro-environment analyses, including both the ECM components and the soluble growth factors and molecules. This holistic approach should better represent the actual *in vivo* conditions and enhance the assessment of cellular response to these conditions. Flaim *et al.*⁷⁵ developed one of the first models for this type of analysis, which consisted of a platform for 1200 simultaneous experiments studying the effects of 240 different microenvironments on stem cell behavior. These microenvironments were composed of mixtures of both ECM (fibronectin, laminin, collagen I, collagen III, and collagen IV) and soluble factors (wnt3a, activin A, bone morphogenetic protein-4 [BMP-4], and fibroblast growth factor-4). Flaim *et al.*⁷⁵ employed an overall microarray design but also utilized a multiwell platform, in which each well contained several spots where a single ECM arrangement was maintained and soluble factor composition was varied. Growth levels were recorded by measuring the amount of nuclear DNA, while differentiation was again measured using a fluorescence-based reporter gene. This approach could potentially be utilized with any set of environmental factors and cell lines, thereby creating a more natural environment for engineered tissue development.

As mentioned previously, one of the recent advances in engineered tissue development was the design and production of the first large-pore polymer biomaterials that could be analyzed in three-dimensional, high-throughput environments for their effects on cell behavior (developed by Yang *et al.*³⁹). Previous studies, including one performed by Levenberg *et al.*,⁷⁶ had completed similar work including the analysis of hESC growth and differentiation in a three-dimensional 50% PLGA/50% PLLA environment with growth factors. While successful growth and differentiation was found with different growth factors in specific pore environments (250–500 μm), this study did not vary the polymer composition or analyze polymer variations in high throughput.⁷⁶ In their advanced, high-throughput analysis, Yang *et al.*³⁹ found a relationship between increasing poly (desaminotyrosyl-tyrosine ethyl ester carbonate) content and increasing osteoblast adhesion. It was also determined that protein adsorption affects cell responses, including osteoblast adhesion for these polymeric scaffolds. Overall, this analysis demonstrates a tremendous improvement over two-dimensional films or surfaces that have traditionally been used in studying cell–material interactions. This is due largely to the similarity of three-dimensional environments to *in vivo* conditions, as well as the varying cell response to the environment structure and material topography. Even so, in this case, Yang *et al.*³⁹ found that the cell adhesion trends in three-dimensional scaffolds fit with the trends previously determined in two-dimensional screening, which indicates that two-dimensional screening may be used to effectively predict cell response in three-dimensional environments. Additionally, one drawback of this system is that wells of only 0.2 mL of medium may not be enough to support mature, high-density tissue growth greater than 14 days. The small scale also limits cell access to the scaffold and thereby restricts nutrient/waste exchange and deep tissue formation. Even so, these types of platforms can still provide a great environment

for testing early stage cell properties such as adhesion and proliferation in a variety of scaffold dynamics.³⁹ Along with high-throughput platforms, the capacity to use three-dimensional screening for cell–material interactions should also considerably enhance the development of applicable polymeric scaffolding and engineered tissues.

Beyond experimental assessment, computational system analysis of combinatorial polymer virtual libraries has been identified as an area of great potential in analyzing material properties for tissue engineering applications. One recent development in computational methods was designed by Kohn and coworkers⁷⁷ to successfully predict the cell growth response to a biomaterial based on its chemical composition and physical properties such as T_g and contact angle (θ). The model, called the logical analysis of data, was developed using 62 known polymers and was then able to distinguish between high- and low-growth polymers for 50 uncharacterized materials and even correctly characterize several materials that cultivated especially high cell growth. This particular model was used to predict the response of rat lung fibroblasts and foreskin fibroblasts to the set of polymers, but could potentially be applied to other cell types and other biomaterials. While this study demonstrates the potential of computational systems to enhance efficiency in combinatorial analysis by bypassing the physical construction of polymer libraries, this type of modeling software still requires further development, including the addition of more input parameters and output measurements, to become widely applicable across various analyses.⁷⁷

