Review

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Micro/Nano Surface Topography and 3D Bioprinting of Biomaterials in Tissue Engineering

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Understanding of active interactions between cells and biomaterials in nano/micro scales is very important in tissue engineering of malfunctioning organs and tissue defects. Diverse biomaterials such as polymers, and their composites were developed for their applications to tissue engineering and overviewed here in the aspects of both tissue engineering and nano/micro-technologies, including 3D bioprinting. Relationship of micro/nano surface topologies of biomaterials to tissue engineering have been reviewed by employing polymeric materials, which have been recognized as *leading* biomaterials due to its advantageous characteristics of biophysical and chemical properties. Cellular responses such as cell adhesion, migration, proliferation, differentiation, orientation as well as gene and protein expression were examined in terms of diversely designed topographical textures such as grooves, walls, pits, posts, shapes, sizes and gaps distance, or even flat patterns of biomolecular stamp marks with certain aspect ratios. We hope that this review is expected to be helpful for better designing of biomaterials for their applications in tissue engineering.

Keywords: Cell, Biomaterials, Micro/Nano, Interaction.

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1. INTRODUCTION

Organ failures and tissue defects remain as major obstacles to be overcome in medical fields. Since the emergence of tissue engineering strategy, full regeneration of tissue or organ has been tried to replace the dysfunction ones[1] by using biomaterials and advanced technologies, as an example, macro to nano-biotechnologies. Even though many approaches to regenerate completely functional biological tissues in laboratory have been studied by numerous research groups, complexity of *in vivo* tissue structures and homeostasis with blood supply remain as big challenges in mimicking the biological systems of defect tissues and organs. Beside the employment of (stem) cells and adjustment of bio-environmental conditions, designs of biomimetic medial materials similar to extracellular matrix (ECM) as well as temporary physical-mechanical properties have been tried to mimic the functions of (stem)

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cells and biologically functional systems of human body. Many types of biomaterials have been developed to mimic the most optimum characteristics which correspond to natural conditions of tissue growth and remodeling. Control of physicochemical and biological variables was employed for designs of biomaterials such as chemical and biological signals. Other methods such as choices and fabrications of biomaterials, modes of delivery of bioactive molecules, control of interfaces of biomaterials and cells, control of biodegradation of biomaterials and mimics of biological systems have been tried. As examples, while hydrophobicity and hydrophilicity were to control an interaction of (stem) cells on surfaces, local and systemic delivery of bioactive molecules such as growth factors and (stem) cells was important to control effective regeneration of target tissues depending on their locations and defect sizes. Diverse fabrications of biomaterials scaffolds were designed such as fibers, patterns, pores in nano/micro-levels by using nanobiotechnology. Recently, 3-D bioprinting was also applied to designs of controlled scaffolds for tissue engineering. Diverse methods of 3-D bioprintings such as extrusion, inkjet, bioplotting and stereolithograpy

have been reported to construct micro/micro-architecture scaffolds with high precision.[2]

Hydrogels have been employed as a choice of interesting biomaterials according to their advantageous profiles of biocompatibility, inject-ability and biodegradability as well easy handling and right fittings to complex shapes of defects. Furthermore, control of chemical and biological structures and physico-mechanical textures of hydrogels have also advantages in their applications to tissue engineering such as close similarity to ECM, by bringing capability to load water, aqueous body fluid[3] and other biological systems. These characteristics were employed to mimic cellular responses and interactions in macro and micro aspects similar to those of biosystems. Furthermore, micro/nano-properties of hydrogels have affected cellular behaviors on/in biomaterials during initial stages and later tissue remodeling. Several technologies demonstrated the importance and possibility of nano/micro-topographical patterns of hydrogel surfaces in tissue engineering through the methods of either surface patterning or 3-D bioprinting. Here, we are going to review recent research progresses of cellular responses on/in biomaterials with nano/micro-characteristics, focusing on hydrogels.

2. CELLS AND BIOMATERIALS IN TISSUE ENGINEERING

2.1. Cells

One of the most important fundamental elements in tissue engineering is to control characteristics of (stem) cells. Many types of cells have been exploited for studies of tissue regeneration such as either differentiated cells or highly potential stem cells/progenitor cells, as well as animal or human primary cells. Differentiated cells such as neurons have typically their own characteristics and functions from one another. While chondrocytes have shown very specific functions in growth and proliferations, bone cells behaved differently. In contrast, stem cells or progenitor cells have high potentials to grow, proliferate, and differentiate into many types of cells, depending on their environments such as surfaces of biomaterials.

Cells naturally response and adapt to surrounding environments either within intercellular or towards extracellular matrix and then regenerate tissues in defect sites. To certain extents, environmental conditions to support cells adhesion, proliferation, migration, differentiation[4] and ultimately functional performance in tissues or organs have to be fulfilled in tissue engineering and regeneration. The cells behave differently, depending on biomaterials species, surfaces, designs and physicochemical properties as well as biomolecules species and their delivery modes. To apply the cells in tissue regeneration, many research approaches have been conducted and developed by controlling either cells themselves or micro/macro environments such as ECM and biological substances of growth factors and other cytokines.

2.2. Biomaterials

As biomaterials are essential in developing tissue regeneration, many researches have been focusing on development and designs of potential biocompatible biomaterials to mimic ECM of tissues. Polymers among possible biomaterials such as ceramics, composites and metals have been extensively studied as biomaterials resources depending on their applications. As well as choices of diverse polymers themselves, many forms of polymers have been designed such as membranes, films, injectable gel/solution, scaffolds, or nano/micro-particles, depending on the required physical-mechanical properties, mass transport properties and biological interaction of the intended applications.[5] Functional biophysical and mechanical properties of polymeric biomaterials were also designed to mimic the spatial shapes, locations and sizes of ECMs, including the elasticity or strength required to support its functions. Diffusion in or out of the loaded substances or nutrients from environment has to be suitable to represent adequate properties of mass transport. Besides, their surfaces and topologies of the fabricated biomaterials in micro/nano-levels must be useful for control of cellular responses such as attachment, migration, proliferation and differentiation for tissue regeneration.

