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Review

High-throughput screening of cell responses to biomaterials

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ABSTRACT

Biomaterials have emerged as powerful regulators of the cellular microenvironment for drug discovery, tissue engineering research and chemical testing. Although biomaterial-based matrices control the cellular behavior, these matrices are still far from being optimal. In principle, efficacy of biomaterial development for the cell cultures can be improved by using high-throughput techniques that allow screening of a large number of materials and manipulate microenvironments in a controlled manner. Several cell responses such as toxicity, proliferation, and differentiation have been used to evaluate the biomaterials thus providing basis for further selection of the lead biomimetic materials or microenvironments. Although high-throughput techniques provide an initial screening of the desired properties, more detailed follow-up studies of the selected materials are required to understand the true value of a 'positive hit'. High-throughput methods may become important tools in the future development of biomaterials-based cell cultures that will enable more realistic pre-clinical prediction of pharmacokinetics, pharmacodynamics, and toxicity. This is highly important, because predictive pre-clinical methods are needed to improve the high attrition rate of drug candidates during clinical testing.

Contents

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1. Introduction

Biomaterials have been used for a variety of biomedical applications for hundreds of years. For example, wood and gold were used in dentistry as tooth implants as early as 700 AD (Anderson et al., 2004a; Langer and Tirrell, 2004; Ratner et al., 1996). Over the years, many other biomaterials have been investigated ranging from natural biomaterials (i.e. collagen, hyaluronic acid, alginate and dextran) to synthetic polymers (i.e. metals and ceramics). In this era of biomaterials research, many materials were selected based on trial and error, availability, and macroscopic properties. Since the late 1800s, synthetic polymers have been increasingly used for biomedical purposes (e.g. Dacron in vascular grafts and poly(methyl methacrylates) in hip replacements. Synthetic materials are desirable in that their chemical, biological, and mechanical properties can be easily engineered. For example, by using simple modifications such as changing the polymer concentration and crosslinking density, the rigidity, and biodegradability of the materials can be tailored. Furthermore, synthetic materials can be modified with desired biological properties. An example of this is the conjugation of peptides such as arginine-glycine-aspartic acid (RGD) moieties to regulate cell adhesion (Burdick et al., 2004). Synthetic biodegradable polymers such as polylactides, polyglycolides and their copolymers are Food and Drug Administration (FDA) approved biomaterials with a number of biomedical applications including resorbable sutures, tissue engineering scaffolds, and drug delivery devices. The biomedical use of biomaterials has generated a large industry that includes major products (i.e. catheters) and medical devices (i.e. contact lenses, renal dialyzers, stents, and intraocular lenses) (Lysaght, 2000).

In addition to biomedical devices, biomaterials have also been used as tissue culture materials for growing cells in culture and for providing scaffolds for tissue engineering applications. Biomaterials can provide the cells with the desired matrices that mimic the native environment of the cells. For example, natural components such as collagen and polysaccharides have been used for a long time as tissue culture substrates. Furthermore, biomaterials such as hydrogels provide a three-dimensional (3D) environment in which cells can be encapsulated. For tissue engineering, biomaterials have been used as scaffolds (Hubbell, 2004; Langer and Vacanti, 1993). Polylactides, polyglycolides and their copolymers have become widely used for cell-based tissue engineering research (James, 1996). These polymers are degraded to non-toxic end products, carbon dioxide, and water. The rate of the polymer degradation can be adjusted by the stereochemistry, molecular weight, and copolymer composition over a wide range of time periods. These polymers can be processed to several forms including porous scaffolds to incorporate modified microenvironments for the cell culture (Lavik et al., 2005; Stevens et al., 2005). However, despite numerous advantages, polylactide-based biomaterials have a limitation such as they form low pH microenvironments upon degradation that may be harmful to the cells. Although other biomaterials have been developed for tissue engineering, there are no universally optimal materials for cell cultures, because the optimal microenvironment conditions are significantly different with various cellular applications such as cell growth and differentiation.

Generating optimal biomaterials that can be used to control cell–microenvironment interactions is important for a number of cell-based applications. These include drug discovery and development, fundamental biological study, and tissue engineering. Of particular interest is the ability to regulate stem cell fate decisions due to their potential importance in regenerative medicine, cell-based therapy, and pharmaceutical research. Biomaterials which mimic the matrix composition of the body are important regulator of stem cell differentiation towards specific cell lineages (Chai and Leong, 2007). Biomaterials provide sites for cell adhesion and initiation of matrix generated signal transduction pathways. Also, biomaterials regulate stem cell differentiation through matrix elasticity and biomechanical cues (Engler et al., 2006).

