Published in final edited form as:

Ann Biomed Eng. 2012 June ; 40(6): 1339–1355. doi:10.1007/s10439-012-0510-y.

Patterning Methods for Polymers in Cell and Tissue Engineering

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Abstract

Polymers provide a versatile platform for mimicking various aspects of physiological extracellular matrix properties such as chemical composition, rigidity, and topography for use in cell and tissue engineering applications. In this review, we provide a brief overview of patterning methods of various polymers with a particular focus on biocompatibility and processability. The materials highlighted here are widely used polymers including thermally curable polydimethyl siloxane, ultraviolet-curable polyurethane acrylate and polyethylene glycol, thermo-sensitive poly(*N*-isopropylacrylamide) and thermoplastic and conductive polymers. We also discuss how micro- and nanofabricated polymeric substrates of tunable elastic modulus can be used to engineer cell and tissue structure and function. Such synergistic effect of topography and rigidity of polymers may be able to contribute to constructing more physiologically relevant microenvironment.

Keywords

Patterning; Biocompatible polymers; Topography; Rigidity; Cell–biomaterial interface

INTRODUCTION

The rapid evolution of cell and tissue engineering has necessitated the use of various materials such as ceramics, metals and polymers as tissue engineering scaffolds for specific cell types. It has been widely recognized that polymers possess a number of advantages as tissue engineering scaffolds in terms of biocompatibility, transparency, and processability. For example, ceramics (e.g., oxides and nitrides) are bioinert with high elastic modulus but their use is limited due to inherent brittleness and opaqueness. Metals also present high stiffness and resilience, but some are susceptible to corrosion. Moreover, both materials lack bioactivity and thus researchers have increasingly employed polymers as materials which can suitably reproduce the physiological extracellular matrix (ECM) environment with the added benefit of increased cell adhesion and biocompatibility.

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Associate Editor Michael Shuler oversaw the review of this article.

The design and preparation of biomimetic polymer scaffold in terms of physical, chemical and biological similarity to native ECM plays a critical role in constructing optimal microenvironments for cells and tissues. For the last few decades, many characteristics of ECM microenvironments have been replicated by using various methods in terms of rigidity,[73,115] chemical concentration,[84] shear stress[56] and micro/nanotopography.[24,62] Among these characteristics, the rigidity and topography of biomaterials has been of major interest for mechanotransduction of cell responses.[74,150]

The elasticity of materials and its importance in the biomedical sciences have long been of interest to researchers. Elastic materials such as polyacrylamide allow for an elastic substrate whose modulus spans several orders of magnitude, similar to that of human tissues.[115] The most striking demonstration was reported by Engler *et al.*,[27] showing that the differentiation of mesenchymal stem cells (MSCs) was directly correlated with the stiffness of the substratum. This seminal work, together with a series of following studies, reveals that elasticity could be a key factor in controlling various responses of cells. Despite the potential of the findings, the materials used in the differentiation studies were too soft to emulate the full spectrum of material rigidity found in the human tissue. Furthermore, the integration of micro- and nanostructures for tissue engineering scaffolds has been a challenge due to poor processability of the polyacrylamide, limiting the widespread fabrication of well-organized *in vivo* like structures for tendons, ligaments, collagen fibers in brain and muscle fibers.[171]

In the cellular mechanotransduction studies, polyacrylamide or gelatin gels were widely used since these materials have shown the ability to control biochemistry and mechanics independently.[115] For example, the elastic moduli of polyacrylamide and gelatin gels can be controlled in the range of 150 Pa–150 kPa[29,80,108,115] and 1–100 kPa,[45,130] respectively. Despite these biocompatibility and tunable elastic moduli, such low mechanical properties render them too soft to fabricate micro- or nanoscale structures with high fidelity. Specifically, when fabricating micro and nanostructures in softer materials, a rounding of corners or shrinkage of height usually occurs due the lack of material rigidity, resulting in a loss of pattern fidelity. The mechanical properties of the material also affect the resolution of the feature sizes that can be fabricated. It is noted in this regard that, to construct well-defined microscale structures with high complexity mimicking that of *in vivo* tissues, at least few hundreds kPa of elastic modulus is required.[39] Consequently, more rigid polymeric materials have been introduced that can be structured with heat, ultraviolet (UV) or solvents. These materials are capable of creating well-defined micro and nanopatterns with smaller feature sizes than those of softer materials. For example, polydimethyl siloxane (PDMS), a well-known silicon elastomer used in soft lithography, has elastic modulus in the range of 0.6–3.5 MPa,[17,110,144] which allows a patterning resolution down to few hundreds of nanometers with moderate fidelity.[66,67] This relatively low elastic modulus still limits the application into well-defined cell and tissue scaffolds with small scale (down to ~100 nm), as the human tissue microenvironment in which cells reside in consists of various sizes of well-organized matrix structure ranging from 50 nm to sub-microns.[28,160] For this reason, a range of other polymeric materials are required for engineering a more relevant *in vitro* microenvironment.

In this review, we address the patterning methods and material properties needed, with a particular focus on mechanical properties and biocompatibility to fabricate well-organized, topographically patterned cell culture substrates. The polymers covered in this review could be classified into four categories: thermo-curable, UV-curable, thermoplastic and conducting polymers. Furthermore, the materials will be compared in terms of elastic modulus, tunable mechanical properties and pattering methods to overcome inherent patterning limitations of each polymer.

CURRENT ISSUES IN CONSTRUCTING BIOMIMETIC POLYMER SCAFFOLDS

As mentioned earlier, the elastic modulus of a material plays an important role in regulating cellular behavior as seen from directed differentiation of stem cells into various cell types in accordance to differential rigidity.[27,135] In Fig. 1, the elastic moduli of tissues in human body as well as various synthetic biomaterials used in cell and tissue engineering are summarized. Human tissues have their own rigidity based on specific cell types and structural organization,[73] ranging from few kPa to few tens of GPa (arterial wall,[1,114,143] brain,[34,82,120,152] breast,[76,136] cancellous bone,[1,105,133] cartilage,[114,143] cornea,[163] cortical bone,[1,133] heart,[36,156] kidney,[26] liver,[94,165] prostate,[76] saphenous vein,[1] skin[114,143] and tendon/ligament[60,114,143]). The liver and breast display very low elastic modulus around ~1 kPa, which is similar to the modulus of polyacrylamide (150 Pa–150 kPa) or gelatin (1–100 kPa). On the other hand, the cortical and cancellous bones have very high rigidity of around ~10 GPa, which corresponds to poly(methyl methacrylate) (PMMA) (2–4 GPa).

In order to construct physically similar microenvironment *in vitro*, one must consider appropriate mechanical properties of materials used. For example, although natural polymers such as collagen, gelatin, alginate and agarose gels have relatively high biocompatibility for implantation into human body and similar elastic moduli of physiological soft tissues, they are too compliant to construct micro and nanostructures with high fidelity. In the case of large scale structures such as microvilli of gastrointestinal tract epithelium (size ~500 μm),[149] the aforementioned materials can provide physiologically relevant structures with low patterning resolution. In the case of mimicking nanoscale features such as matrix fibers in myocardium,[70] however, such low modulus of the materials can pose a potential problem in constructing smaller pattern sizes with diameters of a few hundreds of nanometers. For this reason, it would be beneficial to recognize the limitations of each material in terms of structuring capability, and find alternative methods for the creation of physiologically relevant micro and nanostructures.