Quantitative structure activity relationship (QSAR) analyses have been previously used in the pharmaceutical industry, but are recently being applied to the study of cell–biomaterial interactions.⁷⁸ Recent models have utilized this quantitative analysis to predict cellular response and protein adsorption to biodegradable polymeric biomaterials.^{78,79} The model predicted the biological activity for six polyarylates with an average percent error of only 15.8% compared to the measured values using a quantitative system based on five parameters—the number of tertiary carbons, the number of branches in the pendent chain, the molar refractivity, the polar surface area, and the logarithm of the octanol–water partition coefficient.⁷⁸ A subsequent model again utilized five input parameters chosen through QSAR analyses and put into an artificial neural network (ANN) platform to predict the protein adsorption and biological response for 77% and 71% of the polymeric test materials, respectively.⁷⁹ Kohn and his coworkers⁸⁰ also utilized an ANN model to predict fibrinogen adsorption and cell growth for the previously presented 112 polyarylate library.

More recently, larger scale attempts at computationally predicting cell–material interactions have been pursued. A study performed by Kholodovych *et al.*⁸¹ analyzed the cellular response to a combinatorial library of 2000 polymethacrylates using a polynomial neural network (PNN) as opposed to the traditionally used ANN algorithms. The benefits of the PNN tool include its ability to assemble the architecture (i.e., the precise number of neurons in the input, output, and hidden layers) in response to the characteristics of the data and to handle very small or very large data sets.⁸¹ These qualities will likely make PNN algorithms, in conjunction with QSAR analyses, extremely useful in the future for computationally screening many diverse cell–biomaterial interactions. Even so, despite this progress, the computa-

tional prediction field still needs further development to become fully applicable across the wide range of polymeric materials and the complex variety of cell lines. Additionally, at this point in development, prediction results from computational systems must be rechecked in actual, full-scale physical experiments to confirm the cell–material interaction. However, computational systems will certainly help to rapidly assess and identify optimal material formulations, beyond traditional experimental techniques.

Overall, the field of high-throughput assessment of cell–material interactions has experienced a tremendous amount of progress as a variety of platforms and analysis techniques have been designed to better identify successful microenvironments. Even so, this field is still considered to be in its early stage.⁴ There are still significant shortcomings to these systems; namely, the systems are *in vitro* and do not perfectly imitate *in vivo* conditions, as well as the limitations in drawing conclusions from high-throughput, very low-volume conditions (i.e., cell–material interactions must be retested in higher volume conditions to demonstrate accuracy). In moving forward, researchers will need to continue to develop platforms and microenvironments that emulate actual *in vivo* conditions (i.e., scaffolding combined with soluble factors and fluidic environments), as well as move from the micro-scale to the macro-scale to fully approach tissue engineering applications.⁴

Microfluidic Devices for the Rapid Development of Engineered Environments

One effective method to assess cell–material interactions is the use of microfluidic systems. Microfluidic systems can be used for a variety of applications and simulations, but for tissue engineering, these devices are primarily used for HTS, characterization of cell–material interactions, and the optimization of the conditions that precisely regulate cell behavior and fate.^{2,82} Like many other microscale techniques, microfluidics are inexpensive and able to operate with fewer cells and reagents.² In addition to these advantages, microfluidic devices provide a fluid, dynamic environment that is much more similar to *in vivo* conditions compared to traditional static platforms, such as those described in the previous section.

One microfluidic platform that has gained use involves the generation of gradients of soluble or bound factors to assess the factor's influence on interacting cells.⁸³ For instance, in 2005, Chung *et al.*⁸⁴ produced a microfluidic system that delivered a gradient of growth factors, including epidermal growth factor, fibroblast growth factor-2, and platelet-derived growth factor to human neural stem cells. In response, these cells proliferated and differentiated into astrocytes proportionally to the concentration of growth factors. Proliferation was directly proportional to growth factor concentration and conversely, differentiation occurred in an inversely proportional relationship with growth factor concentration. For this experiment, the actual microfluidic device was produced using the techniques of rapid prototyping and soft lithography. The gradient flow of multiple factors allows for the rapid optimization of culture conditions while using low concentrations of relatively expensive growth factors and cells.⁸⁴ These benefits of gradient-generating microfluidic platforms should considerably enhance the process of optimizing

culture and scaffolding conditions for a variety of tissue engineering applications. Also, microfluidics allow precise control over the timing of multiple soluble factors.