Advanced cell models may be useful tools in drug discovery and development. For example, FDA has addressed the need for better test models that would enable better selection of drug candidates to the clinical phases. Currently, approximately 90% of the drug candidates fail in the clinical phases due to unexpected problems related to drug toxicity, inadequate efficacy, adverse effects, or pharmacokinetics. Liver toxicity is a major reason for discontinuation of the drug development, and it would be highly beneficial if such problems could be avoided by better predictions. Current cell models and animal models do not provide accurate predictions, thus these models need to be improved and optimized. The difference of species is a major concern in the case of animal models. In principle, generation of desired cell phenotype in biomaterials within 3D cell culture systems would provide improved and more accurate profiling of the compounds, but generation of such models is not a trivial task.

In this review, we discuss the potential of the high-throughput methods in optimizing biomaterials and microenvironments for cell-based studies. These methods have not been utilized extensively yet, but they have the potential to optimize cell culture conditions and control microenvironments for drug discovery, chemical testing, and tissue engineering.

2. Concept of high-throughput screening of biomaterials

The approaches to synthesize biomaterials in a high-throughput manner and to test the resulting materials for

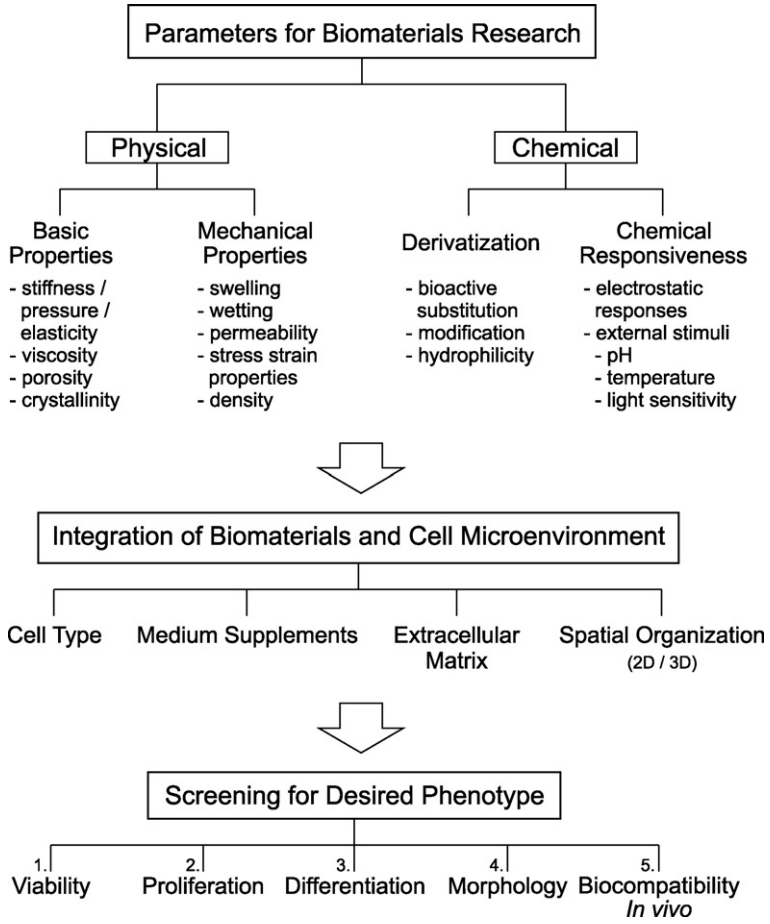

Fig. 1 – The design of biomaterials that can significantly affect cellular behavior and microenvironment.

inducing desired responses such as cell adhesion, growth, and differentiation have the potential to revolutionize studies of cells in response to materials. There are numerous parameters of biomaterials that can affect the cellular behavior in a controlled manner (Fig. 1). Complexity of the interacting parameters is the main motivation for the high-throughput screening (HTS) of the systems. The screening of biomaterials in a high-throughput manner can be used to probe the influence of various physical, chemical, and biological properties on the cellular behavior. Previously the parameters were individually tested, but the complexity of biological systems requires a more thorough analysis in which interactions based on combinations of various parameters are studied.

In addition to the biomaterial optimization, various environmental factors such as extracellular matrix (ECM) can be integrated to the screening procedures. Exploring these combinations will eventually enable screening of the cellular responses such as cell viability, proliferation, differentiation, morphology, and compatibility. Furthermore, HTS studies of cell–biomaterial interactions can be used to perform experiments in a cheaper and more efficient manner.

3. Cell interactions with biomaterials

Synthetic biomaterials provide significant richness and complexity in their properties. Properties of the synthetic materials can be regulated based on their physical and chemical properties (Fig. 1). Physical characteristics of synthetic biomaterials affect the mechanical and macroscopic features of the polymers, whereas the chemical properties can modify the biological responsiveness (Allen et al., 2003). The microenvironment of cell growth and differentiation can be further modified by the addition of bioactive components such as ECM molecules, environment conditions (i.e. temperature, pH and glucose), and soluble signaling molecules (DeMali et al., 2003; Shin, 2007).