Micro/nano technologies led to better physical properties of biomaterials in many biomedical applications, from addressing discovery of subcellular interaction at the states of single biomolecule mechanisms to finding possible ways to control biomimetic constructions.[6] Nano/microparticles played significant roles as carriers of many bioactive substances essential to modulate the behaviors of cells and regeneration of tissues, such as growth factors, small-molecule drugs, siRNAs, and therapeutic proteins, as they could be employed for enhancement of cellular uptakes.[6] Nanotechnology also led to fabrication of variably nanostructured scaffolds, which could encourage the development of more sophisticated biomimetic scaffolds.[7] Electrospinning in nanoscales, molecular self-assembly and nano-patterns have been developed by using different polymers such as chitosan, collagen, poly(ethylene oxide), poly(vinyl chloride), many other natural and synthetic polymers and their composites. Nano/micro-patterns were also applied to control the surfaces of polymers, ceramics and metals. Depending on their topologies, degradability and tensions of surfaces such as patterns, sizes and groves and species of biomaterials, the cells behaved differently in adhesion, proliferation as well as tissue formation by the effects of interactions of the cell surface receptors on biomaterials.

2.3. Polymeric Biomaterials

Polymers have been featured in biomaterials by their unique characteristics, leading to the largest explorations in the areas of tissue engineering, drug delivery and other medical devices. Due to the characteristics of easier fabrications adjustable to desired shapes as well as

supportive physical and mechanical strength, both natural and synthetic polymers were considered as choices of promising biomaterials depending on their applications and locations, showing functional properties of biodegradation and biocompatibility.[8] Natural polymers such as hyaluronic acid (HA), collagen, alginate, chitosan, cellulose and gelatin were studied extensively, and at the same time poly(ethylene oxide) (PEO), poly(lactide-*co*-glycolide) (PLGA) and polycarprolactone (PCL) and other synthetic polymers have been also developed and reported as outstanding polymers in biomedical materials

The polymeric biomaterials have been applied to diverse areas of medical applications such as tissue engineering, drug delivery and other medical materials. Among many candidates, the biocompatible polymers with hydrophilicity such as HA, chitosan and PEO have been primarily considered as hydrogel materials due to their capacity to carry water in relatively large amount. To induce biofunctionalities of biomaterials, diverse chemical modifications of hydrophilic polymers have been developed. As examples, methacrylate HA (HA-MA) was synthesized by grafting methacrylate groups to the side chains of HA (Fig. 1(a)).[9] HA gel was fabricated in pattern forms by lithography technology by exposing UV light on the HA-MA surfaces with a crosslinking agent through photomask. Hexagonal porous scaffolds were also successfully constructed by 3D printing of PEO derivatives featured by the methods of two-photon polymerizations.[10] Collagen-biphasic calcium phosphate complex was obtained by chemical cross-linking, where initially studied for skin reconstruction and then applied to the study of other tissue engineering such as bone and cartilage.[11] In other studies, Matrigel was also used as a biomaterial for 3-D cell printing, which enabled to seed individual cells onto certain patterns.[12, 13] Wang et al. found that sodium alginate (Fig. 1(b)), which has been well-known for its successful applications for incorporation of many types of (stem) cells via ionic interactions intra/inter side chains of alginates, had also a potential as a substrate for proliferation of bone marrow cells.[14] Furthermore, chitosan in diverse forms such as films, fibers and hydrogels was also applied in tissue engineering.[15] Non-woven cellulose fabric showed evidence of development of cartilage tissue in a study by Muller et al.[16] Gelatin micro/nano-spheres crosslinked by glutaraldehyde were used as a carrier of bioactive molecules using basic fibroblast growth factor (bFGF) as model biomolecules to induce adipogenesis in regeneration of fat tissues.[17] On the other hand, there have been many attempts to explore synthetic polymers by capitalizing on their properties such as easy handling for clinicians as well as their better biochemical stability and customized designs of biomaterials. PEO-poly(propylene oxide) (PEO-*co*-PPO) copolymer has been developed as a thermos-reversible polymer, where it is in liquid state at lower temperature and reverses in gel state at higher temperature. Among its applications, PEO-*co*-PPO copolymer as an injectable gel has been *in vivo* tested subcutaneously by loading chondrocytes and showed promising results in cartilage formation.[18] PCL and the functionalized nanofibrous PCL combined with Matrigel demonstrated induction of proliferation and neurite outgrowth, thus providing suitable conditions for regeneration of nerve tissues.[19] Printing of electrospun micro-fibrous PLGA on acellular bladder matrix led to regeneration of bladder as a representative of hollow organs.[20] After *in vivo* implantation, it showed regenerations of many tissue structures such as urothelium and layers of smooth muscles with collagen-rich layers.[20] Grafting of poly(N-isopropyl acrylamide) (PNiPAAm) with HA (PNiPAAm-HA) by dithiocarbamate reaction showed cell non-adhesiveness, indicating the possibility of its applications to biomaterials for cell non-adhesive matrix or tissue adhesion prevention.[21] Poly(propylene fumarate-*co*-ethylene glycol) (P(PF-co-EG) (Fig. 1(c)) hydrogel demonstrated promotion of chondrocyte proliferation which might be possible to induce to articular cartilage regeneration, although it is still lack of induction of proteoglycan synthesis.[22]

3. HYDROGELS FOR TISSUE ENGINEERING

Some of representative hydrogels were provided in Figure 1. Their water-absorbable characteristics were very beneficial to enable nutrients and other bioactive molecules to be delivered into required target tissues and defect sites by local delivery or body fluid when applied for tissue regeneration.[23] Besides, the hydrogel's mild-elastic textures and structures could be controlled similar to those of the ECM of biological tissues. Moreover, hydrogels as supportive matrix in tissue regeneration have possibility to control the interactions of cell surfaces in diverse scales by control of their chemical structures to induce cell adhesion.[23]

Fabrications of hydrogels were extensively explored through physicochemical alteration to obtain desirable characteristics especially to fulfil physical and biological requirements in tissue regenerations. Diverse techniques such as introductions of functional groups, modifications of side chains through grafting, diverse copolymerizations, chemical or physical crosslinking within the same or different hydrogel polymers were carried out to enhance their properties and to mimic human tissues in nano/micro-levels. Chemical cross-linking of polymer networks was also developed by diverse methods and mechanism such as radical reaction, cross-linkers, light with high energy, and enzymatic reaction.[23, 24] Physical crosslinking of polymers was also obtained by molecular interactions such as ionic, hydrogen, or hydrophobic interactions.[23, 24]