For the last decade, the effects of rigidity and topography in cell and tissue engineering have been explored independently. Rigidity has demonstrated direct differentiation potential for stem cells with response to diverse stiffness of surfaces.[27] Similarly, topography has also affected stem cell differentiation as seen from the differentiation of stem cells into osteoblasts with topography and dimensionality similar to that of real tissues.[23] Although both cases have revealed the differentiation capability of each physical cue, the real tissues *in vivo* have well-organized texture and topography as well as specific rigidity. For example,

the brain has collagen fibers with an elastic modulus range of 20–100 kPa and with fibrils of a diameter of 200–500 nm.[41,152] For this reason, the construction of topographically patterned substrate with proper rigidity is of great importance to investigate synergistic effects on cell behavior and function.

Recently, several studies have been reported in the context of synergetic role of rigidity and topography. These studies demonstrated different cell migration, spreading, alignment,[15,145,159] and shape,[117,123,173] as compared to that in the presence of single physical cue of rigidity or topography. Despite the capability of tuning rigidity and topography in the studies, the chemistry of materials was usually heterogeneous; the combined effects from the chemistry and mechanical cues were not decoupled. To investigate such synergistic effect more systematically, the chemical consistency is required with tunable modulus, while incorporating high biocompatibility or bioinertness into the patterned polymer scaffold. Such combinations of appropriate environmental cues potentially provide a new direction for increasingly advanced and sophisticated *in vitro* tissue engineering platforms.

CLASSIFICATION OF PATTERNING METHODS FOR TOPOGRAPHICALLY DEFINED POLYMER SCAFFOLD

Patterning methods for synthetic polymers can be classified into two categories: template-free and template-assisted methods. Each category is further classified based on the patterning principle. In Fig. 2, three representative methods that have been frequently used to form a topographically defined substrate are included in each category: (i) electrospinning, self-assembly, and wrinkle/crack formation for the template-free method (or bottom-up method) and (ii) photolithography, electrochemical deposition, soft lithography, and nanoimprint lithography (NIL) for the template-assisted method (or top-down method). Here, photolithography is included without detailed descriptions for its excellent maturity and popularity in patterning fields. Also, chemical patterning such as microcontact printing is not included in soft lithography as it does not create a surface topography. Therefore, referring to soft lithography, mold-based approaches are only considered such as replica molding (RM), soft molding (SoMo), and capillary force lithography (CFL). A number of extensive reviews are available for the details of each patterning technique.[24,62,68,73,74,134,167,171]

Figure 2 summarizes the existing patterning methods available for each synthetic polymer. It is noted that each polymer could be used in single or multiple patterning methods depending on its properties. For example, UV-curable polymers such as polyurethane acrylate (PUA), polyethylene glycol (PEG) acrylate, Norland Optical Adhesive (NOA), poly(*N*-isopropyl acrylamide) (pNIPAM) have mostly been used in soft lithography in the form of RM and CFL, while thermoplastic polymers such as polymethyl methacrylate (PMMA) and polystyrene (PS) being used in multiple methods from electrospinning to NIL. It is therefore important to recognize the limitation, properties, and uses of each material in various patterning methods.

In Fig. 3, representative template-free patterning methods are displayed with various polymers, which include electrospinning, microphase separation of block copolymer, and PDMS stretching for reconfigurable wrinkles and cracks. These bottom-up patterning methods are capable of creating well-ordered surface textures in a simple and cost-effective manner for various cell and tissue engineering applications. In the first example, the electrospinning of PLA fibers was used to investigate the effect of alignment and orientation of fibers in wound healing. It was observed that different wound healing speed was observed presumably due to the contact guided growth following the fibers (Fig. 3a). In the second example, self-assembly of PS-*b*-PMMA block copolymer *via* microphase separation was used to assess different actin filament expression on various topographically patterned surfaces (Fig. 3b). In the third example, wrinkles or cracks were formed on rigid film supported on soft PDMS substrate. On a wrinkled substrate (PDMS), cardiac-like cellular morphology was generated (Fig. 3c, (i)–(iii)), while on a cracked channel elongated cellular shape was formed upon cyclic stretching (Fig. 3c, (iv)–(v)).

Similarly, template-assisted patterning methods are briefly summarized in Fig. 4 along with their exemplary applications. In the template-assisted methods, polymer thin films are typically processed into desired shapes by applying a variety of external stimuli such as oxidation, pressure, heat, and UV irradiation. In the first example, patterns of electrochemically deposited conducting polymer (PPy) were used to apply an electrical stimulus to the cultured cells. With contact guidance by PLA:PLGA fibers, the patterned surfaces induced highly oriented undifferentiated myoblasts (Fig. 4a). In the second example, the most well-established soft lithography was illustrated in the form of replica molding. This method is a simple and low-expertise route to 2D or 3D topographically patterned surfaces with thermo-curable materials. Here, an array of high aspect-ratio micropillars was used to measure traction forces exerted by the cultured cells (Fig. 4b). In the third example, NIL is presented with PMMA polymer, which was used to investigate the role of pattern ordering on osteogenic differentiation (Fig. 4c).

SYNTHETIC POLYMERS AND THEIR PROPERTIES/USES IN PATTERNING METHODS

In this section, various synthetic polymers will be described with a particular focus on their properties in terms of biocompatibility and processability and their uses in various patterning methods. Their elastic moduli, patterning limit, and available patterning methods are summarized in Table 1.

Thermally Curable Polymer

Polydimethyl Siloxane (PDMS)—PDMS is one of the three primary reference biomaterials chosen by National Heart, Lung and Blood Institute (NHLBI) with the two other polymers of low-density polyethylene (LDPE) and fluorinated ethylene propylene (FEP).[9] According to the references on hemocompatibility, biocompatibility, inflammatory behavior *in vivo* studies, PDMS causes only mild inflammatory reaction when implanted without irritating the skin, and induces no adverse effect on animal models such as rabbits

and mice.[9] Additionally, a recently reported dry adhesive skin patch made of PDMS pillars demonstrated negligible skin irritation.[77]

PDMS was first introduced by Whiteside's group in the early 1990s in the form of soft lithography in order to massively produce micro- and submicron-scale structures.[129,167] Traditionally, micropatterning utilized inorganic hard materials in photolithography at the expense of higher costs and laborious fabrication processes. Since the introduction of PDMS in micropatterning, one can directly fabricate various two-dimensional or three-dimensional patterns in a cost-effective and low-expertise fashion, which has dramatically improved the patterning capability in a typical laboratory setup. Microstructures of PDMS are made by mixing the prepolymer and cross-linker with an appropriate ratio (usually 10:1), followed by backfilling into a pre-patterned master and curing at 60–70 °C in an oven for an hour or two.[129] Depending on the amount of curing agent and curing time, PDMS has tunable elastic modulus in the range of 0.6–3.5 MPa.[4,72,106,110] As a result, when the pattern scale is smaller than 1 μm, the resolution decreases significantly.[39] Although the patterning ability of PDMS is limited to 500–800 nm, the resolution can be further enhanced by increasing the ratio of cross-linker or adding a hard modulator (hard PDMS, E = ~9 MPa), which allows for sub-100-nm pattern resolution.[17,118]

One important application of micropatterned PDMS involved the use of microscale PDMS pillars for measuring traction force of cells *via* observing the bending of structures.[31] In this study, the rigidity of pillars was modulated by varying the aspect ratio of microposts with the identical PDMS materials. Then, the amount of deflection or bending of the micropillars was easily measured when a shear force is applied to the top, where the bending was related to the magnitude of the applied shear force.

