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Three-dimensional nano-biointerface as a new platform for guiding cell fate

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Three-dimensional nano-biointerface has been emerging as an important topic for chemistry, nanotechnology, and life sciences in recent years. Understanding the exchanges of materials, signals, and energy at biological interfaces has inspired and helped the serial design of three-dimensional nano-biointerfaces. The intimate interactions between cells and nanostructures bring many novel properties, making three-dimensional nano-biointerfaces a powerful platform to guide cell fate in a controllable and accurate way. These advantages and capabilities endow three-dimensional nano-biointerfaces with an indispensable role in developing advanced biological science and technology. This tutorial review is mainly focused on the recent progress of three-dimensional nano-biointerfaces and highlights the new explorations and unique phenomena of three-dimensional nano-biointerfaces for cell-related fundamental studies and biomedical applications. Some basic bio-inspired principles for the design and creation of three-dimensional nano-biointerfaces are also delivered in this review. Current and further challenges of three-dimensional nano-biointerfaces are finally addressed and proposed.

Key learning points

(1) Three-dimensional nano-biointerface is an emerging and important direction of cell biointerface design.
(2) Three-dimensional nano-biointerfaces are where most exchanges of materials, signals, and energy between cells and their microenvironments take place.
(3) Learning from the interior disciplines of biological systems, some basic bio-inspired principles have been applied to design three-dimensional nano-biointerfaces.
(4) Three-dimensional nano-biointerfaces provide a novel platform to guide cell fate in a controllable and accurate way, bringing a series of new phenomena and developments of cell-related fundamental biological studies and advanced biomedical applications.

1. Introduction

Cells inhabit a complicated microenvironment including extracellular matrix (ECM) and neighboring cells. Extensively spatiotemporal stimuli in cell microenvironments regulate cell fate and tissue development. Cells are inherently sensitive to local signals from macroscaled to nanoscaled and chemical to physical, such as molecules and structures, soluble and fluidic factors, and mechanical stimuli.[1–3] Cells also communicate with the outside through material transfer, molecular recognition, mechanical transduction, and electrical sensing. This intricate situation raises great challenges and opportunities to design cell biointerfaces for biomedical applications.[4]

The design of cell biointerfaces has been predominantly focusing on two aspects, chemical and topographical.[1,3–6] In recent years, the consideration of mechanical aspect is also gradually involved. Along with the development of micro/nanofabrication and engineering techniques, it is able to design cell biointerfaces from simply two-dimensional to complex three-dimensional with spatiotemporally chemical/physical factors.[1,3,5–7] From the chemical aspect, briefly speaking, by molecule-scaled engineering of two-dimensional biointerfaces (smooth biointerfaces without surface topography), fundamental cell activities have been systematically investigated in the presence and absence of chemical clues. ECM proteins have been patterned to control the size, shape, viability, and differentiation of cells, as well as cell–cell contacts.[1,7] Chiral molecules and groups have also been applied for studying the influence of surface chirality on cell behaviors.[8] From the topographical aspect, on the other hand, micro/nano-patterns such as grooves, columns, and ridges on biointerfaces exhibit different interaction modes with cells compared to smooth

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biointerfaces.[3] Using elastic micropost arrays, the contractile force and the mechanotransduction mechanism of cells can be studied with highly spatial resolution compared to flexible smooth substrates.[7] In addition, micro/nano-structures such as nanofibers have been applied for cell culture and scaffolds.[4] These extensive studies benefit the understanding of fundamental cell behaviors and cell–material/cell–microenvironment interactions, and promote the development of tissue-engineering materials for advanced biomedical implants and devices.

Along with the exploration of cell biointerfaces in the last few decades, the importance of three-dimensional nano-topography has gradually increased, from a supplementary understanding of chemical-aspect cell–material interactions to an essential direction of biological studies and applications. That is because three-dimensional nano-topography is an important part of cell microenvironments, like the nanoscaled villi and podia of cell surfaces and the nanofibers of ECM. Most exchanges of materials, signals, and energy between cells and their microenvironments take place at three-dimensional nanostructured cell interfaces. Learning from the interior disciplines of biological systems is an optimal way for biointerface design and applications. On the other hand, some important biological directions in recent years, such as stem-cell related regenerative medicine, nerve-cell related neuroprosthetics, and rare-cell related cancer diagnosis, have raised higher requirements for precisely nanoscaled control of cell–material interactions. These factors all together promote the creation of more advanced and smart biointerfaces at the three-dimensional nanoscale. Many novel three-dimensional nano-biointerfaces (biointerfaces with three-dimensional nanoscaled topography) have been developed with the help of advanced nano-chemistry, nano-fabrication, and nano-engineering methods and technologies. Different from traditional two-dimensional biointerfaces, these novel three-dimensional nano-biointerfaces own a series of unique properties brought by the intimate interactions between cells and nanostructures. These unique properties and capabilities make three-dimensional nano-biointerfaces a powerful platform to guide cell fate in a more controllable and accurate way, such as the enhancement of cell adhesion, the control of cell adhesion/detachment and stem-cell differentiation, the help of cell

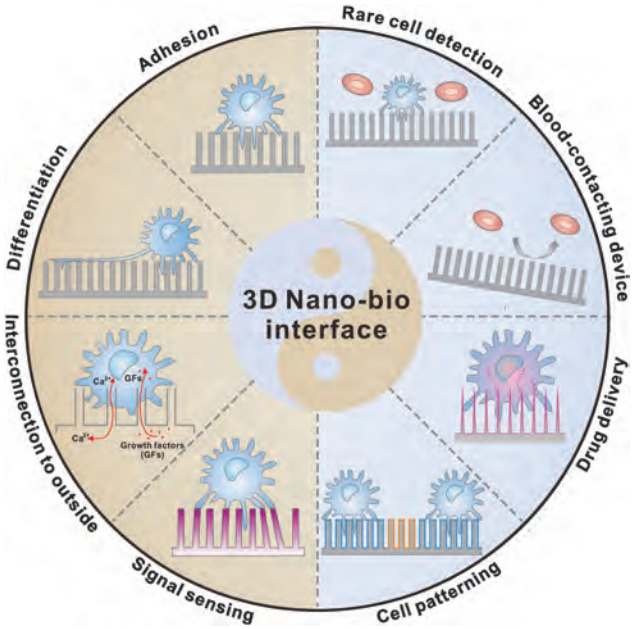

Fig. 1 Explorations of three-dimensional (3D) nano-biointerfaces for cell-related fundamental biological studies (*i.e.*, enhancing cell adhesion, controlling stem-cell differentiation, helping cell interconnection to outside, and promoting cell sensing to external signals) and advanced biomedical applications (*i.e.*, increasing rare-cell detection efficiency, improving anti-platelet-adhesion capability of blood-contacting device, helping drug delivery into cells, and realizing effective and long-term cell patterning).