Many functional hydrogels have been developed which are responsive to specific stimuli such as temperature, pH and biomolecules such like glucose or

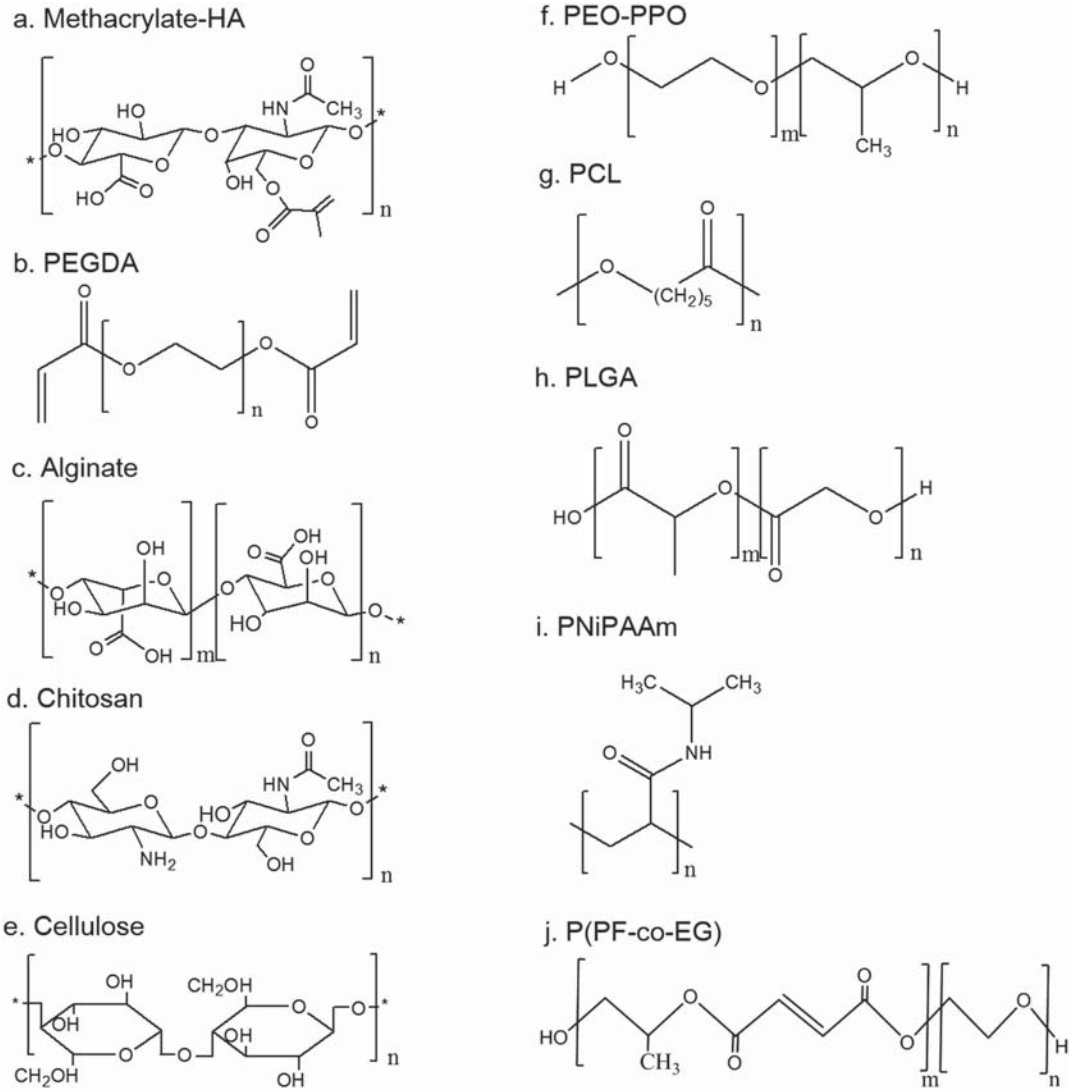

Figure 1. Examples of some polymeric biomaterials for tissue engineering. (a) Adapted with permission from [9], R. A. Marklein and J. A. Burdick, *Soft Matter* 6, 136 **(2010)**. © 2010; (b) Adapted with permission from [10], A. Ovsianikov, et al., *Biofabrication* 2, 014104 **(2010)**. © 2010; (c) Adapted with permission from [94], P. M. Kharkar, et al., *Chem. Soc. Rev.* 42, 7335 **(2013)**. © 2013; (d) Adapted with permission from [95], I. Y. Kim, et al., *Biotechnol. Adv.* 26, 1 **(2008)**. © 2008; (e) Adapted with permission from [16], F. A. Müller, et al., *Biomaterials* 27, 3955 **(2006)**. © 2006; (f) Adapted with permission from [18], A. Gutowska, et al., *Anat. Rec.* 263, 342 **(2001)**. © 2001; (g) Adapted with permission from [19], L. Ghasemi-Mobarakeh, et al., *Mater. Sci. Eng. C* 30, 1129 **(2010)**. © 2010; (h) Adapted with permission from [20], M. Horst, et al., *Biomaterials* 34, 1537 **(2013)**. © 2013; (i) Adapted with permission from [21], S. Ohya, et al., *Biomacromolecules* 2, 856 **(2001)**. © 2001; (j) Adapted with permission from [96], J. E. Mark, Physical Properties of Polymers Handbook, 2nd edn., Springer **(2007)**, p. 948. © 2007.