One of the important characteristics of PDMS is its high elongation at break (~160%).[17] This property enables an application of cyclic stretching (stretching and releasing) onto single or multiple cells (colonies) with desired tensions and frequency. Since some human tissues such as muscle,[102] heart,[12] cartilage,[8] ligament and tendon[164] are inherently exposed to mechanical loads, it is potentially beneficial to observe the effect of external forces onto the mechano-sensitive cells. When a uniaxial stress was applied to mimic uniaxial stretching *in vivo*, some cells have shown elongation and orientation to the direction of stretching.[13,93] With this stretching-induced alignment, the aligned ligament cells showed more efficient calcium wave propagation compared to the randomly oriented cells,[59] and human patellar tendon fibroblasts (HPTFs) expressed more α-smooth muscle actin protein according to the alignment angle.[164]

In order to create self-organized micropatterns of PDMS, alternative methods such as wrinkle and crack formation have been used in some applications. Wrinkling is a mechanical instability occurring on a thin, stiff film adhered onto an elastomeric substrate. When an elastomeric substrate is treated with oxygen plasma or UV/Ozone, or deposited with metal layer or diamond-like carbon upon stretching, a multi-layered structure is formed with a thin stiff film on a soft substrate. Upon releasing, the stiff surface is buckled while the underlying substrate is relaxed, resulting in a spontaneous formation of well-ordered wrinkles.[170] The wavelength and amplitude of wrinkles can be modulated by adjusting the thickness and

modulus of thin film[35] as well as the alignment *via* controlling the direction of mechanical strain.[170] The wrinkled pattern has been used as a scaffold for heart cells, in which a certain degree of alignment and protein localization of mouse and human cardiomyocytes were observed.[99]

Cracking is a result of mechanical fracture occurring on a thin film adhered onto an elastomeric substrate. Similar to wrinkle patterning, PDMS surface treated with oxygen plasma or UV/Ozone can give rise to cracks upon stretching in response to the applied mechanical strain. In the case of oxygen plasma treatment, few hundreds of nanometer scale cracks are generated[50,109] whereas UV/Ozone treatment induces few micron-range cracks.[69] The crack formation and propagation has been studied mainly from a mechanics aspect, so that an application to tissue engineering has been rarely reported. With reconfigurable cracks, cellular elongation of mouse myoblasts was demonstrated upon cyclic stretching.[177]

UV-Curable Polymers

The major advantage of UV-curable polymers is a short-processing time by using UV-exposure (λ = 250– 400 nm) of few tens of seconds. In most UV-curable polymers, incorporated or trapped oxygen retards cross-linking by radical scavenging in the course of photo-crosslinking.[58] To prevent such inhibition effects upon curing, a flexible and transparent support such as polyethylene terephthalate (PET) or polycarbonate (PC) sheet, or other engineering plastics can be used as a blanket or backing support of polymer structures.[18] Therefore, either free-standing structures or structures supported on a backing support can be fabricated. Although the process of UV-curable patterning is simple and well-established, cell and tissue engineering applications could be restricted due to significant auto-fluorescence of plastic films, limiting the imaging of tissues.[128] Thus, for biomedical research, a few hundreds of nanoscale patterns on cover glass are recommended to reduce auto-fluorescence.

Polyurethane (PU)-Based Materials—PU is a versatile UV-curable polymer whose chemical structure can be readily modified. Commercially available PU-based polymers include polyurethane acrylate (PUA, Minuta Tech. Inc., Korea) and NOA (Norland Optical Adhesive, NY, USA). PUA is a UV-curable polymer that was first introduced in 2004.[18] Similar to other UV-curable materials, PUA can be cross-linked in tens of seconds upon UV-exposure, resulting in a transparent and flexible thin polymer structure with or without a backing plane. Since PUA has several notable characteristics such as transparency for optical imaging, chemical stability for long-term cell culture and tunable surface energy for easy molding, it has been successfully utilized as a cell culture platform either in single cell studies[83,121,122] or various tissue engineering applications for diverse cell types such as human embryonic stem cells (hESCs),[86] human mesenchymal stem cells (hMSCs),[175] fibroblasts,[63,71] cancer cells,[78] neurons.[55]

The most distinguished characteristic of PUA is that its modulus can be tuned between 20 and 320 MPa, by adjusting the amount of soft and hard modulators.[16,174] By utilizing the modulus-tunability and patterning method of CFL, the effect of rigidity has been investigated with the identical patterns without losing chemical consistency.[174] Also, various

multiscale, hierarchical structures can be fabricated with the aid of partial curing kinetics, which would be useful to recapitulate complex, hierarchically organized structures.[57]

NOA is the brand name of polyurethane-based UV-curable adhesive which is commercially available. Due to high transparency and simple curing process, it is usually used as an optical adhesive in fixing glass lenses[38] or for micro lens arrays.[22] As compared to PUA or other UV-curable polymers, the curing process of NOA is not affected by the presence of oxygen. For this reason, it can be cured even in an open environment without the use of a transparent blanket. Furthermore, NOAs adhesion properties onto glass substrate are moderate to good, thus not requiring any type of pretreatment or an adhesion promoter. When using NOA as a patterning material, a flexible mold with low surface energy is needed. Although the detailed chemical structures and additives are not known, NOA shows a wide range of elastic modulus (6 MPa–2.5 GPa, available from the provider's website).[116] As a cell culture scaffold, sub-100-nm NOA patterns have been used for culturing endothelial cells,[95] fibroblasts,[104] human embryonic stem cells,[111,112] and breast cancer cells[101] without significant adverse effects.

Polyethylene Glycol (PEG) Acrylate—PEG is a Food and Drug Administration (FDA)-approved UV-curable hydrogel that has been frequently used for drug delivery and tissue engineering.[161] Due to its minimized adverse effects, it is widely used for tissue implantation surgery.[46] Furthermore, it can also be used as a material to prevent cell adhesions for microchips.[64] Similar to PUA, PEG acrylate patterns are fabricated onto glass substrate in the form of a thin, structured film with UV-exposure of few tens of seconds. However, as PEG is a hydrogel, it has swelling problems upon exposure to water or media. As such, the patterns are easily delaminated from the substrate.[132] To prevent delamination, the substrate surface can be treated with an adhesion promoter (phosphoric acrylate or acrylic acid dissolved in propylene glycol monomethyl ether acetate (PGMEA), 10 vol.%).