Microfluidics have also helped identify ideal cell microenvironments through the development of control over soluble chemical distribution within a scaffold. This strategy was explored in contrast to previous research, which has largely focused on adjusting the chemical and mechanical characteristics of the scaffold.⁸⁵ In 2007, Choi *et al.*⁸⁵ described a method for managing this distribution using microfluidic channels constructed within the cell/biomaterial combination to complete convective mass transfer of the solutes. The solute exchange is completed in two steps involving interfacial convective mass transfer between the flowing solutions and the walls of the microchannels and molecular diffusion between these walls and the bulk of the scaffold.⁸⁵ This model included a calcium alginate hydrogel and chondrocyte cell lines and utilized lithography to develop the microfluidic structure. This precise control of soluble chemical distribution could help prevent necrosis from developing at the center of thick engineered tissues as well as guide cell growth toward regions within engineered tissue.⁸⁵ Even so, these models still must overcome numerous challenges, including the organization of multiple cell types into conditions similar to those found *in vivo* and the inclusion of multiple stimuli types, including mechanical forces.⁸⁵

Khademhosseini and coworkers⁸⁶ have also developed microfluidic channels in cell-seeded agarose hydrogels using soft lithographic techniques. This work showed that cell viability in large engineered tissue constructs can depend largely on the distribution of nutrients and oxygen through vasculature-like channels and proved that microfluidics can help overcome the difficulty in transport and exchange of materials, namely, nutrients, that typically causes cell ne-

crosis in large three-dimensional engineered tissues. While further development is necessary, this method will certainly enhance the progress toward engineered three-dimensional scaffolds with artificial vasculature that allow for the exchange of nutrients, waste, and signaling molecules. The technology could also be used for assessment and screening of multiple environments in single constructs. This work has been expanded to produce three-dimensional cardiac organoids on a patterned HA platform.⁸⁷ In this model, seeded cardiomyocytes grew parallel to the pattern direction and subsequently detached from the scaffold to develop into contractile cardiac organoids. For confirmation, contractile properties were assessed and quantified using imaging technology. This method displays the double advantage of both growing and aligning the myocytes on a biocompatible scaffold and allowing for natural detachment of the contractile cells without requiring enzymes. In addition to a variety of other studies, these methods, namely the microfluidic patterning, could potentially be used in biodegradable scaffolds to organize cell growth for the purposes of tissue engineering and full tissue replacement.⁸⁷

Other researchers have also used soft lithography techniques to develop hydrogel-based microfluidic platforms. For instance, in 2007, Figallo *et al.*⁸⁸ used soft lithography methods to design a micro-bioreactor array (MBA), which consists of layers of poly(dimethylsiloxane) and glass and maintains microfluidic channels throughout the system. The microfluidic channels are able to supply the perfusion of a controlled medium and enhance the growth of cells in high density, including the advantage of purging residual nonpolymerized reactants. A schematic of the device, as well as representative cellular outcomes are shown in Figure 3. The miniaturization of the bioreactor allows for better use of expensive media factors while still maintaining this advantage of a fluid,