Cellular behavior is regulated by extrinsic signals from the microenvironment such as cell–cell contact, cell–ECM interactions, and cell–soluble factor interactions (Garcia, 2005; Goodman et al., 1996). The manner in which biomaterials interact with cells depends partially on its source. For example, natural biomaterials (i.e. collagen, laminin and glycosaminoglycans), which are native part of the native ECM, comprise of many signaling domains that can interact directly with cell surface molecules. The properties of these matrices are highly dependent on the nanoscale organization of the polymers as well as the ratio of the ECM constituents. For example, the ECM of the basement membrane is rich in collagen IV, while the cartilage ECM is comprised mostly of glycosaminoglycans. In addition to regulating the chemical composition of the matrix, various components of the native ECM also regulate mechanical properties such as stiffness. Thus, recreation of the native

ECM in a rapid and scalable manner involves the possibilities to change the concentration of components in the artificial ECM.

The response of cells to biomaterials is often initiated by cell contact and adhesion to the biomaterial surface. In most cases, the cell adhesion to substrates or ECM components is regulated by proteins that are either part of the matrix or have adsorbed onto the surface of the biomaterials from the culture media (Fittkau et al., 2005; Yamada and Kleinman, 1992). These proteins (i.e. fibronectin, laminin and collagen) interact with specific receptors on the surface of the cells (DeMali et al., 2003). After adhesion, cells divide and the integrin binding of the ECM proteins also initiates activation of the intercellular adhesive complexes, which is a prerequisite for the formation of the cell–cell junctions and functional cell phenotypes (Fittkau et al., 2005; Massia and Stark, 2001). Given the multi-dimensional aspects of the cell–microenvironment interactions, the development of biomaterials that signal the cells in a desired manner is time-consuming and labor intensive. However, HTS studies can address this challenge, because they facilitate the optimization of the microenvironment conditions. This approach was demonstrated by Flaim et al. (2005) who investigated different ECM combinations in a high-throughput microarray.

4. Gradients for screening biomaterials and soluble factors

Materials that contain gradients of various properties (either chemical, mechanical or biological) provide a powerful method for screening biomaterials and studying cellular behavior. The material gradients are generated on planar surfaces by changing the surface potential of these substrates. A number of techniques can be used to generate biomaterials with gradients in either one or two directions.

In one-way gradients, the material properties change in only one direction and therefore each 'line' on a material provides a unique testing condition. Alternatively, in two-way gradients, distinct properties can vary in two different directions so that each spot of the surface has distinct properties. An example of the fabrication of one-way gradient materials was demonstrated by Simon et al. (2005) who manufactured strip-shaped gradients of poly(L-lactic acid)(PLLA) and poly(D,L-lactic acid)(PDLLA). The concentration gradients of PLLA and PDLLA in the mixtures were generated so that the fraction of PLLA in the material varied from 0.25 to 1.00 over a distance of 45 mm (Fig. 2A). Interestingly, PDLLA formed a smooth surface with about 10 nm height of roughness. The roughness increased up to 80 nm with increasing PLLA fraction (0.6–0.8), and then decreased to 30 nm in the PLLA rich end of the gradient. They also analyzed the cell adhesion and proliferation on the gradient materials. No difference in cell adhesion was seen, although there were distinct differences in the rate of cell proliferation across the material. This method is suitable for the evaluation of polymer combinations that are compatible with each other. The requirement is that they can be dissolved in the same solvents and no phase-separation should take place during the process.

Two-dimensional (2D) polymeric gradients have also been generated by simultaneous variation of the polymer composition and processing temperature. For example, Meredith et al. (2003) used blends of biodegradable polymers such as PDLLA and poly(ε-caprolactone) and changed the mixing temperature to generate a 2D polymeric material. Adhesion of osteoblasts onto various mixtures was monitored with the final aim to discover materials with optimal expression of alkaline phosphatase.

Biomaterial gradients have also been used to analyze the importance of the surface energy and hydrophilicity of the materials (Kennedy et al., 2006). In this approach, self-assembled monolayers of *n*-octyldimethylchlorosiloxane were generated on the glass substrates. By UV exposure, the monolayer was partly converted from methyl-terminated layer into hydroxyl and carboxyl terminated species. The length of UV exposure was used to adjust the degree of conversion. Thus, different sectors of hydrophilicity and hydrophobicity were generated, and the cells were grown on these surfaces after fibronectin coating. The study demonstrated higher rate of cell proliferation in hydrophobic regions of the surface. Since proteins are known to preferentially adhere on the hydrophobic surfaces, fibronectin probably adhered more effectively on hydrophobic than hydrophilic areas of the gradient surface.