proteins. Temperature-responsive hydrogel has particular characteristic in tissue engineering denoted as phase transformation where the reversible transition occurs between swelling and shrinking, hydrophilicity and hydrophobicity, or solubility and insolubility of polymers. Temperature-responsivity has been considered to be advantageous to generate less invasive delivery of injectable liquid state in room temperature for gel formation at higher temperature.[18] Gelatin has been reported as a polymer with sol–gel transition temperature at around 30 °C, according to its modification.[25] PNiPAAm has been studied as the most popular thermo-responsive synthetic polymers at around 32 °C.[26] PEO-PPO[27] and poly(ortho ester)–PEO[28] graft copolymer are other examples of thermogels applied in tissue regeneration. Furthermore, pH-sensitive systems were also designed to be responsive to acidic/basic environment dependent on their pKa values to switch their transition states. Derivatives of chitosan have been synthesized to have pH-responsive features. Chitosan combined with PLA was

fabricated for new tissue formation via induction of cell growth through delivery of bioactive molecules, such as growth factors, anti-inflammation agents or antibiotics.[29] Thiolated-chitosan showed the smart properties of not only pH-responsiveness but also muco-adhesiveness, that enhanced permeation of carried bioactive substances and induced scaffold interconnection to the surrounding tissues in implant sites.[30] Carboxymethyl chitosan.[31, 32] containing phosphatidylethanolamine demonstrated similar trends of their pH-responses as well. Poly(2-diethylamino ethyl methacrylate)/poly(2-aminoethyl methacrylate) in nanoparticles for delivery of calcein and ovalbumin[33] also showed pH-sensitiveness. Interestingly, when a temperature-sensitive polymer was copolymerized to a pH-sensitive one, for instance poly(N-iPAAm-*co*-acrylic acid), it showed combined responses to both temperature and pH in narrow variations.[34]

In further step, researchers attempted to modulate hydrogel behaviors against biomarker molecules responsible to specific functions or conditions. Glucose-responsive hydrogel was firstly developed for insulin delivery in diabetic treatment. Correspondingly, the same principal was applied to develop biodegradable scaffolding for pancreatic tissue regeneration, where the glucose-sensitive hydrogel was designed to sustainable delivery of insulins during new pancreatic tissue formation. Miyata et al. summarized three types of glucose-responsive hydrogels,[35] such as glucose oxidase-loaded hydrogel,[35] lectin-loaded hydrgel[35, 36] and hydrogel with phenylboronic acid moieties.[35] In similar principal, other biomolecules such like antigens[37] and integrin binding proteins[38] may act as a stimulus to alter hydrogel responses. Involvement of arginine-glycine-aspartic (RGD) integrin binding-motif induced adhesion of many cell types on scaffold surfaces.[38]

In situ hydrogels obtained by the mechanism of Michael type addition reactions of thiols and -enes have been applied in tissue engineering of bone and cartilage. Noh et al. reported development of diverse hydrogels in injectable forms by using natural polymers such as chitosan, hyaluronic acid and chondroitin sulfate (CS) for regeneration of tissues. They incorporated *in situ* bioactive agents such stem cells, bone morphogenic protein-2 in the hydrogel, showing tissue regeneration *in vivo*. Garbern et al. used pH-temperature-sensitive hydrogel, random copolymer of poly(NIPAAm-*co*-propyl acrylic acid-*co*-butyl acrylate), to deliver bFGF into infarcted rat myocardium with outstanding results such as sustained and local bFGF delivery, improved angiogenesis, increased capillary and arteriolar densities, regional blood flow and cardiac function.[39] Two growth factors, insulin-like growth factor-1 (IGF-1) and transforming growth factor-β1 (TGF-β1) were delivered to injured cartilage tissue in water-soluble hydrogel, oligo(PEG fumarate).[40, 41] Chitosan-PEO hydrogel was used to incorporate recombinant human bone morphogenic protein-2 (rhBMP-2) and human bone marrow-derived stromal cells which was subsequently implanted *in vivo* for 8 weeks with clear tissue regeneration.[42] Porous carboxymethyl cellulose-PEO hydrogel was applied to grow smooth muscle cells with excellent results of *in vitro* cells adhesion and migration into scaffold's pore channels.[43] Hydrogel disc of CS-PEO also demonstrated excellent biocompatibility in *in vitro* evaluations.[44] HA-based hydrogel was used to fabricate micro/nano-patterned disc for its applications for bone tissue engineering.[45] Hybrid hydrogel scaffolds composed with collagen–CS–HA were also found as suitable chondrocytes carriers for cartilage regeneration, with properties of temperature-sensitive and mild gelations.[46]

4. INTERACTION OF HYDROGEL AND CELLS

4.1. Hydrogels and Cells

Interfacial contact between hydrogels and cells involves molecular interaction to surfaces, such as attachment or detachment, adhesion, spreading, migration, proliferation and ultimately differentiation of cells, leading to tissue regeneration. Initial stages of the interaction between cells and hydrogel surfaces have been recognized as very critical in tissue engineering. Chemical properties of hydrogel surface determine cell responses as summarized by Roach et al.[47] Many surface functional groups may have been able to enhance adhesion, namely hydroxyl, amine and carboxylic acid, especially for differentiated cells, even though less specific than ECM ligands. Meanwhile, stem cells may response more extensively to those chemical surfaces for adhesion. Specific notes were put on myoblast cells which responded to their proliferation and differentiation.

Physicochemical properties of hydrogel surface have been recently reported. Since human or animal cell sizes were in micro scales, cellular responses may correspond to micro-sized features. Nevertheless, cell molecular response may be affected by smaller size scales of surface topography through its surface receptors and specific ligands, leading to study of the interactions between surfaces and cells in nano-scales.

4.2. Cells and Hydrogels with Cell Adhesion Domains

Cells interact with their surrounding matrix through transmembrane receptors binding to specific ligands which subsequently induce cellular chain reactions or moreover with neighboring cells guiding to consecutive responses.[47] Diverse classes of adhesion transmembrane proteins are selectins, immunoglobulin superfamily, cadherin and integrins.[48] While cadherin, a family of transmembrane proteins, is responsible for contact interaction of cell–cell, integrins are adhesion domain of cells, consisting of α-β dimers. Integrins and cell adhesion domains of ECM are the main target to discuss about cellular interaction to ECM or on the surface of biomaterials in tissue

