Review

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Recent advance in surface modification for regulating cell adhesion and behaviors

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Abstract: Cell adhesion is a basic requirement for anchorage-dependent cells to survive on the matrix. It is the first step in a series of cell activities, such as cell diffusion, migration, proliferation, and differentiation. *In vivo*, cells are surrounded by extracellular matrix (ECM), whose physical and biochemical properties and micromorphology may affect and regulate the function and behavior of cells, causing cell reactions. Cell adhesion is also the basis of communication between cells and the external environment and plays an important role in tissue development. Therefore, the significance of studying cell adhesion *in vitro* has become increasingly prominent. For instance, in the field of tissue engineering and regenerative medicine, researchers have used artificial surfaces of different materials to simulate the properties of natural ECM, aiming to regulate the behavior of cell adhesion. Understanding the factors that affect cell behavior and how to control cell behavior, including cell adhesion, orientation, migration, and differentiation on artificial surfaces, is essential for materials and life sciences, such as advanced biomedical engineering and tissue engineering. This article reviews various factors affecting cell adhesion as well as the methods and materials often used in investigating cell adhesion.

Keywords: surface modification, cell adhesion, cell behaviors

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1 Introduction

Cell adhesion is critical in life systems, ranging from organisms to individual cells. It plays an important role in cell communication, cell regulation, organ formation, and tissue maintenance [1–5]. As a complex dynamic process, cell adhesion consists of adsorption of proteins to the surface and the expression of specific peptide sequences. *In vivo*, cells are surrounded by extracellular matrix (ECM). ECM is a three-dimensional (3D) network structure composed of proteoglycans, glycosaminoglycans, adhesion proteins, and fibrin. It provides a wide range of biochemical and mechanical signals for cells and affects a variety of cell behaviors [6–8]. Cells adhere to specific surfaces through integrins, and those that do not adhere usually die. ECM contains proteins recognized by integrin and other cellular receptors (such as arginine-glycine-aspartic acid [RGD] ligands, fibrinogen, vitamin c protein, collagen, and fibronectin [FN]). These ligands regulate cell physiological processes, including adhesion, migration, growth, secretion, gene expression, and apoptosis, which are triggered by ECM [9,10]. Cell adhesion is also associated with a range of pathological diseases [11], such as arthritis, cancer, osteoporosis, and atherosclerosis. The adhesion of cancer cells is usually lower than that of normal cells, resulting in the destruction of tissue structure [12,13]. This morphological feature is generally considered a sign of malignant tumors. Research on cell adhesion *in vitro* has been widely concerned in the fields of cell biology, biomedicine, and tissue engineering [14,15], covering biomaterials with implantable sensors, artificial bone and tooth replacement, skin regeneration, organ transplantation, and so on. Exploring how to control the behavior of cells on artificial surfaces is the key to many biomedical and biotechnological applications. In recent years, researchers have devoted themselves to creating structures close to natural ECM to regulate the gene expression of cells *in vitro*, thus regulating cell adhesion, activity, proliferation, and differentiation. Surfaces that control cell adhesion are also arousing more and more interest, and various materials and surfaces have been prepared to mimic natural ECM [16].

Although the aforementioned methods have been summarized by other researchers, there is an urgent need for a comprehensive and systematic review of engineering-facilitated surface-modified cell adhesion techniques. On that account, this review focuses on recent advances in surface-modified techniques related to cell adhesion. In Section 2, we present a series of factors influencing surface properties related to cell adhesion. Section 3 is about surface-modified methods are demonstrated in detail, followed Section 4 which discusses about how they work and how they are used in real-world situations. Finally, in Section 5, we summarize the current challenges and future directions of surface-modified methods in relation to cell adhesion.

Figure 1: Cell morphology of bone marrow formed along grooves and influenced by surface roughness (reproduced from ref. [27]).

2 Factors influencing surface properties related to cell adhesion

2.1 Effect of substrate surface topography on cells

Substrate surface topography has been proved to affect cell adhesion [17–19]. Biological tissues in the body have a variety of surface morphological features such as fibers, pores, and pits. Various kinds of micromorphological features have a specific effect on cell behavior, which we call "contact guidance" [20,21]. Related studies have shown that micromorphology mainly affects the morphology of whole cells, while nano-morphology mainly regulates the subcellular sensing mechanism [22].

2.1.1 Roughness of substrate surfaces

Surface roughness is an important factor affecting cell adhesion behavior [23–25]. Some early studies have found that surface roughness may affect cell adhesion behavior regardless of the cell type and matrix materials (Figure 1). Depending on the irregularities of the material surface, surface roughness can be divided into several different grades: macroscopic roughness, microscopic roughness, submicron surface roughness, and nanometer roughness. Different surface roughness has different effects on the cells. Macroscopic roughness has little effect on cell adhesion behavior, because cells have enough space to spread and grow between macroscopic irregularities. Surface roughness on micron and submicron scales has dual effects on cell adhesion and growth but in a positive way. For example, Zhao et al. found that the number of MG63 cells on titanium discs with submicron surface roughness is lower than that on flat nanostructures [26]. On the other hand, nano-roughness is considered to be the closest to natural tissue morphology, and it is an ideal factor that has a positive effect on cell adhesion, growth, and maturation. For example, in human venous endothelial cells, increasing the roughness of the biomaterial surface at the nanometer scale can enhance cell adhesion and growth on the rough surface.

2.1.2 Micropores on substrate surface

On the substrate of cell growth, the micropore morphology on the surface is another crucial factor affecting cell adhesion, migration, proliferation, and differentiation. Pore size is the main factor affecting cell adhesion [28–30]. Previous studies have shown that nanoscale pores are prone to the formation of collagen fibers and ECM (Figure 2). Larger pores affect cell seeding, distribution, migration, and further neovascularization *in vivo*. Murphy et al. studied the effect of the surface pore size of polycarbonate on cell adhesion and differentiation [31]. The adhesion of MG63 human osteoblasts to a membrane 0.2–8 µm in aperture was observed. Studies have shown that MG63 cells adhere better on the surface of the membrane with pores 0.2–1 µm in size; while on the membrane with larger micropores (3.0–8.0 µm), the cells are spherical with a small amount of filling and foot. In addition, the cells grown on the membrane with pores 5.0–8.0 µm in size have a higher degree of differentiation and reach the highest degree of differentiation on pores 8 µm in size. Hatano et al. found that, compared with a

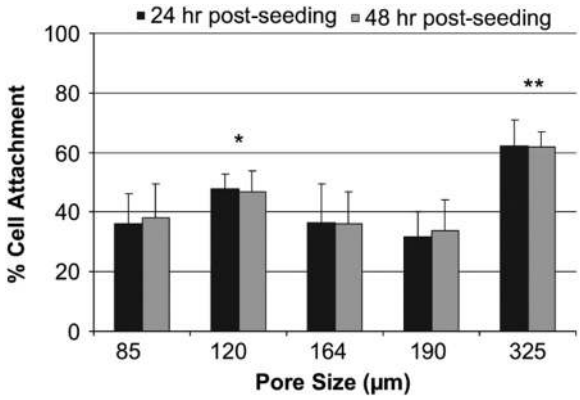

Figure 2: The relationship is shown between mean pore size and cell attachment. Cells cultured on scaffold with pore size of 325 μm shows the highest percentage of cell adhesion (reproduced from ref. [31]).

smooth surface, osteoblasts attached and proliferated more effectively on a rough surface (0.81 μm in aperture) [32]. Various experiments have shown that nanopores on the substrate can affect cell adhesion and activity [33]. However, the size of nanopores should be within the appropriate range. For example, cells on a smooth surface will form clamps at the edges, which obstruct the spread of nutrients, hinder the removal of cell waste, and damage the functions of these cells. Pores with a large size will reduce the adhesion of cells.