Microfabricated microfluidic devices can be used to generate concentration gradients. The microfluidic device manipulates and controls the laminar flow and diffusive mixing. Burdick et al. (2004) developed hydrogel gradients in a microfluidic platform (Fig. 2B). They infused two distinct monomer solutions with photoinitiator into poly(dimethylsiloxane) (PDMS) channels from opposite directions to obtain one-way biomaterial gradient within the channels. In that study, both the concentration of RGD protein and the density of biomaterial was studied. Upon UV light exposure, the solutions were photopolymerized to form cross-linked poly(ethylene glycol) (PEG) hydrogels and gradients of RGD peptides within the hydrogel to study cell adhesion. The endothelial cells showed preferable adherence to the sites with RGD peptides.

In addition to the biomaterial gradients, the microfluidic systems are also useful methods to study cell responses to the gradients of soluble factors. In this case, the cells were cultured in the channel system and the compound was transiently infused to the chip to generate concentration gradients (Pihl et al., 2005). This set-up gives rapid information about the concentration versus cellular behavior relationships with minute amounts of the compound. Similar approach was used to study stem cell differentiation in a microfluidic device treated with poly-L-lysine and laminin. Human neural stem cells were exposed to stable concentration gradients of growth factor mixtures of epidermal growth factor (EGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) (Chung et al., 2005) (Fig. 2C). The cell growth and differentiation were real-time observed. It was demonstrated that the high concentrations of growth factors resulted in increased proliferation and the low concentrations of growth factors induced astrocyte differentiation.

Therefore, gradients are useful tools for the screening of the biomaterials and studying cellular behavior in a high-throughput manner. It is notable, however, that the exact

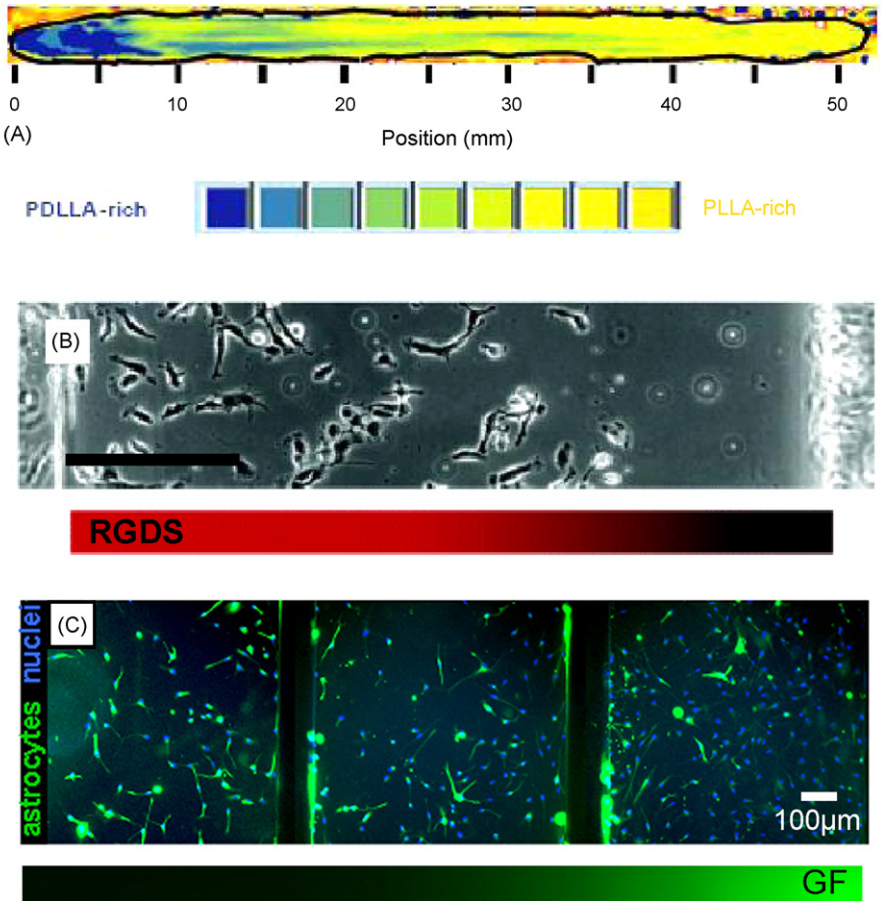

Fig. 2 – The gradients generated in polymer blends and microfluidic devices. (A) PLLA-PDLLA gradient in a strip-shape film. A qualitative gradient in color is visible in the film, with blue corresponding to PDLLA rich regions and orange corresponding to PLLA-rich regions (Simon et al., 2005). (B) A microfluidic device generates hydrogel gradients through UV photopolymerization. Endothelial cells were attached on high RGDS concentrations (Burdick et al., 2004). Scale bar is 200 μm. (C) Differentiation of human neural stem cells exposed to gradients of growth factor (GF) mixtures in the microfluidic device. Human neural stem cells in low grow factor concentration compartment more differentiated into astrocytes (GFAP, green) than high growth factor concentration (Chung et al., 2005).