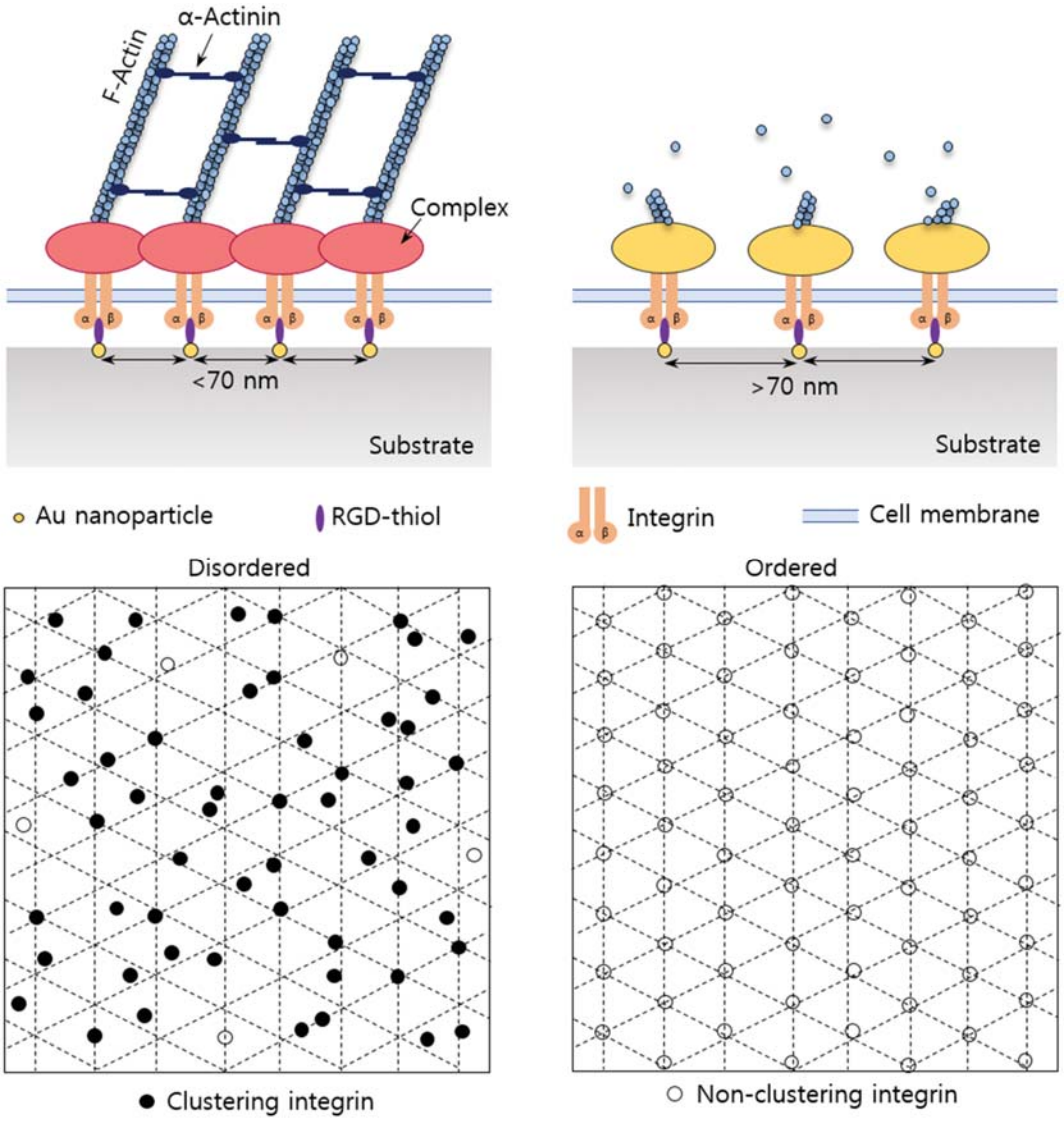

Figure 2. Integrin clustering in MSCs due to RGDs disorder placed <70 nm in distance to activate actin fibers, while in ordered RGDs placed >70 nm in distance. Adapted with permission from [97], J. Huang, et al., *Nano Lett.* 9, 1111 **(2009)**. © 2009.

regenerations. As examples, one of the well-known specific ligands for integrin receptors is a RGD domain found in the ECM proteins such as collagen, fibronectin, vitronectin, and laminin, thus indicating a possibility that cells could attach on solid surface in naoscales.[49,50] Cells build tentacle-like protrusions called lamellipodia or pseudopodia, and filopodia, constructed by actin fibers network in parallel direction to the surface[48] to sense environmental

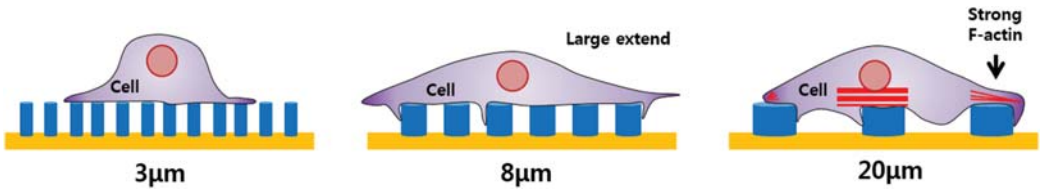

Figure 3. Schematics of cellular responses to mechanical stress of substrates due to pattern sizes. Adapted with permission from [98], H. Sunami, et al., *Biomater Sci.* 2, 399 **(2014)**. © 2014.

surface to attach as described on Figure 3. When two sub-units integrins with α and β dimers bind to RGD domain of ECM or biomaterials surface, complex proteins will be recruited and signal transduction will proceed to nucleus for gene expression, resulting in cytoskeleton (actin fiber) activation[48] (Fig. 2). After attachment, cells can move in static or dynamic. Sawyer et al. studied behaviors of mesenchymal stem cells (MSCs) on fetal bovine serum enriched hydrogel, consisting of HA and RGD domaim.[51] They found that low concentration of RGD-fetal bovine serum (FBS) coating could extensively induce MSCs attachment and spreading, and as expected occurred better in higher concentrations of RGD/FBS coating.[51] Furthermore chemical surface modification of hydrogel network by RGD grafting may also lead to osteoblast spreading, calcification, ECM proteins expression, as well as morphology and cytoskeletal organization in bone tissue regeneration.[47]

5. FABRICATIONS OF PATTERNED HYDROGEL

Topographies of hydrogel surface have been obtained by constructing patterns or by 3D printing in micro/nano-scale resolutions. While the direct construction of hydrogel surface patterns was dominated by the fabrication methods such as photolithography process and micro-molding technique, 3D bioprinting has been currently supported by several techniques such as laser-guided printing, stereo-lithography, inkjet printing and 3D bioplotting. Tables I and II showed summary of fabrication methods for

Table I. Cell responses on micro-patterned hydrogel.