2.2 Effect of substrate surface physical properties on cells

2.2.1 Mechanical properties of the substrate

In vivo, most tissue cells, such as soft brain tissue and hard bone tissue cells, adhere to fiber ECM with different hardness and elasticity [34]. The stiffness of ECM *in vivo* ranges from about 0.1 kPa (brain tissue) to about 100 GPa (bone tissue) [35]. The composition of collagen and elastin in ECM determines the stiffness and elasticity of fiber ECM. Living cells generally perceive the mechanical properties of ECM by applying forces and detecting the resulting gaps and respond to ECM by regulating local adhesion structure, cytoskeleton tissues, and the overall state. The stiffness of ECM affects cell activities ranging from gene transcription, through cytoskeletal remodeling, to intercellular interactions (Figure 3). Engler et al. prepared polyacrylamide (PA) gels with different mechanical properties and studied the relationship between the spread area of smooth muscle cells and the elastic modulus of matrix [36]. The results showed that the elastic modulus of PA gel matrix had a significant effect on cell expansion and adhesion.

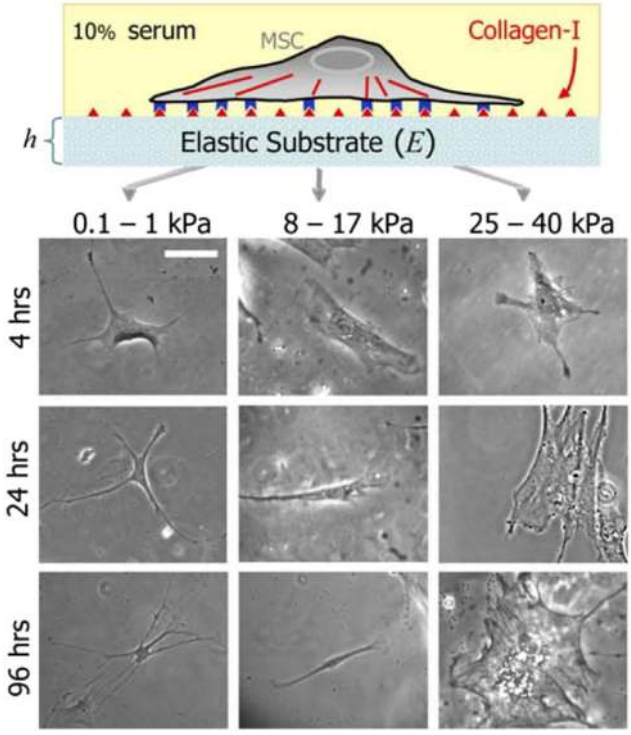

Figure 3: Cell adhesion and morphology were controlled by collagen-I and stiffness of substrate (reproduced from ref. [36]).

Cell adhesion usually increases with matrix hardness. For example, when mesencgymal stem cells (MSCs) adhere to type I collagen-modified polyacrylamide (PAAM) gels, adhesion is marked with parslin. The study also showed that NIH3T3 fibroblasts were more dispersed and adhered better on the harder collagenous type I-coated PAAM gel (7.69 kPa), and that the cell survival rate after centrifugation was >80%, while the softer gel (2.68 kPa) had a cell survival rate of about 30% only.

In addition, the mechanical properties of the substrate surface have extremely important effects on cell structure and protein expression [37,38]. Some studies have found that the essential condition for the formation of fibroblast actin stress fibers is that elastic modulus is greater than 2,000 Pa. On the contrary, neutrophils seem to be insensitive to changes in stiffness in a large range. This indicates that either the mechanical sensing uses the internal stiffness of the cell as the standard or the signal of cadherin in cell-to-cell contact takes precedence over the signal of the cell–matrix adhesion complex.

2.2.2 Wettability of substrate surfaces

The wettability (hydrophobicity and hydrophilicity) of cell adhesion surfaces can affect surface protein adsorption

and cell adhesion [39,40]. According to previous studies, cells are more likely to adhere to hydrophilic surfaces. For example, Wei et al. found that the adhesion of osteoblasts decreased when the contact angle increased from 0° to 106° [41]. When the contact angle is between 60° and 80°, the adhesion of fibroblasts is the highest. The wettability of a surface is greatly affected by the surface functional groups, the surface roughness of the material, and so on. On the other hand, the super-hydrophilic matrix surface (with a contact angle of less than 5°) and the super-hydrophobic surface (with a contact angle of more than 150°) are unconducive to cell attachment and growth. This may be due to the fact that the wettability of the matrix surface affects the type, conformation, and binding strength of the proteins adsorbed from the culture medium, which further influences cell attachment. If the surface is too hydrophobic, the proteins in the ECM (such as FN, vitrein, collagen, and laminin) are adsorbed in a denatured state, and their geometry becomes unsuitable for cell binding. A highly hydrophilic surface inhibits the binding of these cell adhesion mediators, thus hindering cell adhesion behavior. Interestingly, surface wettability exerts different effects on the adhesion of different types of cells. For example, Wei et al. brushed grafted polyhexamethyldisiloxane (PHMDSO) on the substrate to prepare PHMDSO with different surface wettability (from hydrophobic to super-hydrophilic) [41]. It was found that, with the enhancement of the hydrophilicity of the polymer surface, more fibroblasts could adhere and spread widely on the surface. Using some hydrophilic materials, Filová et al. found that the absolute amount of ECM molecules mediating cell adhesion was smaller than that of the more hydrophobic surfaces formed by octadiene [42]. However, more cells adhered to more hydrophilic materials. This may suggest that the number of ECM molecules affecting the adhesion process is not the key factor , but the spatial conformation of the adsorption molecules mediates cell adhesion.

2.3 Cells adhesion influenced by chemical properties of substrate surfaces

Previous studies have concluded that the chemical properties of the material surface can change cell adhesion behavior [43–45]. ECM *in vivo* provides a variety of chemical information to cells to guide their behavior. The chemical properties that affect cell adhesion mainly include surface energy, surface charge, and bioactive factors.

2.3.1 Surface energy of substrate

Surface energy is regarded as a measure of the unsaturated bond energy caused by the hanging bond of the surface material [46,47]. It can affect the activity of cells. For example, when the polymer surface comes into contact with a biological fluid, serum protein adsorption and cell adhesion depend on the energy of the polymer surface. A large number of early studies have reported the relationship between cell adhesion and matrix surface free energy. The surface with high free energy can improve cell adhesion and spreading, while that with low free energy can inhibit cell behavior [48,49]. Surface energy can be changed by plasma treatment. For instance, Ozcan et al. demonstrated that the surface free energy of poly(methyl methacrylate) (PMMA) membrane was enhanced by oxygen plasma, and that fibroblasts and serum proteins were cultured on PMMA membrane [50]. Syromotina et al. modified poly(3-hydroxybutyrate) (P3HB) films first by oxygen plasma and then by ammonia plasma and found that, after plasma treatment, the surface free energy of the modified P3HB film significantly increased (oxygen-modified P3HB = 53.5 ± 0.9 mN/m and ammonia-modified P3HB = 57.4 ± 0.9 mN/m) [51]. However, compared with the untreated and the oxygen-plasma-treated P3HB, the fibroblasts on the ammonia plasma-treated P3HB membrane adhered and proliferated well. It can be found that once a polymer surface is treated by different plasma, cell adhesion is stronger on the surface with a larger polar component of surface free energy. In addition, the surface can affect the number of proteins adsorbed on it through controlling its wettability. Jordi Comelles et al. studied the behavior of cells on polymer matrix and glass (Figure 4) [52]. A linear trend was observed and it was found that serum proteins were preferentially adsorbed on the surface with low energy.