PEG and its related hydrogels have a broad range of modulus tunability depending on their modified chemical structures. Basic PEG has a simple chain structure with a relatively low elastic modulus of ~500 Pa.[3,88] This elastic material is suitable as a model matrix for measuring traction force of cells in a real time manner since it is easily deformed by the morphological change of cells.[88] Thus, the pure PEG could not be used for constructing well-defined micro- or nanoscale patterns. With the addition of acrylate group at both ends of the polymer chain then elevates its modulus to three orders of magnitude (~500 kPa),[10] allowing for the fabrication of submicron to few hundreds of nanometer structures without losing its biocompatibility.[44,54] Further modification could be achieved by terminating the polymer chain with methacrylate group, resulting in the increase of modulus up to 1.6 GPa.[14] With PEG dimethacrylate (PEG-DMA), nanopillars of high aspect ratio (diameter of 750 nm, height of 7 μm) have been successfully fabricated without collapse even in the presence of capillary force.[14] With PEG diacrylate (PEG-DA), 50-nm-wide nanogrooves have been fabricated with high fidelity to be used as a nanopatterned scaffold for rat cardiomyocytes.[65,70]

Due to its biologically inert properties, PEG is also widely used for fabricating an anti-adhesion surface for cells. For example, microscale PEG patterns have been fabricated to

obtain single or multiple cell aggregates.[61] On non-adherent PEG substrates, co-culture of heterogeneous cells such as hepatocytes and fibroblasts[87] and differentiation of mesenchymal stem cells within confined geometry[127] have also been demonstrated.

Poly(N-isopropyl acrylamide) (pNIPAM)—Poly(*N*-isopropyl acrylamide) (pNIPAM or pNIPAAm) is a thermo-responsive polymer which can expand or shrink upon a thermal stimulus. One of the distinctive characteristics of pNIPAM is the ability to change phases in the physiologically relevant temperature range. Namely, the polymer has a lower critical solution temperature (LCST) of ~32 °C which is around the body temperature.[20,139] Above the LCST, it shows a relatively hydrophobic surface, which is related to the packed conformation. In sharp contrast, below the LCST, it demonstrates a hydrophilic surface due to swelling by hydration.[20,47]

In addition to the tunable hydrophobicity, the material shows a dramatic difference in the mechanical property. For example, at 25 °C, its elastic modulus is ~9.8 kPa due to swelling, while at 40 °C its elastic modulus is around ~170 kPa due to dehydration.[151] By utilizing this modulus tunability along with shape deformation, Khademhosseini and coworkers[153,154] have recently demonstrated the use of pNIPAM as an active mold for patterning hydrogels and as microwells for forming and retrieving cell aggregates. Some studies have also demonstrated the patterning of pNIPAM surface with e-beam lithography. In the presence of the fabricated microgrooves of pNIPAM, a cell sheet with aligned cell morphology was obtained.[51] With the help of the cell detachment characteristic above a certain temperature, selective cell removal and subsequent co-culture experiments were also presented.[168,169]

The cytotoxicity of pNIPAM has been studied in a number of drug delivery and tissue engineering applications. When the material was used as a drug delivery vehicle (eye drop) for glaucoma therapy, no difference of cell death rate was observed compared to PBS (phosphate buffered saline).[48,162] When it was used as an embolic material, *in vivo* injection test showed no acute toxicity in mice below the dose of 250 mg kg^{-1}.[103] It was also used as a three-dimensional cell scaffold, presenting no significant problems with the exception of minor inflammation after the injection.[119] Furthermore, many *in vitro* cell culture results and *in vivo* transplantation from pNIPAM plate to human body have shown no distinct adverse effects. Therefore, it can be seen that pNIPAM is a biocompatible material and possesses great potential in cell and tissue engineering applications.

Thermoplastic Polymers

A thermoplastic polymer becomes liquefied or molten upon heating above the melting temperature (T_m). The material also becomes plastic above the glass transition temperature (T_g), allowing for further modification such as drawing, bending and molding at an elevated temperature.[146] Since these materials are solids at room temperature, heat or solvent treatment can be used to make fine structures.

Conventional Thermoplastics (PMMA/PS)—The biocompatibility of PMMA can be evaluated from the implantation studies *in vivo*. For many years, various transplantable parts made of PMMA were implanted into human bodies such as porous membranes onto human

kidneys[124] and intraocular lenses.[98] In these reports, PMMA demonstrated long-term stability and reasonable performance without appreciable adverse effects. In contrast, PS is known to have high cellular adhesion properties but to cause strong inflammatory response upon implantation. Due to this undesirable effect, PS is usually used as a control to decide relative biocompatibility of other materials or compare relative cell affinities between materials.[11,97]

The most well-known fabrication method for thermoplastic polymers is Nanoimprint lithography (NIL, also known as hot embossing), which requires heat above T_g (PMMA: 85 to 165 °C, PS: 95 °C) and high pressure.[43] Since the materials used in NIL usually have high elastic modulus on the order of GPa, they can represent high pattern resolution down to ~10 nm.[89,166] An alternative method for patterning thermoplastic polymers utilizes reduced viscosity of the materials *via* temperature rise or solvent treatment. For example, a thermoplastic polymer layer can be patterned by placing a patterned PDMS mold followed by temperature rise above T_g, leaving behind a negative replica of the mold by capillary action (capillary force lithography, CFL).[147,148] Similarly, a solvent-laden polymer film directly fills into the cavity of PDMS mold by capillary action, which can be termed soft molding (SoMo).[134] In this way, various micro- or nanopatterns of thermoplastic polymers such as PMMA, PS and PLGA have been constructed with high pattern fidelity.[33,84,85] It is noted in this regard that PMMA and PS have relatively high elastic modulus on the order of several GPa, capable of rendering several tens of nanometer patterns with high physical integrity.

In addition to the above template-assisted methods, a template-free method is possible with thermoplastic polymers. One such technique is electrospinning, in which a jet of liquid-phase polymer is ejected from a cone or nozzle, drawn by a controlled electric field, and finally stacked on ground-state plate. It is known that few tens of nanometer to few micrometer fibril structures can be constructed in the electrospinning. For more sophisticated, mesh-like structures, precise control of the electric field is required.[41] Also, by adjusting the composition of solution or melt, the diameter and chemical distribution of the fibers could be modulated.[155,172] An alternative template-free method is block copolymer lithography (BCL),[100] where two nanophase polymer domains are self-assembled into various morphologies such as spherical, hexagonal or lamellar lattice structure. Such a periodic, ordered structure showed increased cell spreading area with the decrease of domain size,[158] and more actin filament formation at smaller feature scale.[157]

Biodegradable Thermoplastics (PLGA/PGA/PLA/PCL)—Biodegradable polymers, more specifically synthetic biodegradable polymers, refer to the polymers that lose their initial integrity within the body tissues over time.[30] These biodegradable polymers include polylactic-*co*-glycolic acid (PLGA), polyglycolic acid (PGA), polylactide (PLA) and polycaprolactone (PCL).[42] Due to their biocompatibility, biodegradability and high rigidity, these synthetic polymers have been widely used for human therapy such as absorbable sutures as well as fixation units for medical surgeries.[107]

A family of PGA, PLA and their copolymers (PLGA) are FDA-approved due to their biocompatibility upon implantation which has allowed clinical applications. However, some

side effects have also been recognized such as production of acid and release of small particles upon degradation.[42] PLGA is a quickly biodegradable copolymer which can tune its properties by varying the relative molar ratio between lactic acid and glycolic acid (50:50, 65:35, 75:25, 85:15 are commercially available). By adjusting the lactoyl content from 50 to 85%, the degradation time can be controlled from 1 to 2 months (50% of lactoyl content) to 5–6 months (85% of lactoyl content) while retaining their elastic modulus at ~2 GPa.[6,42,107] In the cases of PGA, PLA, and PCL, their degradation times are relatively long compared to that of PLGA (PGA: 6–12 months, PLA: >24 months and PCL: >24 months).