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interconnection to outside, and the promotion of cell sensing to external mechno-/electrical signals. It has also shown the potential for many important biomedical applications, such as cancer cell detection, blood-contacting device, drug/gene delivery, and cell patterning (Fig. 1).

As there have been many high-quality reviews about the design of two-dimensional biointerfaces and micro/nano-patterns for traditional cell-behavior study, cell culture, and tissue engineering,[3,5–7] here we specially focus on the new phenomena and developments of three-dimensional nano-biointerfaces in recent years for the controllable and accurate guidance of cell fate. In the following part, the design principles and fabrication methods of three-dimensional nano-biointerfaces will be introduced from two aspects: structure design and surface modification. Some basic bio-inspired principles for fabricating three-dimensional nano-biointerfaces will be delivered in this part, to reveal that learning from the intrinsic characters of biological systems is an effective way for biointerface design. How three-dimensional nano-biointerfaces influence cell activities and functions and communicate with cells, and the applications of three-dimensional nano-biointerfaces in biomedical areas will be further highlighted. The current and further challenges of three-dimensional nano-biointerfaces for guiding cell fate will be finally addressed.

2. Fabrication of three-dimensional nano-biointerfaces

Experiences indicate that learning from natural biological systems is one of the best ways to design a functional interface. To create advanced cell biointerfaces, it is worth seeking inspirations from biological systems that cells live in, and exploring disciplines from cells themselves and cell microenvironments. In human biological systems, topography and chemistry are also two main factors that constitute cell/microenvironment interfaces, and determine cell–microenvironment interactions. There are various three-dimensional nano-biointerfaces in biological systems, such as the material–exchange interface of intestinal villi, the immune–elimination interface of T lymphocytes and cancer cells, and the signal-conduction interface of neurons (Fig. 2).[9–11] At these three-dimensional nanostructured cell biointerfaces, cell activities such as adhesion, recognition, nutrient uptake, and impulse conducting take place along with molecule-scaled chemical interactions. These facts indicate the importance of combining three-dimensional nanostructures with surface chemistry to design functional cell biointerfaces that achieve effective cell–material interactions and controllable guidance of cell fate. In addition, mechanical factors can be integrated with nanostructures by using materials with desired stiffness. Many fabrication methods of nanostructures and various chemical modification processes are required to construct three-dimensional nano-biointerfaces, however these are not the emphasis of this part. Here we would mainly like to introduce some basic principles for fabricating three-dimensional nano-biointerfaces, especially some bio-inspired principles for the design and creation of three-dimensional nanostructures.

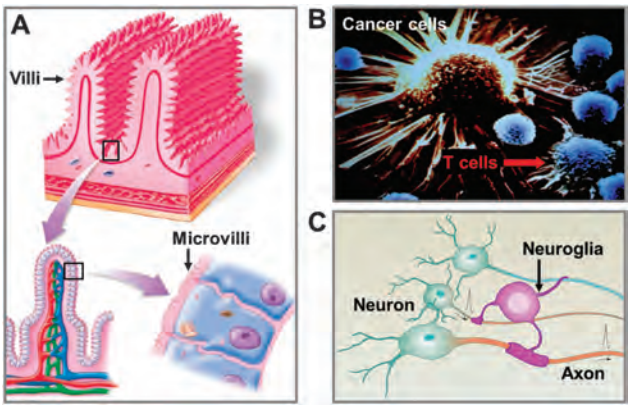

Fig. 2 Illustration of three-dimensional nano-biointerfaces in biological systems. (A) The material-exchange interface of intestinal villi. (B) The immune–elimination interface of T lymphocytes and cancer cells. (C) The signal-conduction interface of neurons. (A) Adapted from ref. 9 Copyright 2012, The McGraw-Hill Companies, Inc. (B) Adapted from ref. 10 Copyright 2002, National Academy of Sciences. (C) Adapted from ref. 11 Copyright 2011, American Association for the Advancement of Science.

2.1 Structure design

Structure design is usually the first step for fabricating three-dimensional nano-biointerfaces. Inspired by the topography features and topographical interactions of biological systems, many bio-inspired three-dimensional nanostructures have been created. Several representative three-dimensional nanostructures, such as nanowires, nanopores, nanotubes, nanoclusters, and nanofibers are demonstrated in Fig. 3.

2.1.1 ECM inspired nanofiber nets. ECM is composed of nanoscaled fiber nets of collagens, elastins, *etc.* Inspired by the fiber-like nanostructures of ECM, many nanofiber nets have been fabricated for the related cell studies and applications. For example, polymer nanofibers have been long-time utilized for cell culture and tissue-engineering scaffolds.[4] Recently, inorganic nanofibers have also been created for rare cell isolation.[12] Enhanced topographical interactions have been shown between cell surface structures and nanofibers, providing an ECM-mimicking topographical environment for better cell-activity controls. Electrostatic spinning is the most common method for fabricating nanofibers. By applying a high voltage on a polymer solution or melt, a charged liquid or melt jet can be formed, and the related polymer nanofibers are finally collected on the grounded collector. By introducing inorganic components (*e.g.*, TiO_2) in polymer solutions, the related organic–inorganic composited nanofiber nets can be produced.[12] After high-temperature calcination, the corresponding inorganic nanofibers are produced with similar morphology as the organic components are thermally decomposed. The nanofiber nets provide a convenient platform for studying cell behaviors, controlling cell functions, and achieving the related biomedical applications.