2.3.2 Surface charge of substrates

The charge property of cell adhesion surfaces is also an important factor affecting cell adhesion [53,54]. A large number of studies have found that cells adhere to positively charged surfaces rather than negatively charged ones. Surface charge can change cell behavior through the chemical functional groups of polymer materials. Lee et al. prepared polyethylene (PE) surfaces (–COOH, $-CH_2OH$, $-CONH_2$ and $-CH_2NH_2$ groups) with different functional groups to study their effects on cell behavior [55]. The results showed that the adhesion of hamster ovary cells to the functional group

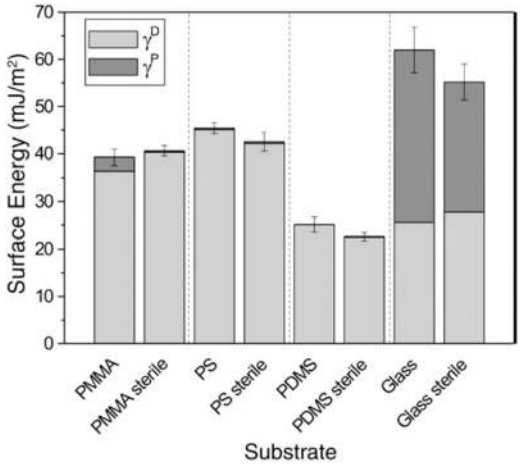

Figure 4: Total surface energy of different materials (reproduced from ref. [52]).

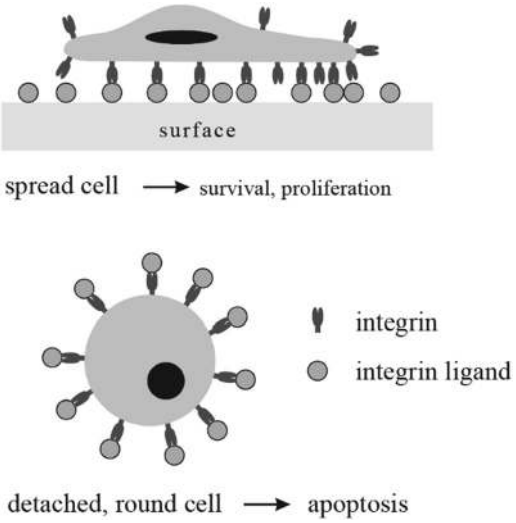

Figure 5: Surface modified by immobilized ligands which act as agonists of the ECM. Cells cannot adhere to the substrate with nonimmobilized ligands leading to apoptosis (reproduced from ref. [62]).

grafted surface was higher than that in the control surface. The adhesion, growth, and expansion rate of cells were optimal on polar surfaces and positively charged surfaces (amino grafted PE). On the other hand, surface charge can regulate protein adsorption, directly bind to integrin, and produce specificity, thus controlling cell adhesion. Thevenot et al. mentioned that the incorporation of negative charge might promote the adsorption of proteins, thus promoting cell adhesion and reaction [56]. Abarrategi et al. reported that the surface with different charge functional groups (–CH_3, –OH, –COOH, and –NH_2 groups) regulated the adsorption of FN and the direct binding of integrins and found that the specific trend of adhesion of MC3T3 osteoblasts to the surface of FN coating would be OH > COOH=NH_2 > CH_3 [57]. This cellular behavior may be modulated by the more favorable geometric conformation of vitrein and FN on positively charged surfaces. Interestingly, the negatively charged –COOH group has a dual effect on cell material adhesion. The –COOH is a high-polarity group that can adjust the wettability of the material surface to a level suitable for cell adhesion. However, Bet et al. reported that the introduction of negatively charged carboxyl into the adhesion matrix reduced the adhesion of human erythroleukemia cells to type I collagen matrix *in vitro* [58].

2.3.3 Bioactive molecules on the substrate surface

Biomolecules in body fluids, such as vitronectin, fibrinogen, and FN, are immediately adsorbed on material surface to form a protein layer, which is conducive to cell adhesion [59–61]. Cells connect with these ECM proteins through a specific RGD sequence, which is a tripeptide component of Arg–Gly–Asp. Many biomolecules are used to modify material surface to improve adhesion. For example, planting adhesion-active proteins on scaffolds, such as type I collagen, vitreous laminin, FN, and laminin, can significantly promote cell adhesion. Liu found that the surface modification of type I collagen scaffold could not only promote cell adhesion and proliferation but also significantly facilitate the differentiation of stem cells into osteoblasts (Figure 5). However, surface protein modification also has many disadvantages, such as poor protein degradability and difficulty in extraction. In this context, the use of RGD sequence as a modification material has emerged as an alternative. RGD is a site that can be specifically recognized by cells, and the surface modified by RGD can significantly increase cell adhesion. In addition, immobilization of some growth factors, such as bone morphogenetic protein (BMP) (an acidic polypeptide) and basic fibroblast growth factor, on material surface can also play an important role in regulating osteoblast adhesion.

3 Surface-modified techniques for cell adhesion

In order to further understand the mechanism of cell-surface interactions, various methods have been developed to change cell adhesion surfaces, as shown in Table 1.

Table 1: Methods of creating cell adhesion surfaces

Techniques	Production methods	Advantages	Disadvantages
SAMs	Ordered molecular structures formed by the adsorption of an active substance on a solid surface in a dilute solution	Higher order and orientation	Need a specific and specially treated solid surface
Polymer brush	Macromolecular structure composed of polymer chains with one end tightly grafted on a curved surface or plane	Significantly improved the performance of the substrate, showing different properties as environmental conditions change	Complex process and easy to lose material's activity
Layer-by-layer assembly	Alternate deposition of interacting species on a substrate with an intervening rinsing step following each deposition	Controlled layered structures, simple, inexpensive, and rapid procedures	Depend on centrifugation, difficult-to-scale, low-throughput assembly
Photolithography	Using various sources of energy, such as ultraviolet light, electron beam, and laser, to create patterns on substrate surface	High precision	Complex operation, high-cost equipment
Electrospun fibers	Under high-voltage bias, the polymer solution or melt is attracted to the material substrate by static electricity	Porous fiber structure, high precision of orientation control	High-pressure conditions, easy to mechanical deformation
Spin coating	Four basic steps: deposition, rotation, rotation, and evaporation	No coupling process variables, high-cost performance, energy saving, and little pollution	Low material utilization rate, constant waste
3D bio-printing	The 2D patterned polymer layer is surface modified with computer-aided imaging technology, while the 3D patterned polymer layer is assembled from the bottom up and printed with liquid biological materials	High precision and speed	Shear force, low-cell survival rate

3.1 Self-assembled monolayers (SAMs) and polymer brush

SAMs were first reported by Zisman in 1946 [63,64]. They are spontaneous molecular assemblies formed by the adsorption of a solution or gas phase to a solid or liquid surface [65]. When molecules in a solution or gas phase are adsorbed and spontaneously organized into a single layer on the surface, a self-assembled monolayer is formed. Polyethylene glycol, protein, and deoxyribonucleic acid (DNA) samples are commonly used surface-mount polymer samples (Figure 6). Polymer brushes can improve the mechanical and chemical properties of the surface and introduce functional groups into the surface. The free movement of the end of the polymer chain in the solution can endow the substrate surface with different micromorphologies and mechanical properties, thus affecting the behavior of cells. An important application of polymer brushes is to make responsive switchable surfaces. For example, thermosensitive polyisopropylacrylamide (PNIPAM) brushes are cellular adhesives above lower critical solution temperature (LCST) and cellular inert below LCST [66,67]. However, these two surface modification methods have some shortcomings. For instance, self-assembled monolayers require the presence of mercaptan on the substrate (only for precious metals or silanes) in order to deposit them. The manufacture of polymer brushes requires complex preparations.