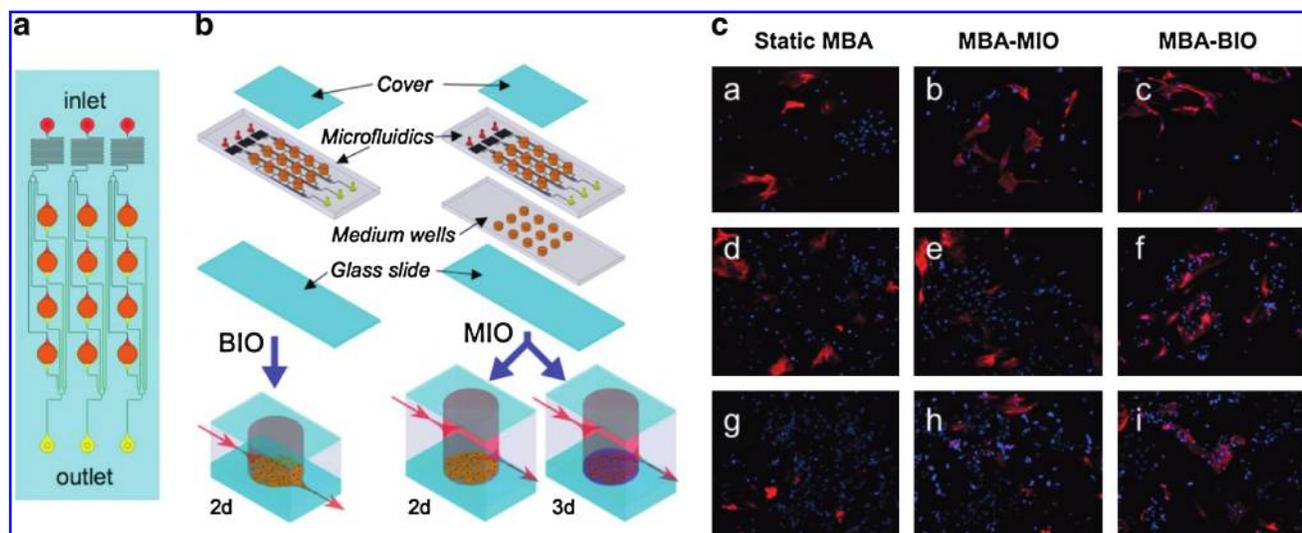


FIG. 3. Micro-bioreactor arrays (MBAs) for identification of ideal cellular environments. Microfluidic device (a) and well schematics (b) for configurations that include a bottom inlet/outlet (BIO) where the flow is directly over the cells and a middle inlet/outlet (MIO) configuration where the cells are in a well with fluid flow over top. hESCs were cultured and stained for smooth muscle actin (red) and DAPI (blue) in three different flow configurations (c): static (left column: a, d, and g), perfused MIO (middle column: b, e, and h), and perfused BIO (right column: c, f, and i) at three different cell densities: 60 ± 6 (top row: a, b, and c), 160 ± 4 (middle row: d, e, and f), and 314 ± 15 (bottom row: g, h, i). This technology is useful to assess microenvironments for tissue engineering, particularly under a range of flow conditions. Reprinted with permission from Figallo *et al.*⁸⁸ Color images available online at www.liebertonline.com/ten.

dynamic system. In different perfusion setups, the MBA system of 12 micro-bioreactors was utilized to compare the expression of smooth muscle actin and cell density (for various cells including hESCs). Additionally, the group was able to analyze cell differentiation by quantifying the expression of cell differentiation markers using an *in situ* and automated image analysis system. As noted by Figallo *et al.*,⁸⁸ this complete system should prove to be quite useful for the study of cell behavior, especially the complex development of hESCs, in fluidic environments.

Other microfluidic platforms have been developed for the purposes of complex cell culture and experimentation in conditions very similar to an *in vivo* environment. In 2007, Lee *et al.*⁸⁹ developed such a model consisting of a cell culture niche, an artificial, microfluidic endothelial (perfusion) layer, and a nutrient transport vessel. This system utilized microfluidics to direct mass transport on the microscale and to help localize cells for increased density and viability in certain areas of the microenvironment. More recently, chip-based microfluidic devices have been developed and utilized to produce microscale scaffolds. For instance, Lee and coworkers⁹⁰ have constructed tissue engineering scaffolds by integrating PLGA microfibers into a microfluidic chip. In this preparation, the microfibers were generated by precipitating streams of PLGA in dimethyl sulfoxide out of a water/glycerol mixture and varying the flow rate of PLGA to produce fibers of different widths.⁹⁰ Neural progenitor cells were then grown on the three-dimensional scaffolds to determine its potential for use in neural tissue engineering.⁹⁰

It is clear that complex environments are crucial to truly assess the potential of approaches in recapitulating the complex microenvironments found *in vivo*. This includes systems that utilize fluid flow, three-dimensional structures, and both bound and soluble cues. Thus, microfluidics provide platform technology for rapidly assessing these environments, whether it is with gradients of cues or through the interface of fluid flow and arrays of polymers. However, much of this previous work has focused on the assessment of known soluble cues (i.e., growth factors) for tissue engineering applications.