concentrations of the bioactive ECM components or biomaterials within the gradients are not known. To address this challenge, a microfluidic device that can generate stable concentration gradients in a spatial and temporal manner has been recently developed. This microfluidic system requires small amounts of media and provides precise concentrations that are essential for the pharmacological characterization and also for the follow-up studies of tissue engineering materials at larger scale.

5. Microarrays for high-throughput screening of cellular microenvironments

Since introduction of the DNA microarrays in the 1990s, different arrays have gained increasing popularity in biosciences. The arrays consist of a large number of small spots, each with defined and different composition. This approach is suitable for testing biomaterials and microenvironments to enable the screening of large number of combinations in a high-throughput manner. A high-throughput microarray that probes the cell–cell, cell–ECM, and cell–biomaterial interactions can be useful for studying the cell adhesion, proliferation, and differentiation (Khademhosseini et al., 2006c). The microarrays can be fabricated by using a robotic DNA spotter and microfabrication techniques.

A robotic spotter has been used for synthesizing biomaterials at nanoliter scale (Anderson et al., 2004b). Microarray with multiple combinations of acrylated monomers was patterned and polymerized to make a polymeric biomaterial microarray. The investigations on the growth and differentiation of human embryonic stem (ES) cells on the microarray demonstrated the differentiation to cytokeratin-positive cells (Fig. 3A). The microarray format enables the rapid synthesis of suitable polymers and thereafter screening of large libraries of multiple biomaterials and microenvironments. Biomaterial microarrays of polylactide-based polymers were developed to screen cell–polymer interactions (Anderson et al., 2005). Human mesenchymal stem cells and 24 different biomaterials (i.e. poly (lactide-co-glycolide) (PLGA)) composite interactions were analyzed. 1152 polymer blends were printed on a glass slide with 500 μm spacing. This polymeric microarray was

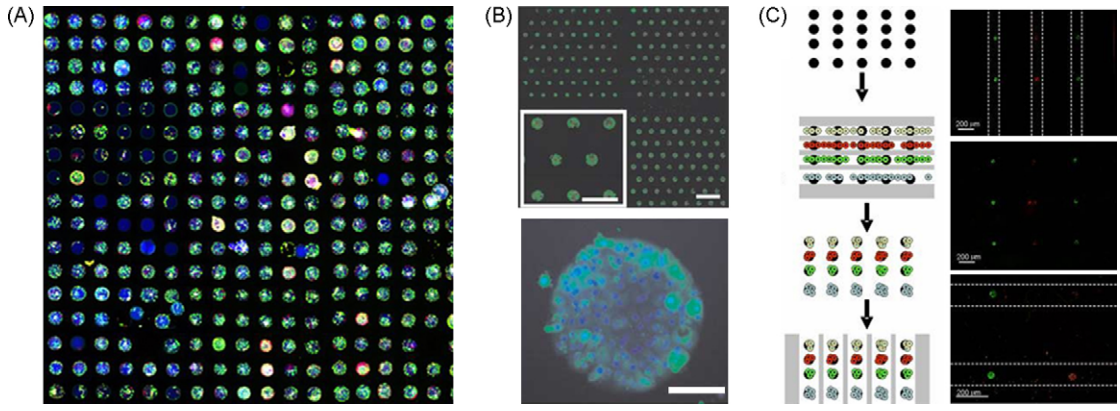

Fig. 3 – Effect of cell–ECM and cell–biomaterial interaction. (A) A biomaterial array for studying differentiation of human ES cells. Human ES cells were stained for cytokeratin 7 (green) and nucleus marker SYTO24 (blue) (Anderson et al., 2004b). (B) An ECM microarray for regulating hepatocyte function and ES cell differentiation. Live/dead staining of hepatocytes using calcein AM and ethidium homodimer-1 reveals ~95% viability (Flaim et al., 2005). Scale bars are 1 mm (top image, inset scale bar is 500 μm) and 50 μm (bottom image). (C) A multiphenotype cell microarray in a microfluidic device. Cells were stained with membrane dyes (CFSE, green) and SYTO (red) (Khademhosseini et al., 2005).

used to study gene expression and differentiation of neural stem cells. Peptide and small molecule microarray made by a DNA microarray spotter was also used to study high-throughput cell adhesion (Falsey et al., 2001). A microarray of immobilized ligands was analyzed with three different biological assays such as protein-binding assay, functional phosphorylation assay, and cell adhesion assay. This array can be used to rapidly screen and analyze the functional properties of various ligands.