Although their chemical structures are slightly different, the fabrication techniques could be identical. For example, a thin film of these biodegradable polymers can be dissolved in a wide range of common solvents such as chloroform, toluene, tetrahydrofuran, acetone and ethyl acetate and spin-coated to be used in a simple molding technique (e.g., SoMo). Alternatively, a thin film can be thermally imprinted above the polymer's T_g (PGA: 35–40 °C, PLA: 60–65 °C, PLGA: 40–60 °C, PCL: 265 to 260 °C), which is relatively low as compared to PMMA and PS.[107] Furthermore, these biodegradable polymers have high elastic modulus (PGA: 7.0 GPa, PLA: 2.7 GPa, PLGA: 2 GPa and PCL: 0.4 GPa), which allows for the fabrication of few tens of nanometer patterns with the existing template-assisted methods.

For template-less structuring with biodegradable polymers, electrospinning is also widely used to prepare fibril structures. It was observed that the ordering and scale of fibers are important for the contact guidance-induced elongation, morphogenesis and migration of cells.[125,172] In particular, cells with inherent anisotropic organization *in vivo* such as skeletal muscle tissue, ligaments, articular cartilage and blood vessels showed high sensitivity to the alignment of fibers.[96] Additionally, bioactive molecules such as growth factors and specific signaling molecules play crucial roles in stem cell differentiation and homing of cells to the specific repair site. Further information on the electrospinning of biopolymers and their applications can be found elsewhere.[96,138,155]

Conducting Polymers

Certain tissues such as cardiac or nerve tissues convey their signals to adjacent cells by conducting electric pulses named 'action potentials'.[7,142] Action potential generation is involved in many crucial physiological processes, including the beating of heart at a desired frequency in a synchronized fashion.[141] Since the transfer of signals is important for observing active cellular functions, the introduction of conducting materials into tissue engineering is required for certain cell types. For instance, when studying neurogenesis or cardiogenesis from stem cells, the cellular functions of differentiated cells can be judged by whether they have similar functions or electrical signals to real tissues.

For many years, conducting polymers such as polyaniline (PANi),[90] polypyrrole (PPy),[37] poly(3,4-ethylenedioxythiophene) (PEDOT)[52] or mixtures of conducting polymers have been used to address this issue. For example, cells cultured on an electroactive surface showed enhanced neuronal differentiation,[75] promoted nerve regeneration,[137] and significant increase in neurite lengths.[140] However, these results were obtained from the cells cultured on smooth surface without the incorporation of topographical effects. Recently,

topographically modified electroactive surfaces have been introduced as a cell culturing platform to support growth of excitable tissue cells. There are a number of available patterning methods such as CFL,[52] NIL[49] or lift-off[25] for patterning conducting polymers. Since most of the conducting polymers are rigid (elastic modulus; PANi: 2–4 GPa,[32] PPy: 1.2–3.7 GPa,[113] PEDOT: 1.1–2.2 GPa[79]) they can form tens of nanometer scale features with high fidelity. It is worthwhile noting that due to their low breaking stress, the patterned thin films need to be handled with care.[91] Furthermore, precise patterning techniques are yet to come for highly controlled active structures.[92] In several studies, the researchers have employed an electrochemical deposition (or electro polymerization) in order to apply an electrical potential during the cell culture on conducting substrates.[126,131,140]

Concerning the neuron culture, the biocompatibility of conducting polymers can be determined from an efficacy test by counting percentage of cells bearing neurites or measuring the length of neurite compared to the control surfaces. There are three factors which can influence toxicity: unreacted monomers, motility and toxicity of dopant ions, and residual solvents.[40] According to the Material Safety Data Sheets (MSDS) available in Sigma-Aldrich, monomers show higher toxicity than dopants, but both of the components are slightly to moderately toxic.[40] Moreover, Schmidt *et al.*[140] demonstrated less adverse tissue response of PPy as compared to PLGA upon animal implantation. From this study, it can be assumed that conducting polymers such as PPy and PEDOT have relatively good biological performance. Furthermore, biocompatibility can be enhanced by adding bioactive factors such as laminin peptide[21] and hyaluronic acid (HA)[19] or coating biocompatible polymers such as PLGA onto the polymer surface.[81] It seems that further studies need to be performed to find an optimal condition between biocompatibility and mechanical, electrical and biological properties.

CONCLUSIONS

In this review, we have described material properties and patterning techniques of various polymers toward topographically defined substrates in cell and tissue engineering applications. As motivated by the pioneering work by Engler *et al.*, there are increasing demands on topographically patterned substrate with tunable modulus in order to investigate synergistic role of rigidity and topography in mechanotransduction of cells.

Here, the patterning methods were classified into two categories of template-free (or bottom-up) and template-assisted methods (or top-down). Then, the existing synthetic biocompatible polymers were described in the order of thermo-curable, UV-curable, thermoplastic and conducting polymers with particular emphasis on biocompatibility and processability. It has been shown that each biocompatible polymer is suited to specific patterning methods depending on its materials properties.

Based on the information provided in this review, an appropriate combination of material and patterning method should be chosen to create diverse and robust cell culture platforms with *in vivo* like cellular microenvironment. Such more physiologically relevant microenvironments may be able to significantly advance tissue engineering research while

providing insight into the effects of topography and rigidity in synergy on cell behavior and function.

Acknowledgments

This work was supported by National Research Foundation Grant funded by the Korean Government (NRF-2011-220-D00035), WCU (World Class University) program (R31-2008-000-10083-0) and Basic Science Research Program (2010-0027955). D. H. Kim thanks Department of Bioengineering at the University of Washington for the new faculty startup fund. D. H. Kim is also supported by a Perkins Coie Award for Discovery. A. Jiao was supported by NIH training Grant T32-EB001650-07.