Screening Molecules for Stem Cell Differentiation

The identification of molecules that control cellular behavior is essential to expand the breadth of cues that may be incorporated into tissue engineering approaches. One emerging field is the use of HTS of molecule libraries for the control of stem cell differentiation. Extensive studies have assessed potential drugs and growth factors looking for soluble cues to direct the fate of stem cells toward a desired phenotype. These attempts have been limited to smaller studies of a limited number of factors due to the techniques in culture and cost of reagents and proteins. Additionally, efforts to recapitulate growth factor cocktails and precise timing of their delivery, as seen during development, have been similarly limited in scope and complexity. With the advent of HTS techniques, smaller numbers of cells can be used to screen a wider range of potential drugs and cytokine delivery schemes to optimize stem cell differentiation. Although many cell types could be investigated, this section will focus specifically on the use of HTS technology toward stem cell differentiation.

To date, few studies have specifically investigated the use of HTS of small molecules for stem cell differentiation. One of

the earliest examples involved a study from the Schultz lab that screened a heterocycle combinatorial library.⁹¹ This library was built using the template of known kinase inhibitor scaffolds, such as purine and pyrimidines, to include nearly 50,000 different small molecules of unknown potential. Originally, this library was thought to be a possible source for new ligands to further investigate the function of new proteins by inhibiting or stimulating their signaling; however, they soon began to investigate their differentiation potential on stem cells. For example, the library was screened with mouse embryonic mesoderm fibroblast C3H10T1/2 cells for osteogenic differentiation.⁹² The initial screen used increased alkaline phosphatase (ALP) expression, which is important to bone mineralization, as a marker for differentiation. Fifteen compounds were identified that stimulated ALP activity, but the molecule they named purmorphamine was the most potent of these. This compound was shown to be more potent than BMP-4 with optimized concentrations, and the combined effect of BMP-4 and purmorphamine were synergistic and not surrogate. The osteogenic nature of purmorphamine was confirmed with increases in the induction of the transcription factor Cbfa1 and histological staining for ALP on several other murine mesenchymal cell lines. Further investigation⁹³ found that purmorphamine stimulated osteogenic differentiation along the hedgehog signaling pathway.

Similarly, HTS was used to identify molecules from this library that drove neuronal⁹⁴ and cardiomyogenic⁹⁵ differentiation of murine embryonic stem cells. Hits were initially screened using transfection of reporter gene activated luciferase plasmids. The use of the library to drive dedifferentiation⁹⁶ also led to the discovery of reversine, which allowed murine myoblasts to dedifferentiate into stem-like cells and then redifferentiate along osteogenic or adipogenic cell lines. This could be important in discovering new autologous sources for cell therapies. These discoveries were largely used to investigate the processes and signaling used during differentiation, not for actual therapy.

More recent studies have screened libraries for suppressors of differentiation rather than looking for new promoters.^{93,97} These can be useful for treating certain disease progressions where it would be therapeutically advantageous to prevent differentiation or in understanding the nature of diseases where normal function is inhibited, but the causes are not understood. The study by Yui *et al.*⁹³ screened over 7500 commercially available compounds by investigating their effects on the normal embryonic development of the dorsal structures in zebrafish. This required the observation and measurement of several individual embryos manually, so it lost some of the rapidity of the soluble factor assays previously discussed, but includes the complexity of the *in vivo* system that two-dimensional culture lacks. This method was able to identify dorsomorphin as a BMP type I receptor inhibitor, which prevents osteogenic differentiation.