In addition, an ECM microarray for mouse ES cell differentiation into hepatic fates has been developed (Fig. 3B) (Flaim et al., 2005). Primary rat hepatocytes only adhered to protein patterned regions. This array composed of different combinations of various ECM molecules was used to study hepatocyte function. ES cell differentiation was indicated by expression of a beta-galactosidase reporter. These miniaturized microarrays can be used for cell diagnosis and screening of cell–ECM and cell–biomaterial interactions. Photochemistry can be utilized in the context of biomaterial microarrays. As an example, polyvinylalcohol was modified with phenylazido groups, to generate a microarray (Ito et al., 2005). The glass plate was spin coated with azido-unit pendant water-soluble photopolymer (AWP). After AWP coating, proteins were micropatterned on the glass substrate using UV light. Cell adhesion on the protein microarray was analyzed and characterized. On the AWP-coated glass substrate, cell adhesion was strongly dependent on the protein concentrations in the microarray.

Although microarrays developed by robotic spotters control cell–biomaterial and cell–ECM interactions, there is a limitation such as expensive equipment. To address this limitation, simple and inexpensive microfabrication techniques such as photolithography can be used. Microfabrication techniques are enabling tools to generate microarrays for controlling cell–microenvironment interactions in a high-throughput manner. Microfabricated microarrays have been recently used for HTS applications (Castel et al., 2006; Diaz-Mochon et al., 2007). To generate microfabricated microarrays, a microfluidic device that incorporates with a microwell array has been developed to control cell docking within microwells (Fig. 3C) (Khademhosseini et al., 2005). Cells were localized within cell-repellent PEG microwell arrays inside the microfluidic channels. Reversible sealing of PDMS molds enabled the patterning of a number of different chemicals and multiple cell types within a microwell array. This microfluidic microarray can be useful for the high-throughput drug screening. The PEG hydrogel microwell array can also control embryoid bodies (EBs) with homogeneus size and shape (Karp et al., 2007; Moeller et al., 2008). To generate microfabricated microwell arrays, PDMS stamps embedded with micropatterns were placed on PEG solutions in a glass substrate. After photopolymerization, PDMS stamps were peeled off and microwell arrays containing various micropatterns were generated on a glass surface. EBs cultured within microwells were easily harvested from hydrogel microwells for the biochemical analysis (i.e. RT-PCR and FACS). This simple yet versatile microfabricated microwell array that can control homogeneous cell size has the potential to direct stem cell fates. In addition, this hydrogel microwell array was used to co-culture with human ES cells and murine embryonic fibroblasts (MEFs) (Khademhosseini et al., 2006b). The self-renewal of human ES cells co-cultured with MEF feeder cells was maintained within a microwell array. In addition to photocrosslinkable hydrogel microwell array, the electropatterning technique has been developed (Albrecht et al., 2005). The dielectrophoretic forces enable the control of cell position and patterning. The dielectrophoretic forces moved cells toward the electrodes and generated an array within the PEG prepolymer solution. This system has the potential to generate 3D hydrogel microarrays for studying cell–cell interactions. Therefore, these high-throughput microarrays fabricated by the robotic spotter or microfabrication technique could be useful for manipulating cell–ECM, cell–biomaterial interactions, and screening multiple combinations of various biomaterials (Mei et al., 2007). These technologies can also facilitate the identification of novel biomaterials from polymer libraries.

6. Functional assays

It is important to control and understand the cellular microenvironment that can affect gene expression and regulation (Anderson et al., 2004c). The study of the cell–biomaterial interaction controlled in a high-throughput manner can be useful for a number of applications such as drug discovery (Yang et al., 2006), chemical toxicity testing (Wang et al., 2007), and tissue engineering (Griffith and Naughton, 2002; Tan et al., 2005). The need for more informative biological assays and models is essential in the development of predictive preclinical methods to the drug discovery process.

The simplest category of cellular assays involves measurement of a single end-point per well or spot. Assays belonging to this category have been utilized in the HTS evaluation of the cell responses to the biomaterials and ECM (Flaim et al., 2005). Differentiation of the cells has been probed by using immunohistochemistry (Anderson et al., 2004b; Flaim et al., 2005; Khademhosseini et al., 2006b; Lavik et al., 2005) and atomic force microscope (AFM) evaluation of cell morphology (Simon et al., 2005) and sizes (Karp et al., 2007). Cell proliferation and cell toxicity have been evaluated with fluorescence staining with cytotoxicity probes (Cabodi et al., 2005; Kudelska-Mazur et al., 2005). These assays are useful for the HTS applications, because they are based on the direct observation of the cells after adding the fluorescent probe molecules and antibodies. They provide basic information about the cell response in the biomaterial environment.