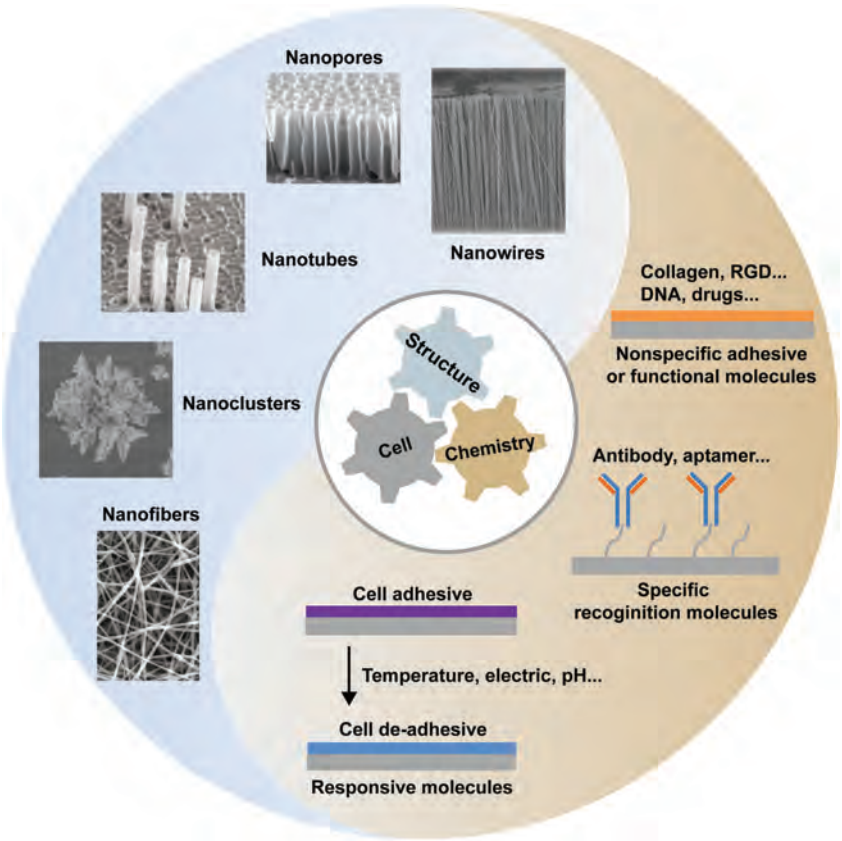

Fig. 3 The fabrication of three-dimensional nano-biointerfaces from two aspects: structure design and surface modification. Some representative nanostructures including nanowires, nanopores, nanotubes, nanoclusters, and nanofibers are demonstrated here. Molecules for surface modification are classified into three parts including nonspecific adhesive or functional molecules, specific recognition molecules, and responsive molecules according to their functions. Images of Nanowires and Nanotubes: adapted from ref. 46 Copyright 2009 and ref. 32 Copyright 2012, American Chemical Society. Images of Nanopores, Nanoclusters, and Nanofibers: adapted from ref. 30 Copyright 2010, ref. 14 Copyright 2013, and ref. 41 Copyright 2011, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

2.1.2 Lymphocyte inspired nanowire arrays. One of the important functions of lymphocytes in the human immune system is to recognize ''non-self'' invaders and generate responses to maximally eliminate them, such as the immune elimination of tumor cells in blood.[10] The elimination of tumor cells by lymphocytes includes the specific recognition of ''non-self'' antigens or proteins on tumor cells, and the topographical interactions between surface nanostructures of these two kinds of cells. These two factors together make lymphocytes eliminate tumor cells highly-efficiently. Inspired by this, three-dimensional nanostructures of silicon nanowire (SiNW) arrays have been developed for highly-efficient recognizing and capturing target tumor cells in blood.[13] The SiNW arrays were fabricated by typical HF/$AgNO_3$ wet chemical etching. Compared with smooth Si substrate, the SiNW arrays showed enhanced topographical interactions with cells, resulting in increased cell recognition and capture capability. This lymphocyte-inspired three-dimensional nano-biointerface provides a different interaction pattern compared with the above mentioned nanofiber nets, and also facilitates many cell-related biological studies and applications.

2.1.3 Fractal morphology of cancer-cell surfaces inspired fractal nanostructures. Fractal morphologies distribute widely in nature especially in human biological systems, such as pulmonary microvasculature and cerebral cortex. Many studies have proved that the surface of cancer cells present fractal morphology. The fractal dimension of cancer-cell surface structures is larger than that of normal cells, which is hypothesized to have a connection with cancer cell–microenvironment interactions and the invasion degrees of cancer. Inspired by this, fractal Au nanostructures have been fabricated on indium tin oxide (ITO) conducting glasses using one-step electrochemical deposition method.[14] By controlling the deposition parameters such as electrodepositing potential and ion concentration, the fractal dimension of Au nanostructures can be adjusted from low to high, providing a programmable platform for systematically studying the influence of fractal dimension to cell–substrate interaction. The results demonstrated that higher fractal dimension resulted in increased specific cell–substrate interactions, suggesting a potential design principle of programmable three-dimensional nano-biointerfaces.

2.1.4 Soft biological system inspired soft nanotube arrays. Cells and ECM are actually composed by soft matter. Previous

studies have demonstrated that the stiffness of materials is very important for biological studies and applications.[2] It is the synergistic interactions of molecules, structures, and stiffness that largely influence cell functions. Inspired by this, soft polystyrene nanotube (PS NT) arrays have recently been fabricated *via* replication using anodic aluminum oxide (AAO) as a template.[15] The diameter of PS NTs is close to that of AAO pores. By controlling the time of template etching, the length of PS NTs can be adjusted from several micrometers to dozens of micrometers. As PS NTs are very soft, they aggregate when the length increases, but without large influence to cell–substrate interactions. This soft nano-substrate provides a soft platform to study the cooperative influence of stiffness, molecule, and topography to specific cell recognition and adhesion compared with rigid SiNW arrays mentioned above.[15] The replication method can also be applied to fabricate other polymer nanostructure arrays, offering an effective method for the fabrication of soft three-dimensional nano-biointerfaces.

The intrinsic principle of structure design is to provide a cell biointerface at three-dimensional nanoscale for close cell–material interactions. Further artificial explorations beyond natural mimicking can provide more experiences and disciplines for nano-biointerface design. Therefore, besides these above mentioned bio-mimicking methods for fabricating nanostructures, many other nanostructures have also been developed for cell-related studies and applications. For example, through the simple spin coating of monodispersed carbon nanotube (CNT) solution, the CNTs formed a paralleled aligned pattern spreading from the center along the direction of centrifugal force.[16] This CNT pattern has been further used for studying cell behaviors. Titanium has been widely used for implant materials. Utilizing electrochemical anodic oxidation method, TiO_2 nanotube arrays can be fabricated on smooth Ti sheets. The diameter and length of nanotubes can be adjusted accurately by changing the electrochemical parameters. Therefore, a series of TiO_2 nanostructures can be fabricated for systematically studying the influence of substrate nanostructures to cells,[17] accelerating the development of advanced implants. These various nanostructures provide a broad arena for cell-related fundamental studies and advanced biological applications.