No	Materials	Fabrication method	Pattern type	Cells type	Cell response
1	PEG pattern on PCL/gelatin substrate	UV photolithography	Square pits	Human MSC	Selective cell adhesion on gelatin fibers, osteogenic differentiation with BMP-2 and bFGF supplementation.[52]
2	Hyaluronic acid	PDMS press molding	Groove	Osteoblast, MC3T3	Cell orientation along groove pattern, better adhesion, proliferation, ECM production.[45]
3	Collagen-polyacrylamide	Silicon molding	Microwell/post: hexagonal, square	MSC	Cells orientation between gaps >15 μm, on surface <5 μm. Cell elongation along gaps on square post and narrow gaps.[59]
4	Tropo-elastin	PDMS molding	Rectangular grating line	Cardiomyocytes	Cell attachment, spreading, alignment, function, intercellular interaction, synchronous cardiomyocytes beating to electrical stimulation.[60]
5	Gelatin	UV photolithography	Rectangular grating	Human umbilical vein endothelial cells	Cell alignment and organization along patterns, formation of circular stable cord structure within >100 μm pattern diameter.[57]
6	PEG coated with Ca-alginate	Photolithography	Circle pillar	Murine embryonic stem cells, human hepatocellular carcinoma	Early stage differentiation, expression of cardiac genes and proteins, spontaneous cell beating.[53]
7	Hyaluronic acid in Matrigel matrix	3D bioprinting/bioplot	Cylinder line	Human MSC mouse EC, Mouse fibroblast L929	hMSCs spheres morphology through cell aggregation, cell mass increasing of fibroblast, endothelial cells dispersion into matrix.[83]
8	Polyacrylamide, fibronectin print pattern	PDMS stamp	Flat, protein fibronectin pattern shape: circle and star	Mesenchymal stem cells	Cell express osteogenesis and myogenesis associated markers, traction stress response towards patterns, special positioning of focal adhesion.[61]
9	Polyacrylamide (fibronectin print pattern)	Lithography PDMS stamp	Circle, square, line stamp/coat	Human MSC	Cell elongation, cellular anisotropy with aspect ratio 5:1 and 10:1, expression of smooth muscle actin, enhancement contractility.[58]
10	P(EO-*stat*-PO) fibronectin print pattern	PDMS stamp	Line stamp/coat	Human dermal fibroblast	Cell adhesion, orientation along pattern.[62]
11	PEG-RGD micro-island pattern	UV photolithography, RGD grafting	Circle, square, rectangular, star, flat RGD stamp/coat	Human MSC	Adipogenic and osteogenic differentiation of single cell in small and large pattern area, respectively.[54] Optimal adipogenic and osteogenic differentiation of cells in circular and star shape, respectively.[55] Cell locating and orienting along within designated pattern.[56]

lithography-based patterning and 3D bioprinting by focusing on hydrogels.

5.1. Photolithography

Like the conventional photolithography process in semiconductor industry, photolithography process was generally used to induce the gelation of polymer solutions through patterned photomask. It enabled the formation of desired shapes of hydrogel, which was useful in understating soft tissue regenerations. That is, hydrogel surface was obtained by cross-linking of polymer solutions through UV exposure on acrylated polymer solutions, as examples acrylated PEG hydrogel,[52–56] methacrylated gelatin[57] and poly(acrylic acid).[58] Patterning of gel may be also created through additional etching process after complete UV lithography. Hydrogels formed by other mechanisms such as simple or spontaneous chemical reactions could be prepared through molding techniques to embody patterns. Most widely used mold material is poly(dimethyl siloxane) (PDMS), which has been designed with previously mentioned photolithography process to build patterned textures (master mold). HA,[45] collagen-poly(acrylic acid),[59] methacrylated tropoelastin (MeTro)[60] were example polymers of hydrogels prepared by pressure molding of PDMS. Patterned PDMS has been also used to imprint functional biomolecules on hydrogel surface (micro-contact printing), such as adhesion peptides[54–56, 61, 62] or functional proteins.[58] Other polymerization or crosslinking methods, for instance, laser deposition and other lithography methods, may be applicable to develop patterns on hydrogels.

5.2. 3D Bioprinting

Three methods of 3D bioprinting of hydrogels were reported such as bioplotting, inkjet, and laser-based printing,[63] in addition to lithography method. PEG[64, 65] and Matrigel[12, 13, 64–66] were printed by laser in diverse dimensions and shapes, where laser was used to guide hydrogels or cells to form desired patterns or shapes with dimensions of around tens to hundreds μm. On the other hand, laser was also applied to induce cross-linking or polymerization by a stereo-lithography technique. Some hydrogels

Table II. Cell responses on micro-topographical surfaces of 3D printed hydrogel.[12, 63]