3.2 Layer-by-layer (LbL) assembly

The LbL deposition method was first proposed by Moehwald, Decher, and Lvov 20 years ago [69]. This method uses self-organized polyelectrolytes alternately adsorbed on the material surface to form polyelectrolyte multilayer (PEM) films [70]. One outstanding advantage of PEM films is that they can maintain the biological activity of biomolecules and may provide a large number of biomolecules. Their growth and internal structure can be adjusted by controlling the process parameters. PEM films can be prepared in aqueous environment under mild conditions, which is a great advantage in the use of biopolymers and bioactive molecules. Therefore, the LbL components are widely used to regulate these parameters, with the purpose of controlling the adhesion behavior of different cells. Croll et al. put forward antiadhesive LbL films

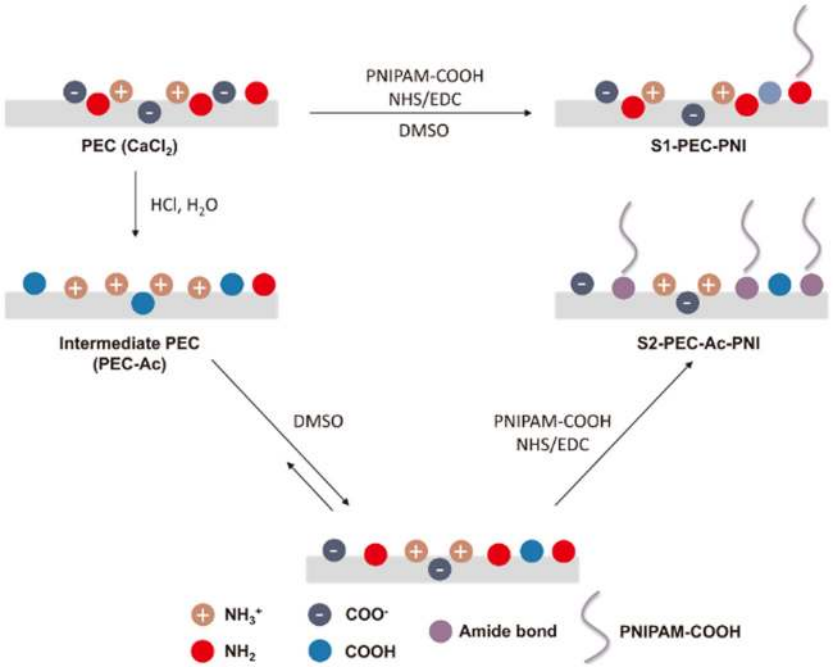

Figure 6: The PNIPAM-COOH was grafted on chitosan's amines for cell adhesion via an NHS/EDC coupling (reproduced from ref. [68]).

composed of high-molecular-weight hyaluronic acid (HA) and chitosan (CHI). Upon covalent grafting of collagen (COL) IV on their top, the films switched to cytophilic to NIH-3T3 fibroblasts [71]. Kinnane et al. proposed an alternative strategy based on "click" chemistry [72]. First, they employed poly(ethylene glycol) (PEG)-acrylate polymers with alkyne or azide groups and constructed low-fouling multilayers through the click-LbL technique. After that, they "clicked" an RGD peptide onto the low-fouling films to promote the adhesion and proliferation of the epithelial cells. The coupled effects of grafting RGD peptide on low-fouling, biopolymer-based CHI/HA films were also studied by Chua et al. and shown to promote the adhesion and proliferation of osteoblasts [73]. The assembly technologies used to assemble such films form five distinct categories: (i) immersive, (ii) spin, (iii) spray, (iv) electromagnetic, and (v) fluidic assembly [74]. These assembly technologies affect both the process properties and the resultant material properties (Figure 7).

3.3 Lithographic surface modification techniques

Photolithography (PL) is the most mature technology used to manufacture precise and complex pattern surfaces with resolution up to hundreds of nanometers [76,77]. In general, to prepare a surface pattern using PL is to transfer the pattern on the mask to a photosensitive photoresist on the substrate and, after chemical treatment, leave the exposure pattern on the surface below the photoresist, or deposit new materials (such as proteins and polymers) on the surface with the desired pattern. The radiation energy types commonly used in PL are white light, ultraviolet light, X-ray, electrons, ions, and so on [78,79]. Ultraviolet light is the most widely used, but the wavelength used during irradiation limits its resolution. Therefore, in a research environment with more flexible requirements and patterns, electron beam lithography (EBL) is the most common choice.

EBL is a maskless lithography technology that scans a beam of electrons through a surface covered by a resist film sensitive to these electrons, thus depositing energy on the resist film in the desired manner [80]. One of the main differences between EBL and PL is that the former uses a much shorter focused electron beam as energy source and is exposed to photoresist, so EBL has a stronger anti-interference ability. Other main features of EBL include ultra-high resolution (below 5 nm) nano-features, a wide range of applications (applicable to a variety of materials), and availability for preparing a variety of patterns (Figure 8). Its disadvantages are low speed, complex operation, costly EBL equipment, and frequent maintenance.

Soft lithography (SL) is a micro-nano processing technology based on elastic seal printing [82,83]. Elastic seals with patterned relief structures are used to generate

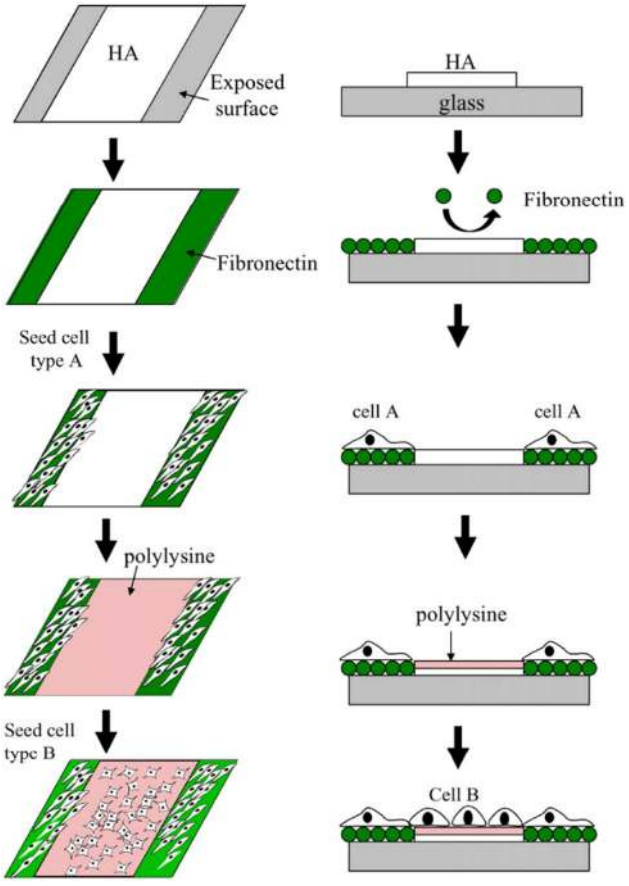

Figure 7: Schematic diagram of HA-PLL deposition on substrate. The HA surface was firstly deposited on substrate to prevent cell adhesion. Then the PLL was applied to the surface and converted HA surface to cell and protein adhesive(reproduced from ref. [75]).

patterns and structures with feature sizes from 30 nm to 100 μm. The process of preparing polydimethylsiloxane (PDMS) seals usually involves pouring PDMS prepolymers onto patterned molds (Figure 9), which are usually made by PL or EBL. After curing, they are stripped from the molds. The surface of these protruding nano- or micro-embossed seals can carry the printed material, which is then transferred to the substrate surface. Therefore, SL is a simple, high-throughput, and inexpensive technology. It provides a convenient, effective, and low-cost method for the formation and fabrication of micro-nanostructures and is necessary in the large-scale production of patterned polymer surfaces. However, there are still many problems with SL, such as how to use elastic materials to reproduce impression images with high precision, and how to control the deformation and distortion of elastic materials.