2.2 Surface modification

Surface modification often acts as the second step to construct three-dimensional nano-biointerfaces after structure design. Sometimes, the as-prepared nanostructures are also used directly without any further modification. The molecules or proteins used for surface modification can be mainly divided into three parts according to their functions: nonspecific adhesive or functional molecules, specific recognition molecules, and responsive molecules (Fig. 3).

2.2.1 Nonspecific adhesive or functional molecules. *In vivo* cells need adhesive proteins, such as arginine-glycine-aspartic acid (RGD) peptide groups, collagens, and fibronectins, to adhere to ECM for survival and functional performance. For cell-related studies, these adhesive molecules are usually simply coated on substrate surfaces to promote cell adhesion. In studies involving cell patterns and cell assays, these adhesive molecules will be patterned to promote cell adhesion at specific positions.[1] In this condition, anti-adhesive molecules, such as polyethylene glycol hydrogels and bovine serum albumin (BSA), are simultaneously coated on the non-pattern areas to facilitate the long-term stability of cell patterns.[7] In studies of drug/gene delivery, some nonspecific functional molecules such as drugs, RNAs, and DNAs, are also connected to the substrate surface to realize their impacts on cells.[18,19] These molecules are always connected onto nanostructures by interactions such as static electrical assembly or other chemical modifications. For these functional molecules, how to realize both efficient connection and the following release from substrate surfaces still remains an important challenge. To develop smart connections and release strategies should be a proper solution for realizing this issue. In other studies, some functional molecules are also utilized for the observation of cell activities, *i.e.*, the detection of molecule expression of cell surfaces. For example, lectins interact with glycans on cell surfaces by the reaction between boric acid and diols.[20] Using this interaction, lectins can be modified on substrate surface to observe the dynamic changes of glycans on cell surfaces, as the status of glycosylation is very important to oncogenic transformation, cell differentiation, and metastasis.

2.2.2 Specific recognition molecules. In some cell-related studies such as rare cell isolation from whole blood and cancer cell detection, the designed substrate should realize specific target-cell recognition while preventing the non-specific adhesion of non-target cells.[12–15] In this case, the modification of specific recognition molecules is required. Antibody is one of the most important specific molecules that recognize cells with the corresponding surface antigen. For example, anti-EGFR (epidermal growth factor receptor antibody) can be used for specific recognition of lung-cancer cells. Anti-HER2 (human epidermal growth receptor 2 antibody) is capable of recognizing breast cells overexpressing HER2 proteins. In order to facilitate the grafting of antibodies to substrate surfaces while maintaining antibody activities, antibodies are usually modified with specific proteins or molecules. For example, biotin modified antibodies can be easily connected to substrate surfaces through biotin–streptavidin interactions.[13–15] However, the high price and easy deactivation of antibodies restrict their universal applications. In recent years, aptamer has been explored for targeting specific cells.[21,22] Aptamers, including natural and synthetic ones, are a class of nucleic acid molecules or peptides that have the ability to bind to specific target molecules, cells, and even tissues. Synthetic aptamers are usually selected from a large library of random oligonucleotides pool for effective recognition of target cells. Compared with antibodies, aptamers are cheaper and more stable, showing an attractive prospect for biological applications. Small molecules such as folic acid also have specific interactions with cancer cells overexpressing folic acid receptors. These specific recognition molecules are connected to substrates by several steps of reactions, including EDC–NHS chemistry and streptavidin–

biotin affinitive interactions.[13] Anti-adhesive molecules or groups such as BSA and polyethylene glycol chains are usually utilized at the same time to avoid the adhesion of non-specific cells for improving the specificity of biointerfaces.[14,15]

2.2.3 Responsive molecules. Smart surface acts as an important direction for cell biointerface design. Responsive molecules are needed for realizing the reversible and smart change of interactions between cells and surfaces.[5,6] After responsive molecules are modified on substrate surfaces, they are usually further connected with adhesive molecules or recognition molecules for realizing related functions.[6,14,23] Responsive molecules can be stimulated by factors such as temperature, electric, light, and pH, realizing the change of surface chemical properties, and the final adhesion and detachment status of molecules, proteins, and cells. For example, various photo-responsive molecules such as azobenzene and spiropyran have been used for controlled protein adsorption–desorption and cell adhesion–detachment. Poly(*N*-isopropylacrylamide) (pNIPAM) is a thermal responsive molecule with a lower critical solution temperature (LCST). It is generally accepted that when the temperature is higher than the LCST, pNIPAM brushes collapse and are hydrophobic, and below LCST the brushes are soluble and become hydrophilic.[23] By changing the temperature, the modified surface can realize different interactions with molecules, proteins, and cells. Increasing investigations indicate that the controlled cell adhesion on pNIPAM surfaces is actually a complex issue.[24] Except the temperature, other factors such as the molecule weight, thickness, and grafting density of pNIPAM brushes influence greatly the protein-mediated cell adhesion. An intermediated grafting density facilitated the maximum protein adsorption and cell adhesion above the LCST.[24] However, those previously stimuli-responsive systems are mostly based on two-dimensional surfaces with microscaled chemical patterns, especially systems for controlled cell adhesion/detachment.[5,6] Until recent years, several stimuli-responsive three-dimensional nano-biointerfaces have been gradually explored for cell-related studies and applications by connecting responsive molecules onto nanostructures, but still at the beginning stage.

The modification methods and processes of three-dimensional nano-biointerface are actually a very important issue. There are probable differences in the specific steps of chemical modifications between two-dimensional biointerfaces and three-dimensional nano-biointerfaces. For example, the high specific surface area of nanostructures may influence the quantity and density of molecules modified. The high surface activity of nanomaterials and nanostructures is supposed to facilitate the coupling efficiency of many chemical agents such as silane. Efficient characterization methods and technologies are prospected to reveal the modification details and quality of three-dimensional nano-biointerfaces to realize their better applications.