A high-throughput siRNA library was used to screen hMSCs for osteogenic suppressors by Zhao and Ding.⁹⁷ MSCs were transfected by a library of 10,000 unique sequences, two per gene, and the ALP activity was then imaged. An increase in ALP activity indicated that the inhibited gene was normally an osteogenic suppressor. This initial screen identified 55 hits for osteogenic prevention, which included genes for a wide variety of kinases, ECM proteins, protein receptors, ion channels, among others. From this initial screen, 12 were

selected to investigate further for prevention of osteogenic differentiation. Interestingly, the different suppressors fell into two types the authors designated “fate specific” or “fate non-specific.” The fate-specific genes were suppressors of osteogenic differentiation because they preferred an adipogenic lineage. The fate-nonspecific type prevented differentiation along any line. These would be important in maintaining the multipotency and proliferation of stem cells, and may be useful for understanding and prolonging a stem cell line *in vitro* or possibly in the dedifferentiation of committed cell lines.

One recent study by Mauck and coworkers⁹⁸ used HTS of hMSC pellets to find molecules that stimulate or suppress chondrogenesis. Since MSCs typically undergo chondrogenesis in pellets, it was necessary to scale down pellet cultures for rapid assessment and culture in round-bottom 384-well plates. Chondrogenesis was assayed by measuring the glycosaminoglycan (GAG) content of the pellets after a week in culture. The setup was first run to evaluate the combinatorial effects of four different growth factors at different concentrations. This allowed for the confirmation of BMP-2 and transforming growth factor- β as being synergistic in chondrogenic differentiation, but also demonstrated how fibroblast growth factor-2 increased cell proliferation and chondrogenic potential of hMSCs. The small volumes of the 384-well plates allow for extensive screening of different growth factor cocktails that are often limited in large scale experiments by the cost of cytokines. Secondly, the National Institute of Neurological Disorders and Stroke (NINDS) library was screened for both chondrogenic inducers and inhibitors. Compounds that led to a 40% reduction in DNA content were removed from the screen to eliminate compounds that reduced GAG content due to their cytotoxicity. This method identified 24 potential inhibitors, including many antimetabolites, antibiotics, and antineoplastics, among others. Outcomes that include hits for molecules that either induce or inhibit stem cell chondrogenesis are shown in Figure 4. Dose response investigation may be useful in these cases to determine if changes in the dose would lead to further cell death or increased effectiveness.

HTS techniques are beneficial due to their ability to quickly screen large libraries of small molecules for desired outcomes. Often, though, the hits are just the beginning, whereby the mechanisms of their action and the optimization

of the dosage must be further investigated. Thankfully, the same HTS setups can aid in the optimization of dosage or the synergies of multiple factors. As always, *in vitro* culture is often limited in capturing effects in an artificial environment that may not translate *in vivo*. The zebrafish experiments⁹³ are interesting in their ability to add a level of complexity, but made it impossible to isolate the effects on specific cells.

Rubin⁹⁹ lamented that often more information could be gleaned from these experiments if the researchers had more diverse sets of compounds and the expertise in chemistry and drug design to further develop initial hits. As such, often researchers are limited by smaller more established sets, such as the NINDS library, that is already characterized for other applications and may already be in use for other therapeutics. This has the benefit of a possibly faster implementation of these therapies if they are discovered, but that the larger research misses out on the true promise of these screens to investigate more diverse and uncharted libraries.

From a tissue engineering standpoint, these small molecule discoveries can be useful in the design of materials, the differentiation of implanted cells, or to discover new cell sources. Scaffolds can incorporate the release of newly discovered small molecules into the environment around these implants to either drive specific differentiation of incorporated cell therapies or to recruit native local stem cells and drive their differentiation for desired outcomes. The molecules, as shown previously,⁹⁶ can be used to dedifferentiate autologous cells toward a stem cell line that can then be expanded to increase cell numbers to therapeutic levels. Then the cells can be differentiated along the desired lineage *in vitro* before implantation.