Scanning probe microscopy-based lithography (SPML) is a lithography technology based on a scanning probe microscope [84]. Because of its ability to control a large number of processes by using sharp probes in contact with the nanometer area of the sample surface, the lithography technology has been widely used. For instance, atomic force microscopy (AFM), pen-immersion nano-lithography, polymer pen-type lithography (PPL), and pen-immersion nano-displacement lithography can be used to produce polymer patterns with nano-resolution and high registration. Scanning probe lithography (SPL) uses scanning tips to generate patterns on ultrahigh-resolution surfaces. One feature shared by all scanning probe-based technologies is the use of sharp scanning probes to produce local modifications on the surface. It is well-known that AFM is embraced by the most current SPL methods. Compared with other technologies such as EBL, SPL is advantageous in that it is a single-step process with resolutions up to 10 nm. Most SPL writing processes are “direct writes” in nature, and no additional development steps are required to produce the desired patterns. SPML combines nanoscale feature size, low technical requirements, and the ability to handle soft matter from small organic molecules to proteins and polymers. In addition, the scanning probe microscope can detect surface characteristics of atomic resolution. Compared with the beam-based method, the imaging and patterning in SPL are orthogonal, that is, the imaging process does not affect the writing structure, nor does it involve partial writing operations.

Extreme ultraviolet (EUV) irradiation is another lithography technology [85]. The photons of EUV are high-energy and low-wavelength photons, ranging from 10 to 124 eV, corresponding to 124 to 10 nm in wavelength. Photons in this energy range can destroy multiple bonds of polymer materials and introduce microscopic and nanoscale structures by direct lithography. Because the EUV photons have limited penetration depth, they can be used for surface modification of polymers without changing the volume properties of the treated materials and are often used to optimize the roughness of polymer materials. For example, polytetrafluoroethylene (PTFE), a hydrophobic polymer material, is widely used in tissue regeneration. This material has inherent low surface energy, which makes it chemically inert to a large extent and very suitable for passive structural applications. However, its disadvantage is also obvious, that is, the surface is incompatible with cell adhesion and easily affected by pathological conditions, such as peeling off the surface of vascular grafts. In order to modify the highly stable surface of PTFE, the EUV irradiation in the presence of nitrogen is used to increase surface roughness. The average surface roughness of a polymer treated with EUV is more than 4 times higher than that of an untreated one, which enhances the hydrophobicity of

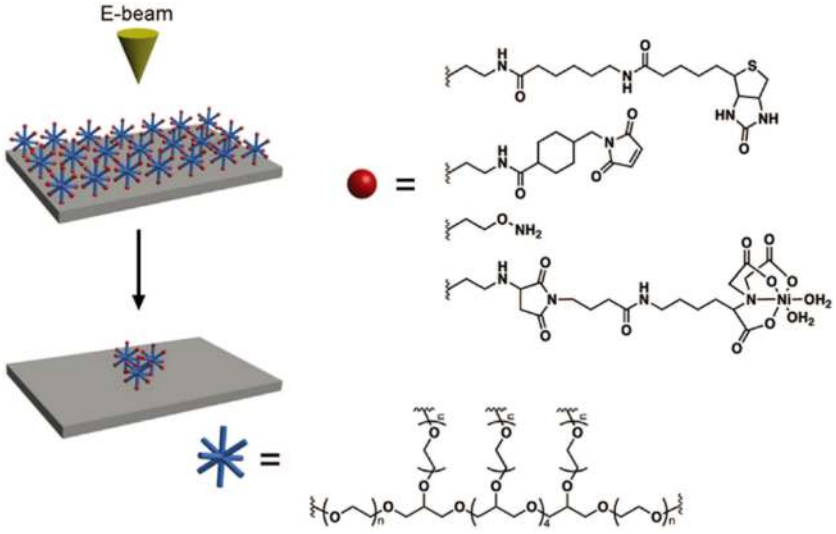

Figure 8: End-functionalized eight-arm PEG polymers were cross-linked in specific patterns using electron beam lithography for protein patterning (reproduced from ref. [81]).

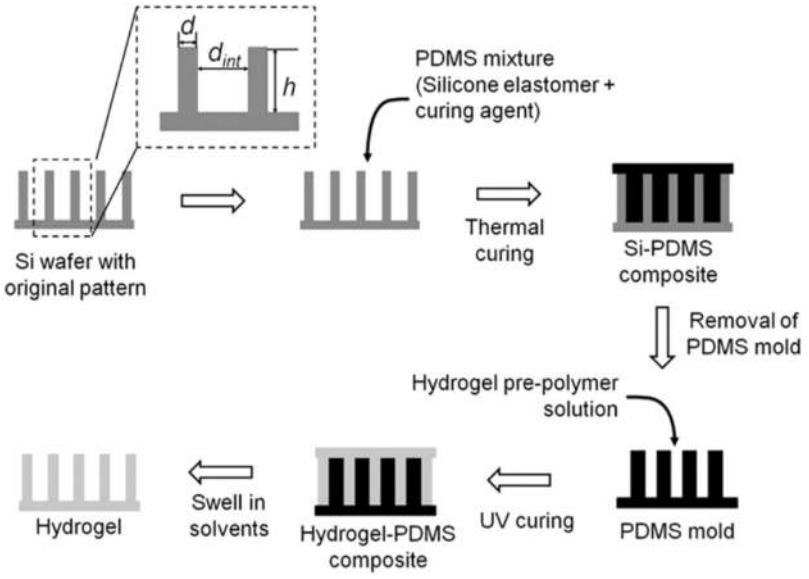

Figure 9: Soft lithography uses elastomeric stamp to replace hard stamp in traditional lithography to fabricate arrays of microstructures onto hydrogel surfaces (reproduced from ref. [82]).

PTFE and significantly improves the adhesion and morphology of L929 fibroblasts.

In practical applications, especially in the fields of biomedicine and tissue engineering, surface modification is an indispensable process to improve the compatibility or biocompatibility of biomaterials. Femtosecond laser processing is a common surface pattern and construction technology [86,87]. For example, the surface is modified through producing grooves and micropores on the polylactic acid micropore structure prepared by 3D fused wire, so as to enhance the functionalization ability of the implant material. The direct laser writing technique with fs laser pulses (temporal pulse width $r = 300$ fs, $\lambda = 1{,}030$ nm, repetition rate $v = 25$ kHz) permits the creation of features with great reproducibility and does not require a clean room. The laser-induced microfeatures improve the surface roughness of the PLA construct and enhance cell adhesion in relation to cell types used for cell growth [88].

3.4 Electrospun fibers

Electrospinning is driven by the free charge on the surface or inside of the polymer liquid [89,90]. The polymer solution or melt is electrostatically drawn to the material substrate under high-voltage bias. Electrospinning can produce fibers with 10 nm to several microns on the substrate. Chen et al. studied the effect of the shape and size of electrospun polycaprolactone (PCL) fibers on the adhesion properties of fibroblasts [91]. The results showed that the cell adhesive rate of scaffolds with a higher specific surface area increased significantly and that nanofibers could promote cell adhesion better than microfibers. One outstanding advantage of electrospinning is that it can produce porous fabrics which can enhance the adsorption of proteins and provide a larger specific surface area for the application of more binding sites on cell membrane receptors. Therefore, electrospinning is often used to create 3D scaffolds with high porosity and spatial connectivity. Another advantage is that it can achieve precise alignment control to produce oriented nanofiber networks through adjusting the electric field and the substrate direction [92]. However, the shape of densely deposited low-porosity fibers hinders the effective penetration of cells. To overcome these limitations, salting out and low-temperature electrospinning are developed to increase the pore size [93]. LbL electrospinning is utilized to form a thick multilayer fiber platform composed of nanofibers and microfibers (Figure 10). However, these approaches still lead to mechanical deformation during transport. Therefore, an ideal engineering fiber platform should have high porosity to provide better nutrient diffusion and inward cell growth as well as reasonable complex structures to mimic natural tissues and maintain mechanical properties.