3. Three-dimensional nano-biointerfaces for controlling cell functions

3.1 Cell activities

Many three-dimensional nano-biointerfaces have been fabricated to study their influence over fundamental cell activities such as cell adhesion, survival, spread, and motility. However, a complicated situation turns up. Some nanostructures facilitate cell activities while some act on the contrary. These different results stem from many factors, such as the type of cells and interface materials, the chemical property and wettability of interfaces, the topography feature of surface nanostructures, and the kind of interface charges. In spite of these complex results, here we mainly focus on recent research about using three-dimensional nano-biointerfaces for enhanced cell adhesion, controlled cell adhesion/detachment, and for stem-cell/nerve-cell control towards advanced biological applications.

3.1.1 Enhanced cell adhesion. Adhesion is one of the fundamental cell activities. To realize the control of cell adhesion at three-dimensional nanoscale is very important for understanding *in vivo* cell behaviors and achieving advanced biological applications. In recent years, several three-dimensional nano-biointerfaces have been designed for regulating cell adhesion, showing enhanced cell adhesion when compared with the corresponding two-dimensional biointerfaces.

To design appropriate cell biointerfaces for strong adhesion and expansion of human umbilical cord blood hematopoietic stem/progenitor cells (HSPCs) is very important for hematological disorder treatments and disease therapies. Three-dimensional nano-topography has shown to be a key factor for the interface design. By 10 day culturing HSPCs on aminated polyethersulfone (PES) nanofibers, enhanced cell adhesion and expansion have been observed compared to the corresponding aminated PES film in the existence of cytokine supplementation.[25] The enhanced adhesion of HSPCs led to the formation of distinct and circular colonies in a radial and outward planar fashion (Fig. 4A). Numerous filopodia were protruded by HSPCs to interact with nanofibers, indicating the enhanced topographical interactions. In contrast, most of HSPCs on the PES film located along crevasses of the film as cells on smooth areas without crevasses were easily washed away, showing a weak adhesion between cells and the PES film. This result indicates the role of three-dimensional nano-biointerfaces in high-quality HSPCs culture, providing a potential platform for effective HSPCs expansion *in vitro*.

By using antibody modified three-dimensional SiNW substrate, highly efficient target cell adhesion on the substrate has been achieved. Compared to the smooth Si substrate, the SiNW substrate showed enhanced cell adhesion under the same incubation conditions.[13] More cells (about 40%) adhered on the SiNW substrate compared with the smooth Si substrate at the same incubation time (Fig. 4B). This enhanced cell adhesion is due to the enhanced topographical interactions between

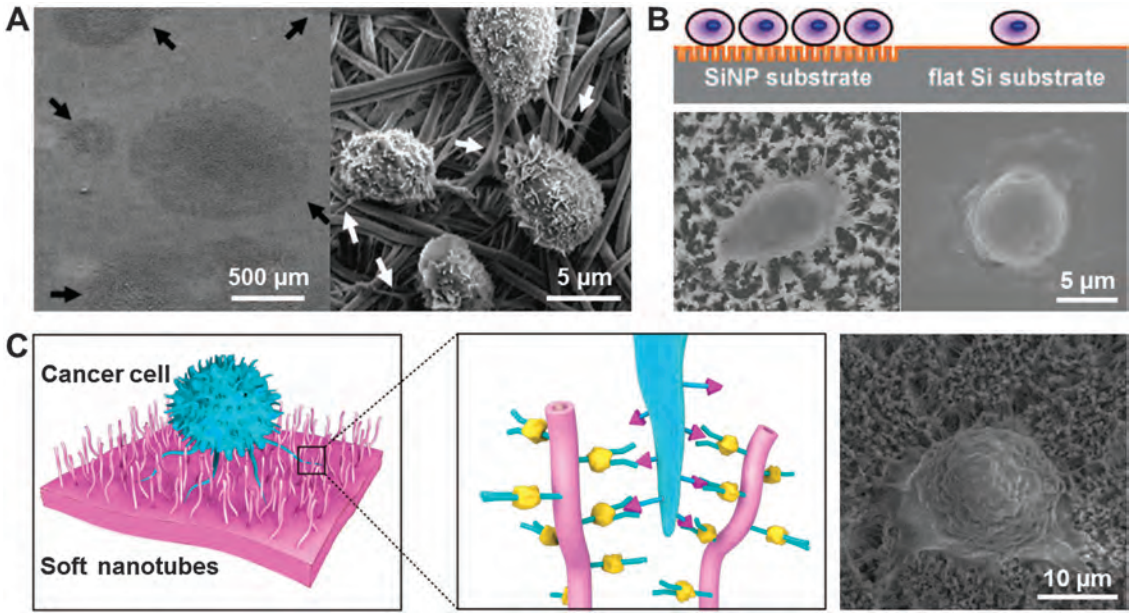

Fig. 4 Enhanced cell adhesion on three-dimensional nano-biointerfaces. (A) Enhanced adhesion of HSPCs on aminated PES nanofibers leads to the formation of distinct and circular colonies. (B) Compared to the antibody modified smooth Si substrate, antibody modified SiNWs capture more cells at the same incubation time, showing enhanced cell adhesion. (C) A soft three-dimensional nano-biointerface based on PS NT arrays realizes enhanced and more rapid cell adhesion compared with rigid SiNWs. Cells on all three kinds of nano-substrates exhibit enhanced topographical interactions between cell surface structures and substrate structures compared to cells on the corresponding smooth substrates. (A) Adapted from ref. 25 Copyright 2006, Elsevier. (B) Adapted from ref. 13 Copyright 2009, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Adapted from ref. 15 Copyright 2013, Nature Publishing Group.

cell surface structures and substrate structures reflected by microscopy characterization. By changing the length of SiNWs from 4 μm to 20 μm, the number of cells adhered on the substrate increased gradually, and reached the maximum when the length of SiNWs was longer than 8 μm.[13] This result indicates that there exists a suitable length for SiNWs at which the topographical interactions between cell and substrate reach the maximum. Utilizing this topographical effect, these three-dimensional nano-biointerfaces can be used for highly efficient rare cell detection, providing a potential tool for advanced cancer diagnosis and treatment.