No	Biomaterials	Cell model	Technology	Cell response
1	PEG, alginate, EDTA, blood plasma, Matrigel; collector slide: agarose	Fibroblasts/keratinocytes, hMSCs, ECs	LIFT, laser-based biofabrication	Cell survival, MSCs differentiation to bone and cartilage.[64] hMSCs stable phenotype.[65]
2	PEG	Ovary cells	Stereolithography	High cell viability, growth, proliferation, density.[67]
3	Poly(oxy ethylene)–poly(oxy propylene), collagen I	Fibroblasts, ECs	3D bioplotting	Cell stability in spatial organization.[79]
4	Collagen	SMCs	Inkjet bioprinter	Long term cell viability.[80]
5	Collagen, Matrigel	ECs, hepatocytes	Laser guided-direct writing	ECs and hepatocytes aggregation along tubular structure pattern.[66]
6	Collagen I, agarose	Embryonic cardiac cells, ECs, ovary cells, SMCs, fibroblasts	3D bioplotting	Intercellular and extracellular adhesion, cell motility.[99, 100]
7	Matrigel	Osteosacroma cells	Biological laser printing	Cells vability.[13]
8	Matrigel	Olfactory ensheathing cells	Biological laser printing	Early stimulated cell growth, cell migration, typical gene expression.[12]
9	Fibrin gel	Neural cells	Inkjet bioprinter	Controlled cellular phenotype and basic physiological function.[81]
10	Fibrin gel	ECs	Inkjet bioprinter	Cell functional in gene expression.[82]
11	Alginate	ECs, fibroblasts, hepatocytes	3D bioplotting, multi-nozzle SFF deposition system	High cell viability.[73, 74] Cell toughness against mechanical process.[75]
12	Alginate, Lutrol F127, Matrigel, agarose, methylcellulose	BMSCs,	3D bioplotting	Long term cell viability, differentiation into osteoblast.[76, 77]
13	Alginate with iron oxide nanoparticles	ECs	3D bioplotting, cell writing system	Cell migration response to magnetic field.[78]
14	Gelatin, gelatin/chitosan	Hepatocytes	3D bioplotting	Long term cell viability, biologically functional.[69, 70]
15	Gelatin/alginate, gelatin/alginate/fibrinogen, gelatin/alginate/chitosan	Neuron cells, Schwann cells, ADSC, hepatocytes	3D bioplotting	ADSC differentiation into endothelial-like cells, functional albumin secreting hepatocytes.[70] Neuron and Schwann cell viability.[72]
16	Poly(propylene fumarate/diethyl fumarate)	Pre-osteoblast cell	Stereolithography	Cell adhesion and proliferation.[68]

were printed in 3D under this method such as PEO-PEG,[67] and poly(propylene fumarate)-diethyl fumarate[68] with resolutions ranging from 150 μm to 250 μm. Biomaterials such as gelatin[69–72] alginate,[73–78] PEO/PPO-collagen[79] and collagen-agarose were employed for 3D bioplotting in diverse dimensions between tens and hundreds μm depending on nozzle sizes used. Besides, collagen[80] was also applicable for inkjet bioprinting, as well as fibrin gel[81,82] with resolution around tens μm.

6. CELLULAR RESPONSES ON MICRO/NANO-TOPOGRAPHICAL SURFACE OF HYDROGELS

Many research groups reported *in vitro* cellular behaviors on or in hydrogel with typical surface topography fabricated by the methods of either nano/micro-patterning or 3D bioprinting. Concise cellular responses towards patterned surfaces of hydrogel were demonstrated in Table I, while those of 3D bioprinted hydrogels were in Table II.

Nano/micro-patterning of hydrogel was fabricated by the methods of surface patterns and biomolecule patterns. Within the studies, behaviors of cells on/inside the patterned surface were also investigated. Among the hydrogels in Refs. [45, 52–62, 83], cells were viable, despite lack of attachment on PEG hydrogel due to its characteristics of non-adhesiveness and thermodynamic chain mobility.[52] In general, rectangular patterns in grooves, lines or biomolecules enabled cells to grow in oriented directions along the patterns.[45,56,57,60,62] as described on Figure 4. These cells responses were promising to guide cells growth in designated directions for tissue regenerations. Moreover, Nikkhah et al. reported that endothelial cells remained stable in the patterns and were considered as prospective for organizing vasculatures.[57] Other pattern shapes such as hexagonals and squares with various sizes of 3–20 μm and gap sizes of 1–20 μm demonstrated orientation of cells growth on the post surfaces when gaps size was less than 5 μm as illustrated in Figure 3, while cells tended to be oriented between gaps when the sizes were more than 15 μm.[59] The cells cultured on the hydrogels with square posts and small gaps were elongated along the directions of gaps.[59] Cardiomyocytes and differentiated cardiomyocyte from embryonic stem cells showed ability to beat either under electrical stimulus or spontaneously after serial co-cultures in patterned hydrogel.[53,60] MSCs underwent differentiation into osteogenic, myogenic, and adipogenic types after cultured either on or in patterned hydrogels.[52,54,55,61] Another unique finding was that cells responded to certain ranges of aspect ratio on biomolecule patterns, by elongation and cellular anisotropy at 5:1 and 10:1 aspect ratio.[58]

Cell culture on or in 3D bioprinted hydrogels was reported to be able to maintain their viability from weeks to months.[69,70,76,77,80] Similar to the behaviors of patterned hydrogel, 3D-printed hydrogels also demonstrated cellular organizations in designated spatial dimensions.[66,79] In more details, Nahmias et al. reported that laser-guided 3D printing of hepatocytes and endothelial cells in vascular structure induced cell aggregations in tubular shapes which imitated hepatic sinusoid organization.[66] Cell migration was also reported on other studies.[12,78] Further *in vitro* evaluations showed molecular function of gene and protein expression of cells.[12,81,82] These findings were definitely useful in developing strategies of tissue engineering.

Table III summarized recent publications related to nano/micro-patterned polymeric substrates for studying *in vitro* cellular responses. Nano-patterned chondroitin