3.5 Spin coating

Spin coating is a simple and high-throughput technique for depositing large-area polymer films on the substrate surface [94]. Film thickness can be controlled with high precision. Currently spin coating is the main technology for the preparation of micron and nanometer organic photosensitive films (Figure 11). Spin coating consists of four basic steps: deposition, rotation, rotation, and evaporation. Seen from maturity, spin coating has many advantages in coating operation, and its biggest advantage is that there are no coupled process variables. By changing the rotation speed or switching to the photoresist with different viscosity, it is easy to change film thickness and obtain a film with uniform thickness. However, the most prominent disadvantages of spin coating are low material efficiency and insufficient utilization.

3.6 3D bio-printing

3D scaffold is a common tool for cell culture [96]. The common methods of making scaffolds include molding, pore-forming agent leaching, gas foaming, fiber bonding, freeze-drying, solvent casting, and so on. However, the preparation of 3D polymer scaffolds by these traditional methods usually results in randomly distributed geometry that seriously limits cell adhesion and diffusion. Recently, some new methods for fabricating scaffolds have been put forward, such as selective laser sintering, melt deposition molding, and stereoscopic lithography technology. The 3D bio-printing surface modification of 2D patterned polymer layers is conducted by micro-/

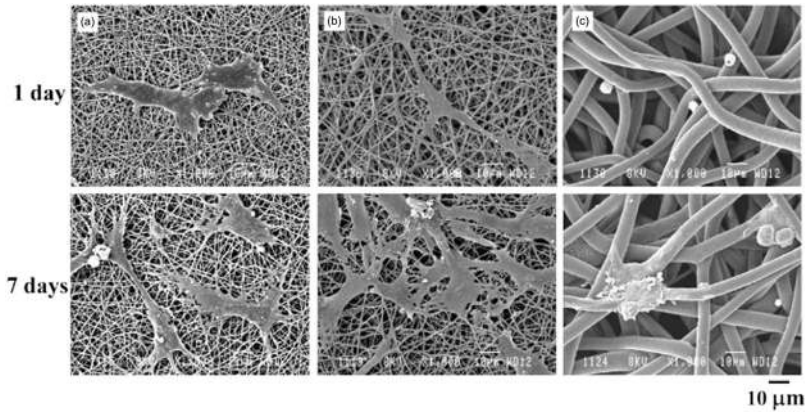

Figure 10: Soft lithography uses elastomeric stamp to replace hard stamp in traditional lithography to fabricate arrays of microstructures onto hydrogel surfaces (reproduced from ref. [82]).

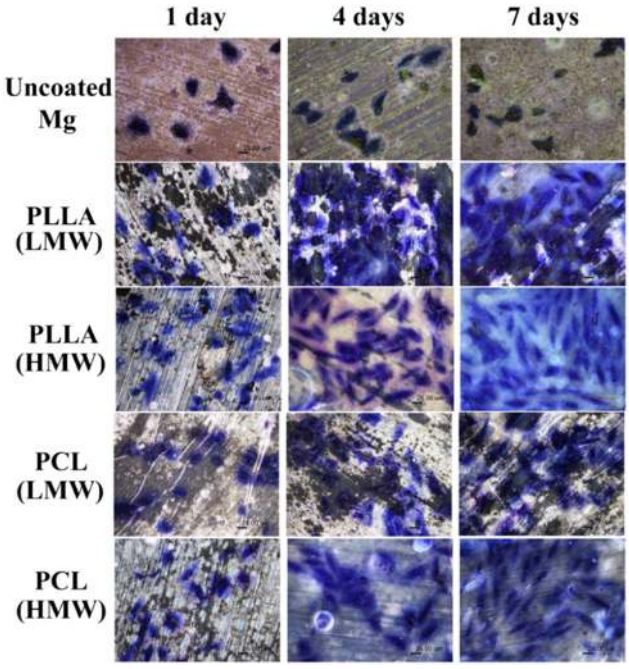

Figure 11: Biodegradable polymer film was coated on Mg samples using magnesium by spin coating, and cells were cultured with these different samples for 1, 4 and 7 days (reproduced from ref. [95]).

nano-manufacturing methods to create 3D structures. Another method is to use the bottom-up assembly of 2D patterned polymer layers. The 3D structures are fabricated by assembling 2D patterned polymer layers from the bottom up. It is a relatively advanced manufacturing method that is widely used for preparing 3D tissue structures for medical and tissue engineering. It creates cell patterns by accurately printing live cells, biochemicals, and biomaterials LbL. One outstanding advantage of this method is that the printed cells can remain alive. The technology also has drawbacks, i.e., the shear force and the impact force of droplets during the spraying process affect the activity of the printed cell fluid. At the same time, the printed cells or molecules must remain liquid before printing and must be cured immediately after printing to maintain a viscoelastic state. This liquid-to-solid transition, which must be prevented from damaging cells, bioactive factors, or other particles, also poses considerable challenges to the development of 3D printing.

4 Materials for cell adhesion

Cells respond differently to different materials. On the surface of cell culture, the biocompatibility of materials is the most important property. This chapter introduces several common biomaterials, as shown in Table 2.

4.1 Silicon

Silicon has special semiconductor properties and is often used as a material for implanting electronic devices *in vivo*. Silicon has been shown to increase the biological activity of various materials without affecting their mechanical properties or inducing cytotoxicity. Up to now, a series of manufacturing technologies have been applied to the surface characteristics of silicon on the micro- and nanoscale [97]. It is found that surface-modified silicon can significantly improve cell adhesion. However, the biocompatibility of silicon *in vivo* is very poor, and the biological stability is also insufficient, so silicon is often covered with surface coating materials in application to improve biocompatibility. Porous silicon is a nontoxic and biodegradable biomaterial with great application potential. Surface modification can not only control the degradation rate but also promote cell adhesion. Low et al. modified the surface of porous silicon by ozone oxidation, silanization, collagen, and serum coating [98]. The adhesion of rat pheochromocytoma (PC12) and human lens epithelial cells to these surfaces was studied. It was found that the two cell lines had more adhesion to collagen coating and aminosilanized porous silicon, while cell adhesion on the surface of ozonation and polyethylene glycol silanization was poor. In addition, silicon substrate is also commonly used as a model scaffold to study the effect of 3D micro-/nano-morphology and surface energy on cell adhesion and growth. Ranella et al. studied the adhesion and activity of fibroblasts on highly rough 3D silicon (Si) surfaces with gradient roughness ratio and wettability (Figure 12) [99]. The results showed that cell adhesion had nothing to do with surface chemical composition or wettability and was better on the silicon surface with less roughness, indicating that the adhesion of fibroblasts was not monotonically dependent on surface energy.