Stiffness is also an important factor in the cell microenvironment that influences cell adhesion. Substrate with tissue-like stiffness facilitates normal cell activities and functions. Recently, an antibody modified soft PS NT substrate was fabricated (Fig. 4C),[15] achieving enhanced and more rapid cell adhesion compared with rigid SiNW substrate. For the SiNW substrate, about 40–60% specific cell adhesion was reached when the incubation time was 45 min.[13] In contrast, the PS NT substrate shortened the incubation time to 20 min with about 70% cell adhesion.[15] Enhanced topographical interactions have also existed between cells and the PS NT substrate, resulting in enhanced cell adhesion when compared with the corresponding smooth PS substrate. This research gives an example of the importance of stiffness to cell adhesion, and opens up a new avenue of soft materials for advanced biomedical applications.

3.1.2 Controlled cell adhesion/detachment. In addition to the enhanced cell adhesion achieved on the three-dimensional nano-biointerfaces, controlled cell adhesion/detachment can also be realized by modifying the interfaces with stimuli-responsive molecules (Fig. 5). Through grafting thermo-responsive pNIPAM on SiNWs, this nanostructured substrate realized the adhesion and detachment of cancer cells controlled by temperature.[23] At temperature higher than the LCST of pNIPAM (about 37 °C), antibodies were connected to the pNIPAM–SiNWs through hydrophobic interactions between hydrophobic pNIPAM and antibody-anchored BSA molecules, therefore achieving target-cell adhesion. While lowering the temperature down to the LCST (about 20 °C), the pNIPAM turned hydrophilic, and the adhered cells were released. The quantity of cells adhered on the pNIPAM–SiNWs was about 2.4-fold more than that on the smooth pNIPAM–Si, confirming enhanced cell adhesion. Compared with the corresponding smooth substrate (about 90.9%), the ratio of cells released from the nano-substrate (about 98.8%) was also higher.[23] This enhanced cell detachment is attributed to the three-dimensional contacting mode between cells and nanostructures. Nanostructures enhance the topographical interactions and provide more contact points for stronger cell adhesion, but restrict cell spreading therefore reducing the contacting area between cell and substrate. In contrast, smooth surface facilitates cell spreading with increased contacting area. The reduced contacting area results in the enhanced cell detachment of SiNWs.

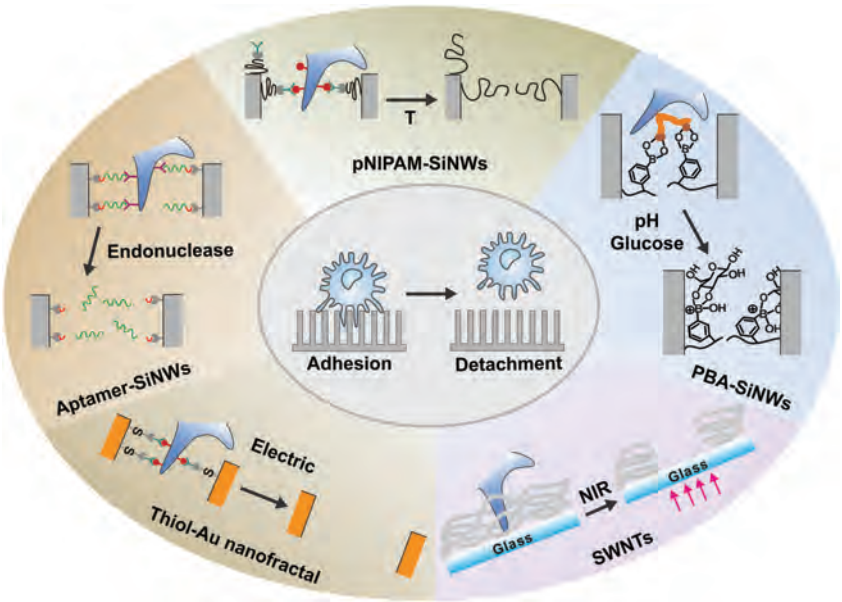

Fig. 5 Control of cell adhesion and detachment on responsive three-dimensional nano-biointerfaces under external stimuli. The adhesion/detachment of cells can be controlled by the chemical responsiveness of three-dimensional nano-biointerfaces, such as the temperature (*T*), endonuclease, electric, or pH/glucose responsiveness of related molecules. In addition, physical responsiveness such as the photothermal and photoacoustic effect of SWNTs can also be utilized for realizing controlled cell detachment under NIR irradiation.

Controlled cell adhesion and detachment has also been achieved on aptamer-connected SiNWs, as DNA aptamers can be designed for target cells and easily digested by endonuclease.[21] In addition, through the control of Au–S bonding and breaking, fractal Au nanostructures on ITO substrates also realized dynamic and controlled cell adhesion and detachment.[14] Biotin–antibodies were connected onto the biotin–thiol modified fractal Au nanostructures though biotin–streptavidin interactions for target cell adhesion and capture. Through the electrochemical cleavage of Au–S bonds, about 98% of biotin–thiol connected cells were released from Au surfaces, achieving controlled cell detachment without damage.

In biological systems, it is always the combination of multiple stimuli that affects cells. It is meaningful to design surfaces with multiple responses to study their influences to cell adhesion. Recently, a pH and glucose dual-responsive three-dimensional nano-biointerface has been fabricated for reversible and controlled cell adhesion and detachment by grafting poly(acrylamidophenylboronic acid) (polyAAPBA) brush from SiNWs.[26] When the concentration of glucose was 70 mM, the surface achieved pH responsive cell adhesion (pH = 6.8) and later detachment (pH = 7.8). When the pH was kept at 7.8, the surface showed glucose responsive cell adhesion (0 mM glucose) and detachment (70 mM glucose). Therefore, dual-responsive cell adhesion and detachment were reached by simultaneously turning from pH 6.8/glucose 0 mM to pH 7.8/glucose 70 mM. With the help of a quartz crystal microbalance, it was revealed that the dual responsiveness of this system is attributed to the competitive binding between polyAAPBA/sialic acid expressed on cell surfaces and polyAAPBA/glucose.[26] Compared with the corresponding smooth surfaces, the enhanced topographical interactions between cells and substrates resulted in enhanced cell adhesion and detachment of the polyAAPBA–SiNWs systems.