ABBREVIATIONS

ECM	extracellular matrix
MSCs	mesenchymal stem cells
hMSCs	human mesenchymal stem cells
hESCs	human embryonic stem cells
HPTFs	human patellar tendon fibroblasts
PBS	phosphate buffered saline
OPN	osteopontin
OCN	osteocalcin
PDMS	polydimethyl siloxane
LDPE	low-density polyethylene
FEP	fluorinated ethylene propylene
PET	polyethylene terephthalate
PC	polycarbonate
PU	polyurethane
PUA	polyurethane acrylate
NOA	Norland Optical Adhesive
PEG	polyethylene glycol
PEG-DMA	polyethylene glycol dimethacrylate
PEG-DA	polyethylene glycol diacrylate
PGMEA	propylene glycol monomethyl ether acetate
pNIPAM	poly(*N*-isopropylacrylamide)
LCST	lower critical solution temperature

PMMA	poly(methyl methacrylate)
PS	polystyrene
PLGA	poly(lactic-*co*-glycolic acid)
PGA	polyglycolic acid
PLA	polylactide
PCL	polycaprolactone
PANi	polyaniline
PPy	polypyrrole
PEDOT	poly(3,4-ehtylenedioxythiophene)
PTS	paratoluenesulfonate
HA	hyaluronic acid
UV	ultraviolet
NIL	nanoimprint lithography
RM	replica molding
SoMo	soft molding
CFL	capillary force lithography
BCL	block copolymer lithography
NHLBI	National Heart, Lung and Blood Institute
FDA	Food and Drug Administration
MSDS	Material Safety Data Sheets

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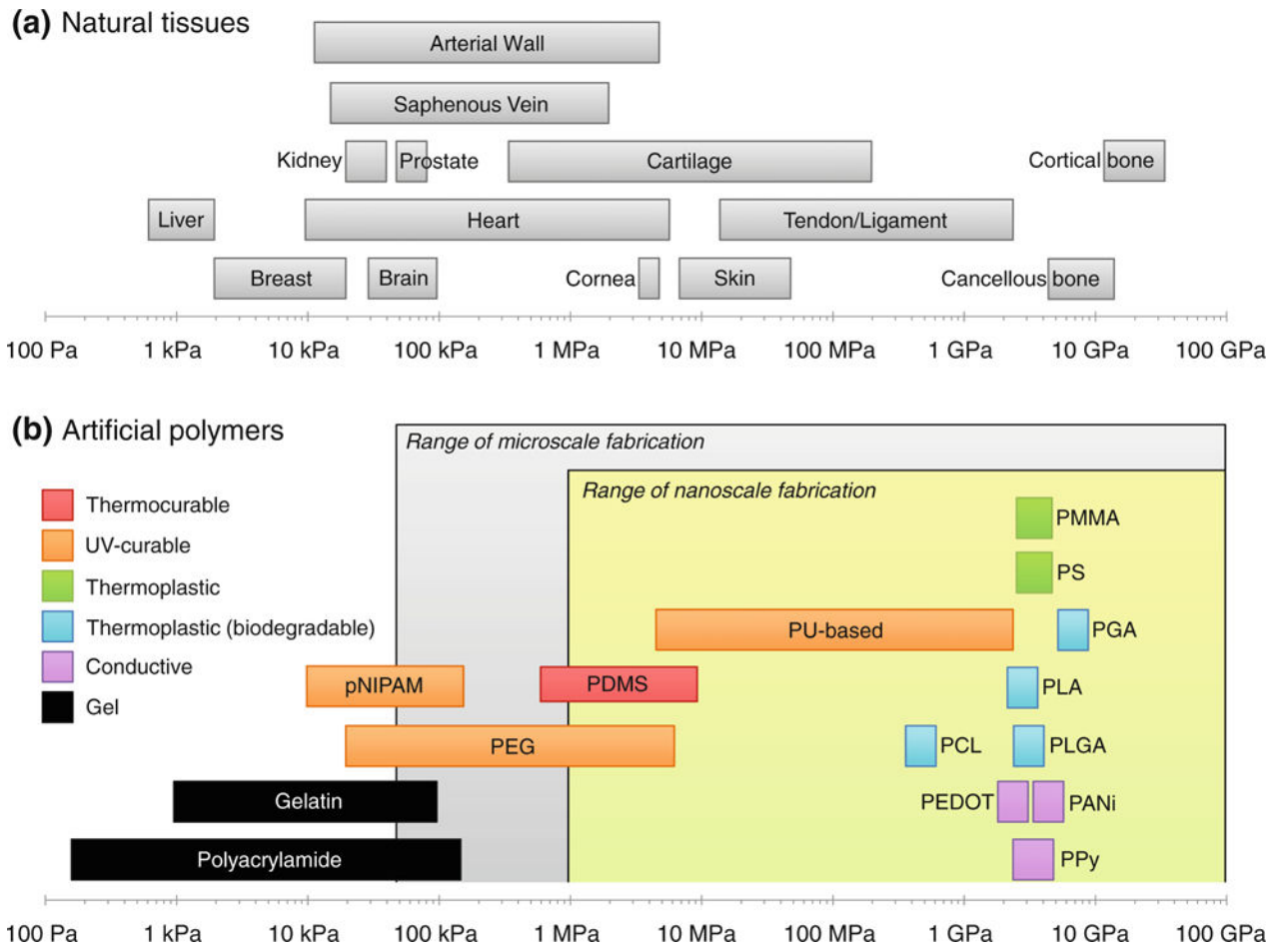

FIGURE 1.

Mechanical properties of natural tissues and synthetic polymers. (a) Range of the elastic modulus of various tissues in human body. Modified from Nemir and West.[115] (b) The same of various biocompatible polymers used for *in vitro* studies with respect to patterning resolution and mechanical properties.