4.2 Metals

A large number of metal materials are used in tissue engineering and regenerative medicine, such as human titanium alloy dental implant, titanium alloy bone transplantation, and stainless steel used in heart scaffold [100–102]. Therefore, studying cell adhesion on metal

Table 2: Several common materials for cell adhesion

Materials	Advantages	Disadvantages	Application
Silicon	Increase biological activity, improve cell adhesion	Poor biocompatibility, unstable	Bone tissue engineering
Metal	High strength, toughness, fatigue resistance, easy processing and forming	Inadequate long-term security and reliability	Hard tissues such as bones and teeth that need to bear higher loads and stents for interventional treatment
Polymer	Biocompatibility, biodegradability, and low toxicity	High production cost and complex synthesis process	Organ repair and transplantation
Nanotubes	Special and excellent performance in mechanics, electricity, magnetism, and optics	Carbon nanotubes are poorly dispersed and difficult to process	Adsorption materials
Bioceramics	Biocompatibility, low toxicity	Low toughness and strength	Dental restoration materials, artificial hips, and other artificial bones, tooth roots, joints, bolts, etc.

surface *in vitro* is of great interest. Because the metal surface has high surface energy, it often enhances cell adhesion. J. Hallab et al. used the jet impact method to determine the adhesion strength of 3t3mc fibroblasts to metal and polymer and identified the colonization of 3t3mc fibroblasts on hs25 (a cobalt-based implant alloy, astmf75), 316l stainless steel, ti-6al-4v (a titanium implant alloy), commercial pure tantalum (ta), PTFE, silicone rubber (SR), and high-density polyethylene [49]. The results showed that the adhesion strength of cells to the metal material was about 5 times higher than that of the tested polymer material. At the same time, many studies have found that the nano-morphology of metal surface also affects cell behavior (Figure 13). For example, titanium surface with random nano-morphology can promote the adhesion of vascular endothelial cells. Therefore, more and more optical techniques, such as laser ablation, are used to change the surface properties of metal materials, thus modifying the adhesion properties of cells on metal surfaces. Many deficiencies in metal materials, including osteolysis, edema, thromboembolism, endothelial overgrowth, infection, tumor, and other adverse reactions, are often caused by fatigue corrosion (such as wear and metal ion dissolution). This points out the direction of metal material improvement in the future.

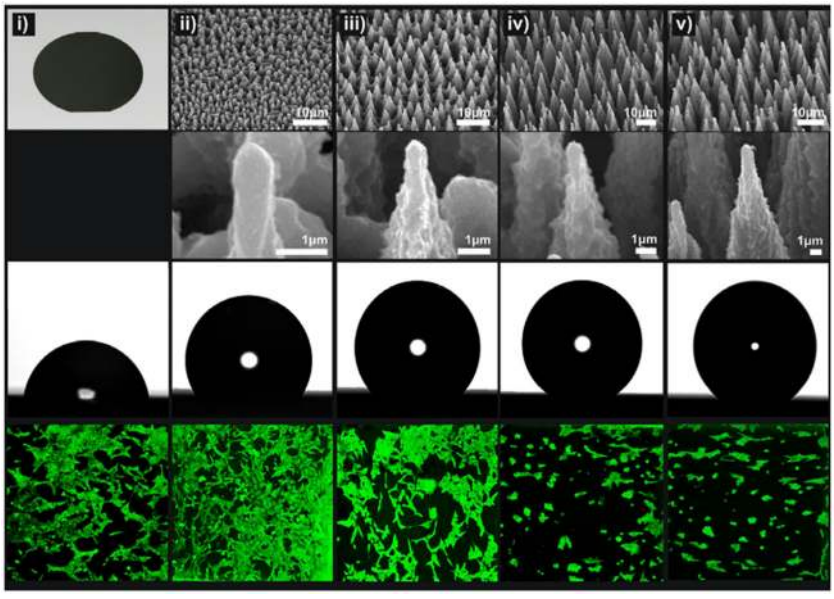

Figure 12: Cell adhesion was regulated by controlling the roughness and wettability of 3D micro/nano silicon structures (reproduced from ref. [99]).

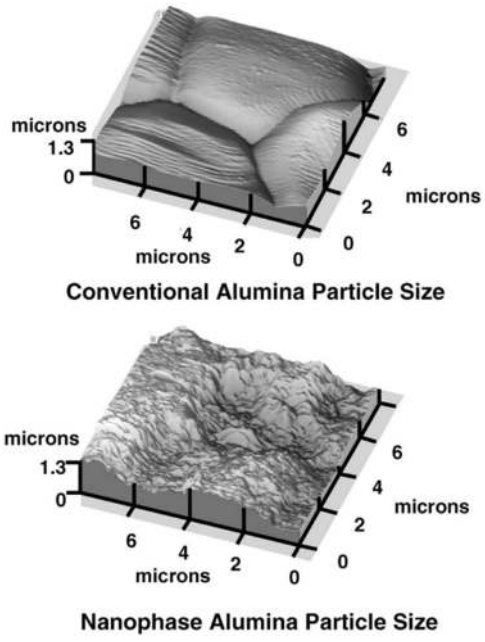

Figure 13: Enhanced adhesion of osteoblasts was observed on nanometer particles of alumina compared to conventional metals (reproduced from ref. [101]).

4.3 Polymer

4.3.1 Polymer gels

Polymer gels are classified into physical gels and chemical gels [103]. Silicon-based elastomer PDMS gel is more common. As a low-cost elastomer material, PDMS gel is characterized by simple preparation process, low cost, and good biocompatibility. Because it can provide a wide range of stiffness values for living cell culture, it is widely used to simulate ECM and in tissue engineering and regenerative medicine. In addition, hydrogel has great application potentials in the 3D cell culture and tissue regeneration medicine. By virtue of its unique similarity to natural ECM in composition and structure as well as its important role in cell proliferation and survival, hydrogel is often the main candidate material for engineering tissue scaffolds.

4.3.2 Polymer thin films

Surface bioactivity (or biocompatibility) is an important property of materials, so it is very important to control the surface properties of biomaterials and to ensure their biocompatibility and bioactivity. Organic films and coatings, especially polymer films, are most commonly used in biomaterials, because they can be combined on the surface of chemical groups or carry out pattern modification to improve biocompatibility and bioactivity [105]. Many studies have shown that surface modification plays an important role in cell and tissue reactions. In addition, the application of thin films is widely regarded as a promising method to change the surface properties of biomaterials. Thin films are widely used in tissue engineering, mainly for their simple processing process, low production cost, and unique ability to control various physical and chemical properties. They are usually used in dental and orthopedic implants, biodegradable scaffolds, and osseointegration of biomimetic materials in the fields of tissue engineering and biomedicine.

4.3.3 3D polymer scaffolds

Cells *in vivo* are surrounded by ECM, a 3D natural structure. For the purpose of maximizing the simulation of the environment around cells *in vivo*, many 3D biological scaffolds have been developed in tissue engineering. These biological scaffolds affect cell behavior in a 3D way, which offers the most suitable culture tool for the real growth environment of cells. Pore size, porosity, shape specificity, binding with natural tissue, degradation according to tissue formation rate, and cost-effectiveness are important factors in the development of scaffolds. The chemical and mechanical properties of 3D polymer scaffolds made of micropores, microfibers, or nanofibers are similar to those of natural tissues. Scaffolds with different softness and hardness have different applications. For instance, stents with higher hardness are often used in bone regeneration; the stents used for bladder, vein, and artery regeneration are mostly soft and elastic materials.