Besides the above chemically controlled cell detachment, physical properties of three-dimensional nano-biointerfaces also provide a suitable approach for controlled cell release. The photothermal and photoacoustic effect of single-walled carbon nanotubes (SWNTs) has been utilized for controlling single-cell collection through the irradiation of near-infrared (NIR) light through a microscope lens.[27] SWNTs were firstly deposited on a glass substrate for cell incubation. After cells adhered on SWNTs, NIR light with a designed pattern was irradiated on SWNTs through the glass substrate, resulting in the decomposition and detachment of SWNTs and the final release and collection of single cells in irradiated areas. The influence of NIR light on SWNTs is speculated through the photothermal and photoacoustic effect of SWNTs that destroys the structures of SWNTs. Other nanomaterials such as graphene and Au nanoparticles with photothermal property can also be applied for designing three-dimensional nano-biointerfaces towards physically controlled cell release. In addition, thermal responsive molecules can be further combined with these nanomaterials to realize NIR-controlled cell adhesion/detachment with more precisely spatiotemporal resolution.

3.1.3 Stem-cell differentiation and nerve-cell regeneration. Stem cells have the amazing ability of self-renewal and differentiation into specialized cell types. The potential applications of stem cells in tissue regeneration, disease treatment, and drug screening have attracted wide attention from scientists and therapeutics. It is important to fabricate cell biointerfaces

to study the inherent nature and disciplines of stem cells, and thereby to control stem-cell fate. Hydrogels combined with ECM proteins and microscaled physical clues have long been applied for understanding stem cell niches. Elaborately three-dimensional nano-biointerfaces have also been gradually designed in recent years for mimicking stem-cell microenvironments more precisely.

An essential issue for orthopaedic repair materials is to regulate the differentiation of stem cells into bone cells rather than tissue cells in the absence of chemical treatment. By using nanoscaled symmetry and disorder, the differentiation of human mesenchymal stem cells (hMSCs) has been directly controlled on polymethyl methacrylate substrates with different nanopit arrangements without osteogenic supplements.[28] It was revealed for the first time that random nano-topography patterns rather than highly ordered ones favor hMSC adhesion and osteoblastic differentiation. Nano-topography induced enhanced osteoblastic differentiation of stem cells has also been demonstrated in other studies. These investigations demonstrate that the nano-topographical strategy is promising for stem-cell based regenerative medicine and tissue engineering.

Topography features of three-dimensional nano-biointerfaces have been systematically explored to control stem-cell fate. For example, the vitality of MSCs cultured on TiO_2 nanotube substrates can be controlled by the diameter of nanotubes (Fig. 6A).[17] Vertically aligned TiO_2 nanotubes with a tube diameter larger than 50 nm dramatically reduced cell activity and caused programmed cell apoptosis. Compared to smooth TiO_2 surfaces, a lateral spacing of 15–30 nm strongly promoted focal contact formation and enhanced cell activities. Using this platform, the influence of integrated nanoscaled topography and growth factors to stem-cell fate has also been investigated, facilitating the further developments of medical implants and materials.

The control of shape and growth direction of MSCs has been achieved by the orientation of monolayered CNTs. The research demonstrates that MSCs can recognize the arrangement of single nanotubes, and exhibits enhanced proliferation and osteogenic differentiation on the aligned CNT patterns when compared with randomly oriented CNT networks (Fig. 6B).[16] In addition, stem cell polarization and alignment have been finely modulated by tuning the density of ordered silicon nanopillar arrays (Fig. 6C).[29] The result shows that morphologies of stem cells can be influenced by pillar density with controlled inter-pillar spacing. A critical interpillar spacing of about 2 μm stimulated the neuron-like differentiation of stem cells. These studies are instructive for the exploration of scaffolds and devices, which demands precise control of cell shape, alignment, and differentiation, by designing advanced three-dimensional nano-biointerfaces.

Nanoscaled topographical clues also have great influence on neuronal behavior such as neurite outgrowth. Nanostructured AAO substrates with variable concave shapes and pore sizes were shown to generate nano-topographical stimuli to neurons in a controllable and systematic manner.[30] It was proved that large concaves with a pitch of 400 nm had an accelerating effect on neuronal polarization and development when compared with small concaves with 60 nm pitch and flat AAO substrate. Superaligned CNT yarn patterned substrates have also been developed to promote the neurite outgrowth of neurons while minimizing neurite branching when compared with the typical flat PS substrate used for neuron culture.[31] This is attractive for the application of topographical scaffold in the regeneration and repair of the central nervous system.

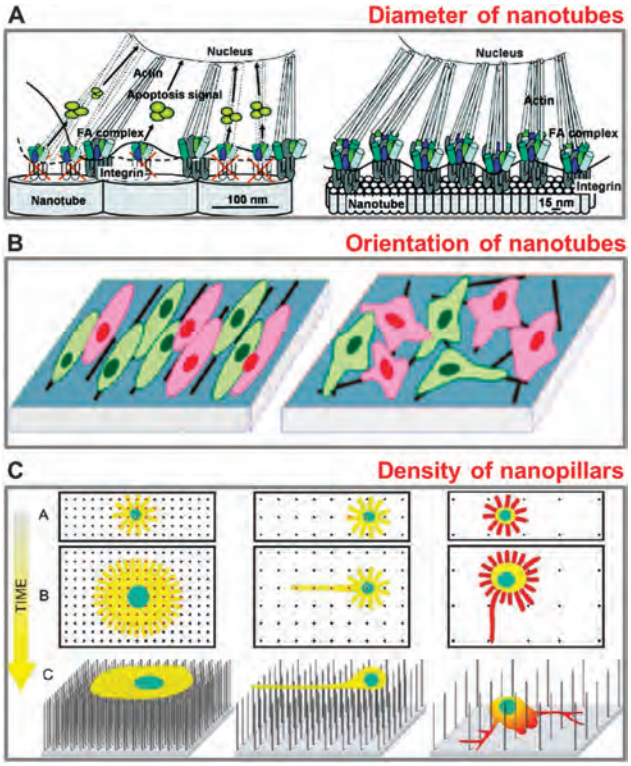

Fig. 6 Three-dimensional nano-biointerfaces for controlling stem-cell fate. (A) The diameter of TiO_2 nanotubes controls the formation of focal contacts and stem-cell vitality. (B) hMSCs recognize the alignment of single CNTs, and exhibit enhanced proliferation and osteogenic differentiation. (C) The polarization and differentiation of stem cells are tuned by adjusting the density of nanopillars. (A–C) Adapted from ref. 17 Copyright 2007, ref. 16 Copyright 2011, and ref. 29 Copyright 2012, American Chemical Society.