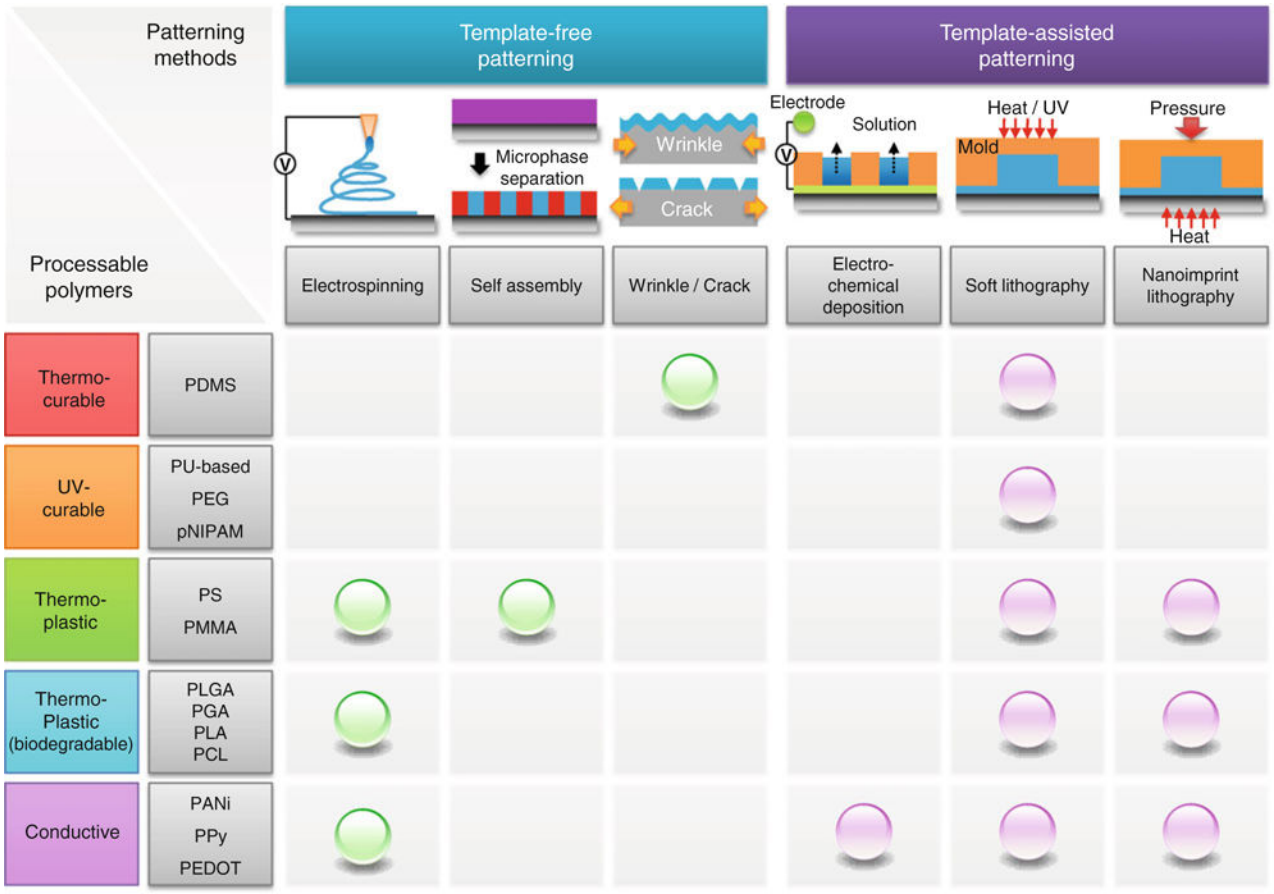

FIGURE 2.

Classification of patterning methods with template-free and template-assisted principles and their availability with the existing various synthetic polymers.

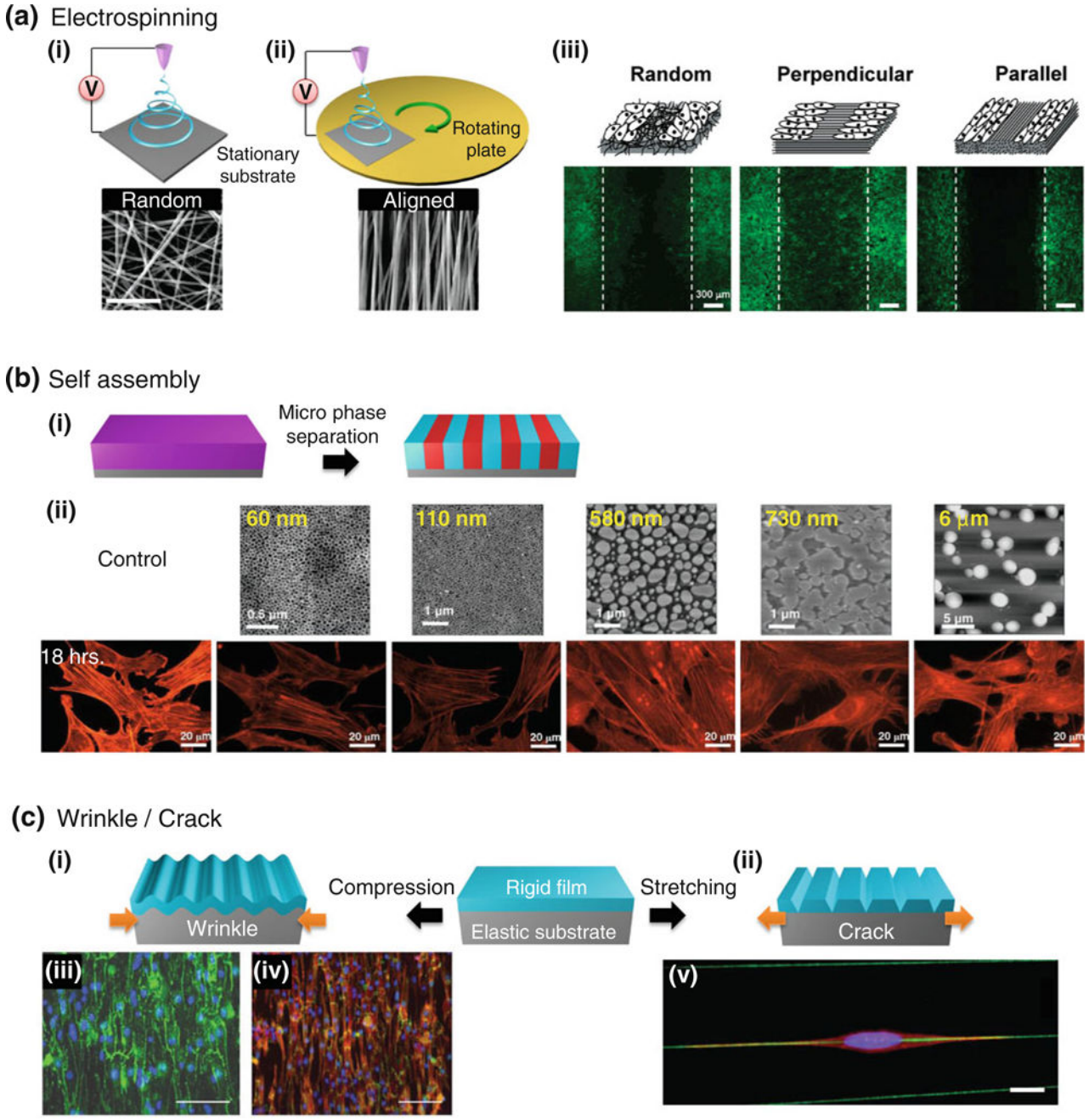

FIGURE 3.

Template-free patterning methods and their applications. (a) Electrospinning of PLA fibers on stationary or rotating substrate, in which random (i) or aligned (ii) fibers can be formed. (iii) Depending on the alignment and orientation of fibers, wound healing speed was different over 48 h time span (actin filaments: green, nuclei: blue). For example, the wound healing was the fastest on perpendicularly ordered fiber matrix. Reprinted with permission from Patel *et al.*[125] (b) Self assembly of PS-*b*-PMMA block copolymer. (i) With neutral interfaces between film-air and film-substrate, vertically aligned nano- to microscale patterns can be fabricated by self assembly of nanoscopic polymer domains. (ii) On

topographically defined surfaces, the degree of actin stress fiber formation (fibroblast) was observed to decrease as the feature size increased. Reprinted with permission from Tsai *et al.*[158] (c) Wrinkle and crack formation *via* compression (wrinkle) (i) or stretching (crack) (ii) of surface modified PDMS. (iii, iv) On wrinkled substrate, neonatal cardiac cells showed alignment. Connexin-43 (green) in (ii) and N-Cadherin (green) and actin (red) in (iii). Scale bars indicate 100 μm. Reprinted with permission from Luna *et al.*[99] (v) An elongated myoblast cell on a crack stained with actin (red), nucleus (blue) and crack with collagen (green). Reprinted with permission from Zhu *et al.*[177]