Summary and Future Directions

Through this review, we have discussed many of the stages involved in research and development for tissue engineering applications and have specifically focused on high-throughput and combinatorial techniques in polymer synthesis, material characterization, cell-material interactions including microfluidic platforms, and small molecule screening to direct cell behavior. While combinatorial synthesis methods have been studied for years and for various applications, these techniques are still developing as a crucial tool

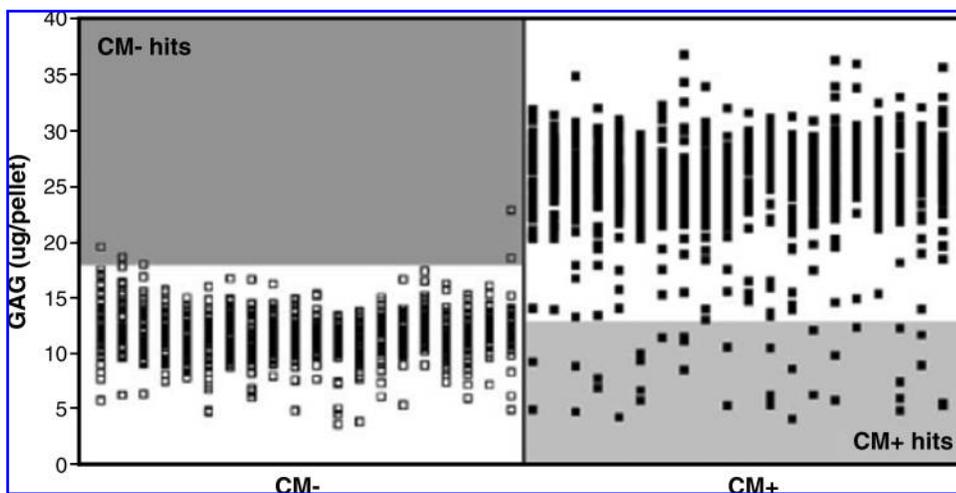


FIG. 4. High-throughput screening of small molecules that influence stem cell differentiation. Screening of a small molecule library (NINDS) to identify inducers (CM⁻ hits, gray area) and inhibitors (CM⁺ hits, white area) of MSC chondrogenesis, measured by glycosaminoglycan (GAG) levels. This technology is applicable to a range of cell types useful in tissue engineering applications. Reprinted with permission from Huang *et al.*⁹⁸

in rapidly producing a wide variety of polymeric materials as potential scaffolds. In the future, miniaturization of polymer libraries to the nanoscale level, the design of three-dimensional combinatorial libraries, and parallel synthesis methods should continue to influence the ease and utility of using libraries to develop polymer scaffolds. In assessing the characteristics of these large libraries, a variety of techniques, from parallelization to fluorescence spectroscopy, have been developed and employed, yet there is still room from improvement in the commercialization and standardization of these methods. Moving forward, computational systems should play an increasingly important role in determining polymer properties and cell–material interactions as they become applicable to a wider variety of cell and/or material combinations.

The high-throughput analysis of cell–material interactions is perhaps the most important step in developing a successful engineered tissue. This field has also seen significant progress as researchers have produced more complex and HTS platforms as well as designing three-dimensional screening environments and including additional factors such as soluble growth factors. Microfluidic systems have also proved to be quite useful in replicating an *in vivo* environment and should be further utilized in studying a range of polymer arrays and soluble factors in high-throughput and under fluid conditions. Ideally, microfluidic platforms will provide structural support; a three-dimensional, dynamic environment for analysis of cell behavior; and pathways to control solute and factor distribution/flow. New imaging techniques will be developed that will help accelerate the identification of optimal environments during screening. Finally, although it is a young field, the application of high-throughput techniques to the screening of molecules for stem cell response has increased the speed of characterizing large libraries of small molecules. These techniques can help a number of engineered tissue factors, but should be most useful in controlling cell differentiation. To this end, future research should maintain a partial focus on the standardization of techniques for measuring differentiation (e.g., the previously discussed fluorescence and transfection tools). Additionally, the further development of analysis methods for the tremendous amount of data produced by these high-throughput systems will be necessary for the field to progress appropriately in the coming years. Overall, all of these platforms and analyses will continue to contribute to the development of the ideal microenvironment for engineered tissue, including the optimal synergy between cells, scaffolding, and extracellular factors. In pursuing this goal, the use of high-throughput and combinatorial techniques should continue to enhance our progress toward *in vivo* testing and clinical tissue engineering applications.

Disclosure Statement

No competing financial interests exist.

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