More thorough characterization and analysis of the cell responses would require the gene expression either by PCR techniques, DNA arrays, or proteomics. In general, these methods such as DNA array and proteomics have a limitation that the analysis of the cellular responses is not easy, because these methods require larger samples. For example, it is often difficult to extract the cellular materials for the analysis methods such as PCR or flow cytometry from the cells that are embedded into the biomaterials (Goodman et al., 1996). However, these methods that can obtain high information contents play an important role in the analysis of the lead biomaterials after scaling up to larger culture size.

Current high-content screening (HCS) assays have some notable advantages. These methods are based on multiple simultaneous read-outs of fluorescence or luminescence (Haney et al., 2006). The methods allow direct quantitative observation of the cells in the HTS system and generation of cell response data versus time. Various functional assays can be developed based on direct fluorescence probe determination, fluorescence resonance energy transfer measurements, luminescence determinations and cells that express transfected fluorescent or luminescent marker proteins. Modern HCS assays allow non-invasive monitoring of the cellular responses from various biomaterials or chemical libraries. These methods may be useful in the development of the optimal biomaterials, evaluation of drug candidates, and manipulation of the biomaterial-based HTS systems.

Another dimension in the cell assays is the cell manipulation by the use of transfections (Brummelkamp et al., 2006). Transfections are based on gene up-regulation (with DNA or RNA) or knock down (with miRNA, siRNA or antisense). This allows system biological approach in the studies of the cell responses. Reverse transfection (i.e. transfer of genetic material from the cell growth matrix into the cells) is commonly used procedure in the cell array research that is related to the biological mechanisms. Such arrays allow probing of the mechanisms of the disease and search of new drug targets. This is a powerful approach, because it allows systematic mapping of the cellular machinery by knocking down individual genes with siRNA and then non-invasively measuring the cellular responses with fluorescent assays. In similar manner small molecules were screened by embedding them into biodegradable biomaterial discs in array format (Bailey et al., 2004). The cells were cultured on these discs and effects of the reverse delivered small molecules were monitored. In this approach no multi-well plates are needed.

Array technologies have been used to find the optimal polymers for the gene transfer (Fig. 4) (Anderson et al., 2004c). In this study, specific monomers were collected from the structure/function data and were used to optimize polymerization conditions for generating a new polymer library of over 500 poly(β-amino esters). The members of the polymer library were screened by using an *in vitro* high-throughput transfection assay with different polymer/DNA ratios. The functional assay must be selected case-by-case, and it depends on the cell type, end-points, and goals of the study.

7. Potential of nanotechnology in high-throughput cell systems

The high-throughput screening systems for biomaterials are designed based on microscale components, such as microwells, microarray spots, and microfluidic channels. As such, they are not nanotechnological devices. However, the combination of nanoscale functional units into these screening platforms may advance the development of the high-throughput cell systems. The surfaces of the systems can be decorated with nanosystems with diameters in the range of 10^{-7} to 10^{-9} m.

The nanotechnological systems are useful addition to the biomaterial based screening platforms that can be used in chemical biology, tissue engineering, and drug discovery. Relevant nanotechnological systems include functional systems for cell recognition and labeling, selective cell inactivation, gene knockdown, gene transfection, and delivery of bioactive components. Although the nanotechnological methods are not yet well developed for these purposes, there are already some examples that show their potential.

Quantum dots are metallic semiconductor beads with diameters in the range of 1–10 nm (Weng and Ren, 2006). They have interesting spectroscopic properties. Particularly, they exhibit narrow and specific wavelengths of fluorescence emission, low quenching upon repeated excitation, long fluorescence lifetimes and exceptionally high quantum yields (Pinaud et al., 2006). Quantum dots can be functionalized with antibodies for specific cell imaging and different sizes of quantum dots can be utilized to obtain several signals at various wavelengths of emission (Giepmans et al., 2006).

Nanoparticles can be used for selective cell destruction. Recently, gold nanoparticles were functionalized with anti-