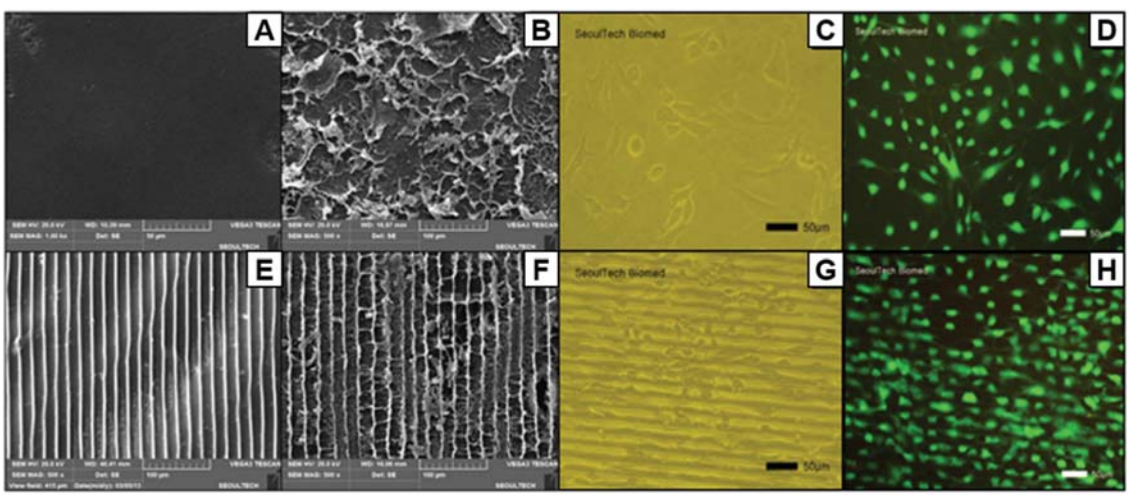

Figure 4. Examples of cells responses on patterned substrates with grooved lines. Reproduced from [45], H. S. Park, et al., *Pure Appl. Chem.* 86 **(2014)**. © 2014. (A–D) images showed cells on non-patterned surfaces of hyaluronic acid, while (E–H) images did cells on patterned ones, after 7 days *in vitro* culture of bone cells. Images were taken by SEM (A–B, E–F), light microscope (C, G) and fluorescent microscope after live/dead assay (D, H).

sulfate-coated PCL in pillar, hole and grill shapes were applied to culture hMSCs, where found that nano-pillars and nano-holes could induce better chondrogenesis as well as hyaline cartilage formation, while nano-grill caused delayed chondrogenesis which eventually resulted in fibrous and superficial cartilage.[84] Micro-patterned PLA was also found having potency to enhance hMSCs proliferation and mitogenicity and to alter morphology into smaller and longer shapes, even though it was not a hydrogel shape.[85]

Osteogenesis tendency on patterned hard substrates was also observed in several studies. Wang et al. and Janson et al. found that nano-groove polystyrene and nano-grating PMMA caused poor osteogenesis.[86, 87] However, pillar pattern shapes, either micro or nano-sized, as well as nano-pore patterns induced significant osteogenesis, mineralization and gene expression.[88, 89] Despite being less significant in osteogenesis, nano-groove patterns could induced noticeable adipogenesis and myogenesis.[86]

Some research groups investigated neuronal development on patterned substrates. Either nano/micro-gratings or grooves were able to orientate cell alignment and elongate cell morphology in accordance to pattern depth, which was essential in neuronal initial differentiation.[90–92] Figure 5 illustrated neurons appearance on patterned substrates. Furthermore, neuronal gene expression was found better on micro-patterned than nano-patterned substrate was.[93] Additionally, hierarchically patterned substrates could lead differentiation into neurons more significantly than astrocytes compared to those of the single-type patterned substrates.[90]

Table III. Cell responses on nano/micro-patterned polymeric substrates.

No	Materials	Fabrication method	Pattern type	Cells type	Cell response
1	Polycaprolactone with chondroitin sulfate coating	Thermal nanoimprinting using silicon molds	Grill hole pillar	Human MSC	MSC morphological and cytoskeletal structure change, cell aggregation and differentiation. MSC chondrogenesis and hyaline cartilage formation enhancement on nano-pillar and nano-hole surface. Delayed MSC chondrogenesis, fibro/superficial cartilage formation on nano-grill surface.[84]
2	PLA	Hot embossing on silicon mold	Isosceles triangle, circle, rectangular pillar	Human MSC	hMSCs proliferation and mitogenicity, cells smaller and higher length morphology.[85]
3	PDMS	Soft lithography	Rectangular grating line combined grating perpendicular	Human embryonic stem cell, murine neural progenitor cells	hESCs neuronal differentiation, neuronal maturation.[101] MNPCs alignment, neurite lengthening, high gene expression level on micro patterned substrate.[93]
4	PDMS	Soft lithography	Rectangular grating	Primary murine neural progenitor cells	Increasing cells alignment, elongation along with deeper gratings.[91]
5	Polystyrene	Electron beam lithography, soft lithography	Grooved line	Rat mesenchymal stem cells	Cells alignment along the groove direction, higher cell alignment tendency along with deeper groove, no significant osteogenesis induction, adipogenesis enhancement, myogenesis induction especially on 900–550 nm groove.[86]
6	Polystyrene	Hot embossing nickel nano-stamp	Pillar pore	Osteoblast MC3T3-E1	Enhanced cell attachment, proliferation and differentiation.[88] Most significant induced cell functions on nano-pore surface.[88]
7	Poly(methyl methacrylate)	UV lithography on silicon mold	Rectangular grating	Human MSC	Aligned actin cytoskeleton and elongated focal adhesion of MSCs, but poor osteogenic differentiation.[87]
8	Poly(styrene-*co*-methyl methacrylate)	Conventional photolithography	Microgroove nanopore	Human neural stem cells	NSCs high alignment, elongation, differentiation tendency to neuronal rather than astrocyte on hierarchically patterned substrate.[90]
9	Silicon	UV photolithography and reactive-ion-etching	Micro/nano-pillar circle	Rat MSC	Significant MSCs adhesion, growth, aggregation, osteodifferentiation, mineralization, osteopontin expression on nanopillar surface.[89]

7. CONCLUSION

Recent studies have shown that not only chemical properties of materials surface affected cellular responses but also physical features were essential for cellular interaction. In micro size ranges, surface patterns in small scales induced cells to grow on their surfaces while those in larger scales seemed to cause orientation and elongation of cells along the patterns. Many studies of 3D printed hydrogel showed that the cells survived after printing and they maintained viability for long term *in vitro* culture. As examples nano-patterned substrates in pillar or pore types showed enhancement of chondrogenesis and ostenogenesis better than the patterns of grill, grating or groove types did. Conversely, rectangular grating or groove patterns induced alignment orientations, elongation, differentiation and maturation of neural stem cells, where additionally, micro-patterns were found better than nano-patterns were.

Either patterned or 3D bioprinted hydrogels was capable to lead cells attachment, adhesion and proliferation, possibly leading to tissue regeneration. Both hydrogel patterning and designated 3D bioprinting enabled cells to organize in stable spatial shapes, which was promising for oriented direction of cells growth to imitate the shapes of patient's tissues and organs. These nanotechnologies could give ideas of better designs of biomaterials for tissue engineering.

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