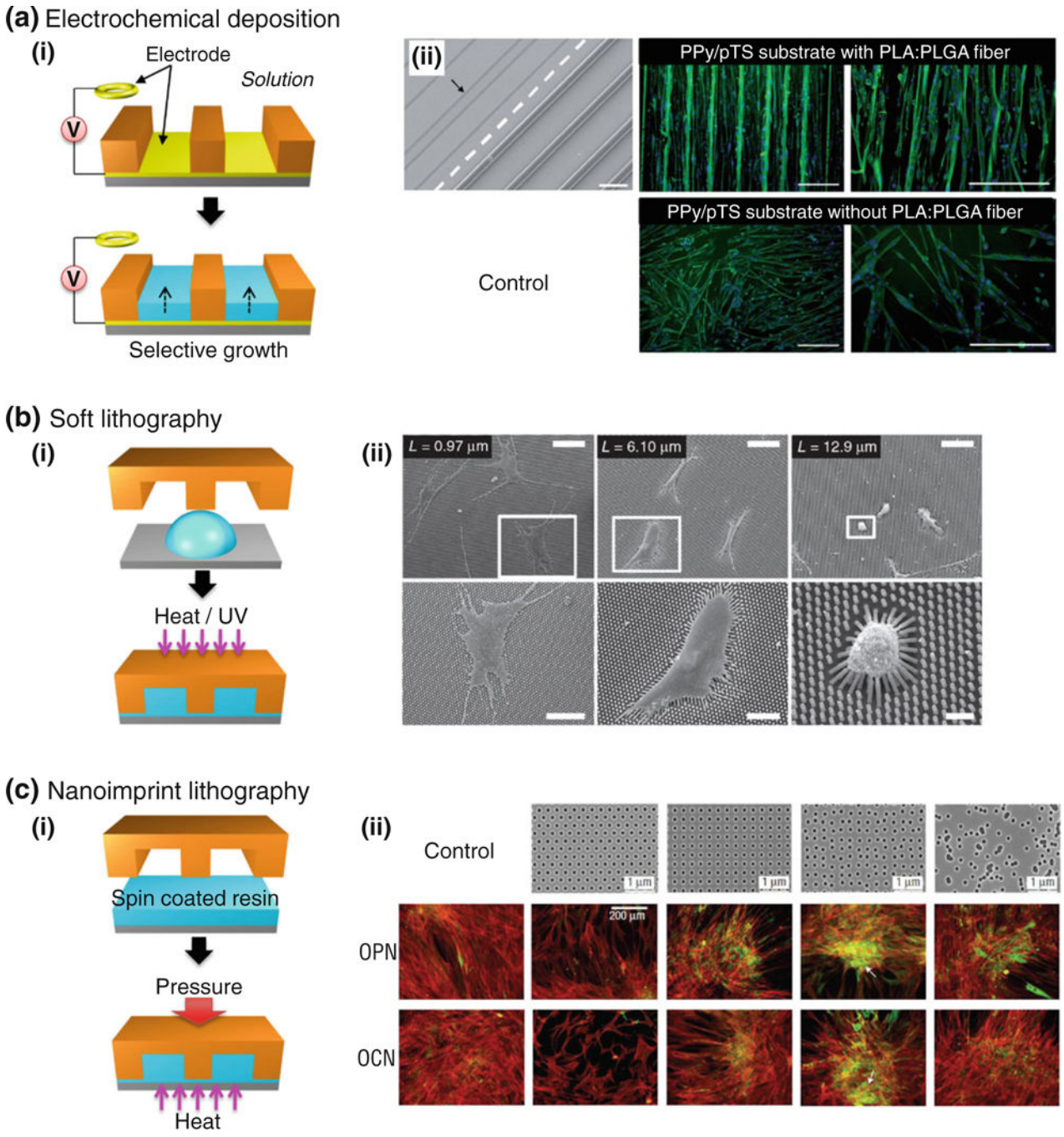

FIGURE 4.

Template-assisted patterning methods and their applications. (a) (i) Schematic illustration of electrochemical deposition. (ii) SEM image of patterned surface and fluorescent images of skeletal muscle cells that adhered and proliferated for 2 days and finally differentiated into myotubes at day 4 (arrow indicates delaminated points of PLA:PLGA fiber). Immunofluorescence images of differentiated, desmin (green)-expressing myotubes on PPy/pTS substrate with (top panel) and without (bottom panel) PLA:PLGA fiber arrays. Cell nuclei are shown in blue. Scale bars are 200 μm. Reprinted with permission from Razal *et*

al.[131] (b) (i) Schematic illustration of soft lithography in the form of UV-assisted capillary force lithography (CFL). (ii) SEM images of hMSCs cultured on various micropost arrays. Bottom panel is an enlarged view from each top panel. Scale bars, 100 μm (top panel), 50 μm (first column, bottom), 30 μm (second column, bottom) and 10 μm (third column, bottom). Reprinted with permission from Fu *et al.*[31] (c) (i) Schematic illustration of nanoimprint lithography. (ii) Effect of ordering on human mesenchymal stem cell differentiation. Top panel: SEM images of PMMA hole arrays with various orderings (diameter: 120 nm, depth: 100 nm, average center-to-center spacing: 300 nm). These arrays include hexagonal, square, displaced square with random displacement. Middle and bottom panels: Immunostaining images of osteopontin (OPN) and osteocalcin (OCN) (actin: red, OPN/OCN: green). Reprinted with permission from Dalby *et al.*[23]

TABLE 1

Summary of material properties of various polymeric biomaterials used in cell and tissue engineering.

Category	Material	Elastic modulus	Patterning limit	Patterning method	References (modulus)
Thermo-curable polymer	Polydimethyl siloxane (PDMS)	0.6–3.5 MPa	500–800 nm	Wrinkle/crack	4,72,106,110
	h-PDMS	9 MPa	<100 nm	Soft lithography	17,118
UV curable polymer	Polyurethane (PU)	6 MPa–2.5 GPa	<100 nm	Soft lithography	16,116,174
	Polyethylene glycol (PEG)	500 kPa–1.6 GPa	<50 nm		3,10,14,88
	Poly(*N*-isopropylacrylamide) (pNIPAM)	9.8–170 kPa	<1 μm		151
Thermoplastic	Poly(methyl methacrylate) (PMMA)	2–3.5 GPa	~10 nm	Electrospinning Soft lithography	5,53,176
	Polystyrene (PS)	3–3.5 GPa	<70 nm	Nanoimprint lithography Self assembly	5,176
Thermoplastic (biodegradable)	Polylactic-co-glycolic acid (PLGA)	2 GPa	<100 nm	Electrospinning Soft lithography	6,42,107
	Polyglycolic acid (PGA)	7 GPa		Nanoimprint lithography	6,42,107
	Polylactic acid (PLA)	2.7 GPa			2,42,107
	Polycaprolactone (PCL)	400 MPa			42,107
Conducting polymer	Polyaniline (PANi)	2–4 GPa	<100 nm	Electrospinning	32
	Polypyrrole (PPy)	1.2–3.7 GPa		Electrochemical deposition	113
	Poly(3,4-ethylenedioxythiophene) (PEDOT)	1.1–2.2 GPa		Soft lithography Nanoimprint lithography	79

Ann Biomed Eng. Author manuscript; available in PMC 2017 May 22.