4.3.4 Nanotubes

Nanotubes prepared from various materials have been widely used in tissue engineering. They can improve the mechanical or conductive properties of the substrate or form specific micromorphologies on the surface. Carbon nanotubes are most widely used in all materials [106,107]. Because of their good mechanical, physical, and chemical properties, they present a great application potential in the fields of tissue engineering and regenerative medicine. Lovat et al. functionalized carbon nanotubes, prepared layered carbon nanotubes, directly introduced hippocampal neurons into the surface of carbon nanotubes, and compared them with glass matrix [108]. The results showed that cell adhesion, dendritic elongation, and

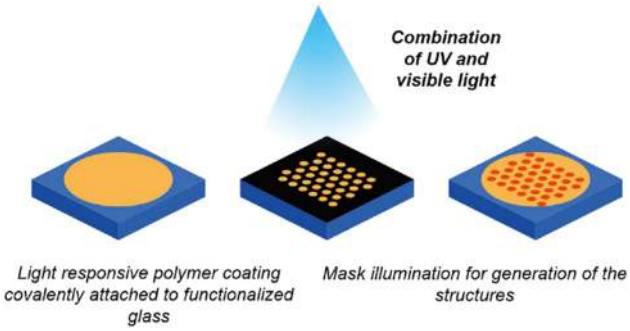

Figure 14: Light-responsive polymer was coated on glass substrate and hierarchically structures were generated by mask illumination (reproduced from ref. [104]).

nerve signal transmission were stronger on the surface of conductive nanostructures than in the control group. Titanium is also frequently used in biomaterials, thanks to its good biocompatibility and common uses of biomedical implants. Oh et al. have shown that the adhesion and differentiation characteristics of human bone marrow mesenchymal stem cells can be controlled by growing titanium nanotubes with different diameters [109]. The behavior of cells on nanotubes was observed by electron microscope. It was found that the change in nanotube size had a significant effect on cell characteristics (Figure 14). Most cells adhered to the 30 nm nanotubes but did not differentiate. Larger diameter nanotubes of 70–100 nm significantly promoted the morphological elongation of cells.

4.3.5 Bioceramics

Calcium phosphate ceramics (CPCs) is a kind of tunable bioactive materials widely used in bone tissue repair and reinforcement [111]. They have the surface properties of supporting osteoblast adhesion/proliferation (i.e., bone conduction) and stimulating new bone formation (i.e., bone induction). Hydroxyapatite, tricalcium phosphate, amorphous calcium phosphate, and biphasic calcium phosphate have inorganic composition and crystal structure similar to those of natural bones, so they are the most commonly used bioceramics in orthopedic surgery. In recent years, with the research and development of tissue engineering, porous CPCs, as a kind of bone tissue engineering cell scaffolds, have been widely used in the repair and replacement of bone defects [112]. At present, many methods of preparing porous ceramics have been developed, such as organic foam impregnation method, foaming method, and adding pore-forming agent (Figure 15). These methods usually need to be formed by high-temperature sintering (>1,100°C), and the ceramics obtained is

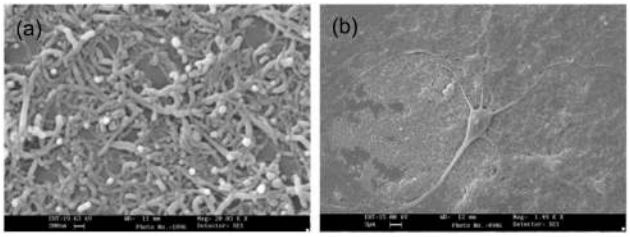

Figure 15: Neonatal hippocampal neuron adhesion (b) and survival were boosted by purified multiwalled carbon nanotubes (MWNTs) deposited on substrate (a) (reproduced from ref. [110]).

relatively dense. This dense ceramic surface will affect the recognition of cells to the material, and even affect the adhesion, spreading, proliferation, differentiation, and functional expression of cells on material surface. *In vitro* studies have shown that the treatment of material surface and the introduction of viscous protein molecules conducive to cell recognition can increase cell adhesion and proliferation. Bioceramic materials also have some shortcomings, such as the inadequate strength and toughness of the materials. However, bioceramics has a broad development prospect because of its biocompatibility and almost nontoxicity.

4.3.6 Dynamic materials

As is well-known, the ECM around the cells in the body is a dynamic 3D structure, and the cells live in the ecological environment [113]. The culture surfaces are used in many *in vitro* experiments only for the purpose of studying cell behavior on a static basis, and the properties of the substrate surface remain unchanged, which is incapable of reflecting the dynamic characteristics of ECM. In this context, studying dynamic surfaces can more accurately restore the real living environment of cells [114]. There are also materials that can achieve the response of the material surface by changing the pH, temperature, light, and other external stimuli. We call this type of material a “stimulus response” or “smart” material. Examples in this regard include temperature-responsive polymers modified with nano-thick PNIPAAm graft layer, photoresponsive polymers modified with nitrobenzyl ester derivatives, UV-mediated rigid modulated hydrogels, and dynamically controllable cell culture surfaces. This provides a way for us to understand how cell functions and fate are affected by continuous changes in cell niche. In addition, a dynamic surface also shows better bionic performance than a static one. In fact, based on their unique advantages, dynamic surfaces are also used in *in vitro* diagnosis, intelligent robot skin, and other intelligent systems. So far, the

dynamic properties of materials are mainly limited to simple physical and chemical properties and unable to completely simulate complex situations in the body. Therefore, the most advanced bionic design standards should be introduced to develop "more intelligent" dynamic surfaces.

5 Conclusion and prospective

Surface modification for cell adhesion and regulating cell behaviors, which could gain a fundamental understanding of how cells respond to these structures, is vital for a broad applications in cells research, drug discovery, and tissue engineering. For instance, PEG diacrylate (PEGDA), a common hydrogel, was widely used for tissue engineering owing to its tunable mechanical properties and biocompatibility. However, the native PEGDA film shows bio-inertness which means that cells cannot adhere to the surface of this film, thereby limiting its applications. In order to solve this problem and fabricate cell-friendly PEGDA film, researchers have attempted to modify the physical or biochemical properties of PEGDA film for cell adhesion. One of the most effective and common methods to achieve this effect is functionalized with proteins such as FN-derived arginine–glycine–aspartic acid–serine (RGDS) sequence to form PEG-RGDS conjugation. Within this context, a general overview of surface modification for cell adhesion was introduced. Influence factors of surface properties for cell adhesion were firstly presented. Then surface modification methods were demonstrated through working principles and functions for cell adhesion. Finally, a large number of material surfaces including silicon, metals, polymer, and ceramics, which could be modified for cell adhesion, were summarized. However, new challenges need to be addressed in future studies.

More accurate and sophisticated 3D surface modification are required. Generally, cells cultured on plain 2D plate were studied *in vitro*. However, *in vivo*, cells were usually viable in 3D structures. The existing surface-treating methods including lithographic techniques mostly confined to modify the 2D surface. With the future of technological developments, cell adhesive features were expected to accommodate the more complex and hierarchical structures.

Smart and adaptable materials are also necessary to be developed for surface modification. Although surface can be modified by methods mentioned above, the morphology, physicochemical, and biological properties may be failure or changed and hard to be maintained for a longtime. For instance, Jeon, Hojeong et al. used direct-write ablation lithography to fabricate nanocrater for directing cell migration and organization [88]. While nanoscale craters could modulate cell adhesion, the super clean experimental environment is required. Once the fabricated surface was contaminated with dust, nanoscale craters are easier to be damaged. To overcome these challenges, materials with natural cell-adhesive properties are expected to be developed, and these materials can be used for cell adhesion without any treatment.

Furthermore, it appeared that the combination of nanoscale and microscale topographies could be superior to using each single-size scale alone for cell adhesion. It is generally known that the microstructures of biological interface and tissues exist, ranging from nanometer to micrometer scale, and these two-size scaled microstructures have an impact on biological functions. Researchers have found that the elongation of endothelial cells was enhanced once nanofibrous matrices deposited on the surface of the micrograting substrates [115]. On the other hand, combination of different technologies is the trend of surface modification technologies. Lithographic techniques were usually used to change the physical properties of surface. Combination with other biochemical methods including LbL deposition method will bring considerable improvement in cell adhesion.

Overall, the significance of studying cell adhesion and a comprehensive understanding of cell substrate interactions *in vitro* have become increasingly prominent such as in the field of tissue engineering and regenerative medicine. Although significant challenges abound, the method and materials for surface modification should be improved continuously to make exciting contributions to fundamental biology.

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