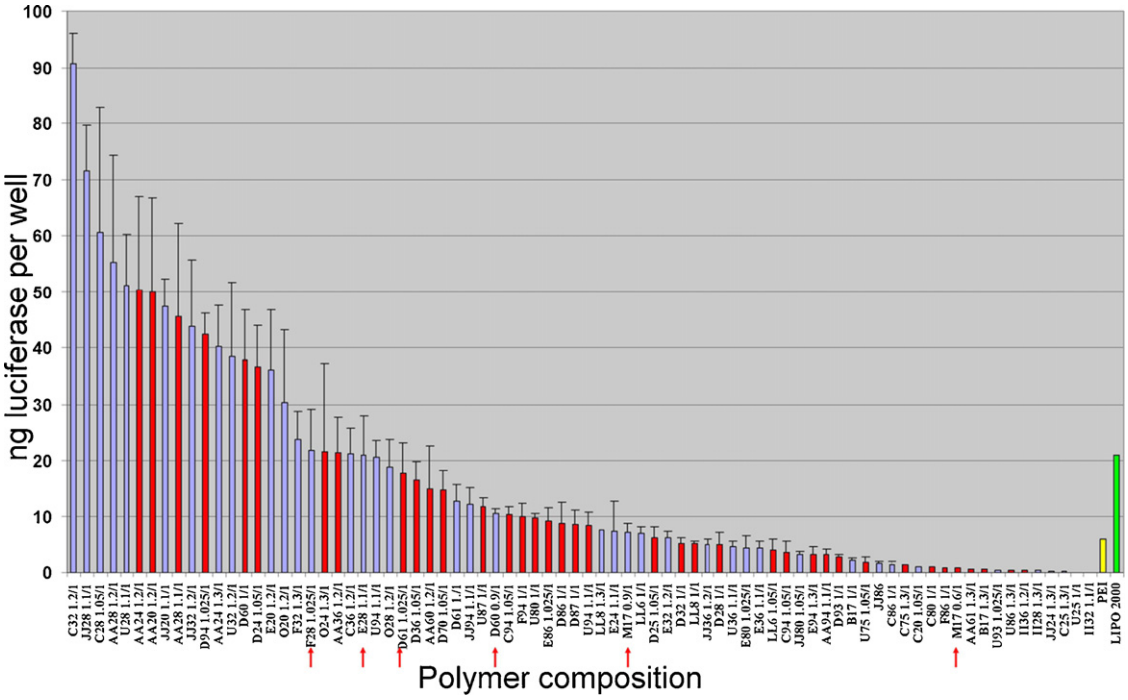

Fig. 4 – *In vitro* transfection potential of poly(β-amino esters) (Anderson et al., 2004c).

bodies that targeted to the cancer cells (Hirsch et al., 2006). Upon light irradiation the gold nanoparticles were heated and the cancer cells are destroyed. Similar principles are applicable also in the high-throughput arrays if the specific role of certain cell type is being explored.

The transfer of siRNA and pDNA into the cells in arrays has been used to silence or upgrade the gene expression. Current nanoparticulates for siRNA and pDNA transfer are not optimally efficient. The progress in the field of non-viral gene transfer, particularly the method to improve the nuclear transfer of the transgene, may provide new tools that can be combined with high-throughput systems (Erfle et al., 2004). In analogy, other bioactive compounds (e.g. drug candidates) can be delivered from immobilized nanoparticulates. Such chemical or genetic intervention can yield large numbers of important data for bioinformatics analyses (Caldwell, 2006).

Controlled localization and activation of the nanoparticulates are important for the design of advanced cell arrays. For example, selective functionalization of the nanoparticles and utilization of magnetic nanoparticles and nanowires may augment their localization within the system in a controlled manner (Tanase et al., 2005). Likewise, the possibilities to activate the particles with external signals (e.g. light and magnetic fields) may provide means of temporal control of the nanoparticle functions (Paasonen et al., 2007).

8. Current state and future prospects

A number of technologies for screening of biomaterials in a high-throughput manner have been developed in last few years (Anderson et al., 2004b; Khademhosseini et al., 2006a). However, the studies in this field have focused on the physical characterization of polymer families and their physical mixtures, mostly polyacrylates, polylactide-glycolides, polyethylene glycols, and hyaluronic acids. These polymer choices have been motivated by the safety of these polymer backbones. The evaluated parameters are related to the physical properties of these polymers such as morphology, mesh-size, and stiffness. In addition, ECM peptides have been screened from a number of biomaterials. However, only small part of these powerful screening methods has been used. New polymers and materials science concepts should be explored with these systems using a variety of physical and chemical methods of material production and characterization.

Most cell-based studies in a high-throughput manner have involved stem cells, osteogenic cells, and hepatocytes. New developments in the cell screening assays such as high-content screening method and high-throughput PCR method could produce more complete biomarker combinations and functional information about the cell phenotypes. However, these methods have not been used in the cellular screening of the biomaterials. Such investigations could provide more precise information to guide further developments. In addition, the successful scale-up of cell culture protocols from the microsystems to macrosystems is not self-evident. The more detailed scale-up studies and further *in vivo* tissue engineering experiments have not been completely performed to test the lead biomaterials and microenvironments.

Overall, the field of high-throughput screening of biomaterials and microenvironments is in the early stage. Upon its maturation such screening methods may lead to more realistic and predictive cell models that augment drug discovery and drug development by providing better insights into the complex pharmacokinetic, pharmacodynamic, and toxicological processes.

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