3.2 Cell interconnection to outside

Cell interfacing tools can provide a helpful solution to understand and control biological processes occurring inside cells or between cells and their microenvironments. Many biological processes, such as intracellular imaging, siRNA knockouts, drug delivery, and cell reprogramming, involve directly approaching cell interior and efficiently delivering biomolecules into the cytoplasm. Therefore, a biointerface that realizes efficient cell interconnection to outside is necessary for promoting material exchanges, altering intracellular microenvironments, and guiding cell activities. Traditional physical approaches such as electroporation and micro-pipetting are restricted by their harm to cell viability and low throughput. In

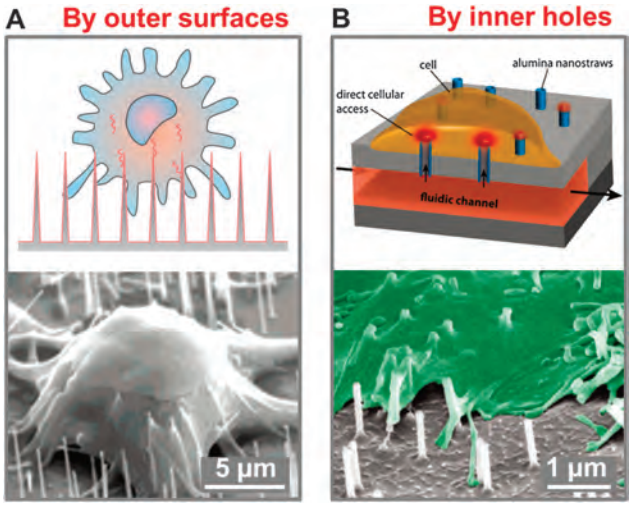

Fig. 7 Three-dimensional nano-biointerfaces for helping cell interconnection to outside by two modes: by the outer surfaces (A) and by the inner holes (B) of three-dimensional nano-biointerfaces. (A) SiNWs penetrate the membrane into cell interior. Molecules connected to SiNW surfaces are therefore delivered into cells. (B) Compared with SiNWs, nanotube arrays also penetrate into the cell interior, providing a convenient and controllable platform for helping cells interconnecting with outside through nanotube's inner holes. (A, B) Adapted from ref. 18 Copyright 2007 and ref. 32 Copyright 2012, American Chemical Society.

recent years, three-dimensional nano-biointerfaces show their great advantages in this field because of their ability to directly interconnect cells to outside in a nondestructive, controllable, and high-throughput way. The interconnection methods mainly include two modes: from the outer surfaces (Fig. 7A) and from the inner holes (Fig. 7B) of three-dimensional nanostructures.

SiNWs with high-aspect ratio and sufficient rigidity were shown to penetrate into mouse embryonic stem cells without any external force just by culturing cells on them (Fig. 7A).[18] The SiNWs can also promote material transportation into cells by directly introducing plasmid DNA connected on SiNW surfaces into cells. The viability and differentiation capability of cells were maintained up to several days on the SiNWs, showing the potential of SiNWs as a new type of stem-cell culture substrates. The nanoscaled diameter of SiNWs, much smaller than that of cells but closer to that of intracellular biomolecules, may provide a way to study molecule-level intra- and intro-activities of cells.

SiNWs are mainly suitable for delivering molecules connected to their surfaces, for example DNA molecules adsorbed by electrostatic interactions, therefore lacking direct and precise control of the delivery process unless a more convenient release scheme is developed. Recently, nano-templated nanostraws with sufficiently small diameter (about 100 nm) provided an alternative approach to delivery membrane-impermeable materials into cells through directly fluidic intracellular access to the cell interior (Fig. 7B).[32] This three-dimensional nanostraws penetrated cell membrane nondestructively, acting as a permanent fluidic pipeline, to deliver small molecules, proteins, and genetic material into cell cytoplasm in a continual and highly-efficient manner with temporal and concentration control. This method may provide a powerful tool to monitor and control the inner clockwork of cells in the future.

When interconnecting cells to outside through nanostructure penetration, how to decrease the negative effect of penetration on the viability of cells is also very important. A biocompatible and biostable nanomaterial is the first consideration. Before cell incubation and related experiments, these nanostructures should be sterilized thoroughly. The diameter of nanostructures has been proved to influence greatly cell viability.[18] Cell apoptosis occurred within a day when the diameter of SiNWs was larger (about 400 nm). In contrast, cells on SiNWs with a diameter of about 30 nm could survive for more than 5 days.

3.3 Cell sensing to external signals

3.3.1 Mechanotransduction. It is believed that adherent cells sense the physical and stiffness clues of substrate through integrin-mediated mechanotransduction. Binding and clustering of integrin to ECM lead to the formation of focal adhesions (FAs), through which the traction forces are transmitted from cytoskeleton to ECM and then fed back. Stiffness influences greatly the mechanotransduction of cells as well as cell behaviors and functions.[33] The level of tension generated by cytoskeleton against the substrate is proportional to substrate stiffness. The resulted intracellular stress fields finally determine markedly cell activities such as apoptosis, shape, and signal transduction.

During the study of mechanotransduction of cells, flexible substrates of hydrogels or silicon elastomers are utilized for detecting subcellular forces by monitoring the surface wrinkling caused by cell contraction. Elastic micropillar systems further provide a more direct measurement of forces,[7] and the feedback relationship between forces and FAs with high resolution. However, those micropillars have large pillar diameter and pillar spacing, limiting cell motility and spatial resolution of forces. In addition to that, the ECM surrounding cells is composed of nanoscaled topographies to which cells adhere. Therefore, it is necessary to develop elastic post arrays with nanoscaled topography features to better understand nanoscaled signal sensing and mechanotransduction of cells. Recently, using an advanced photolithography technique, "nanopost arrays" have been fabricated possessing high array density with smaller post diameter down to 750 nm (Fig. 8A).[34] A range of post diameters from 0.75 to 1.5 μm and center-to-center spaces from 1.5 to 4.5 μm have been systematically investigated on their influence to cell spreading and traction force. The nanopost arrays were shown to have no restrictions to cell spread and motility, similar to the result of flat culture plates. Four out of nine nanopost arrays were demonstrated to be capable of measuring traction forces, showing the potential application for advanced traction-force measuring tools.

In addition to nanopost arrays, other nanoscaled topographies have also been explored for studying the mechanotransduction of cells. By designing polydimethylsiloxane (PDMS)

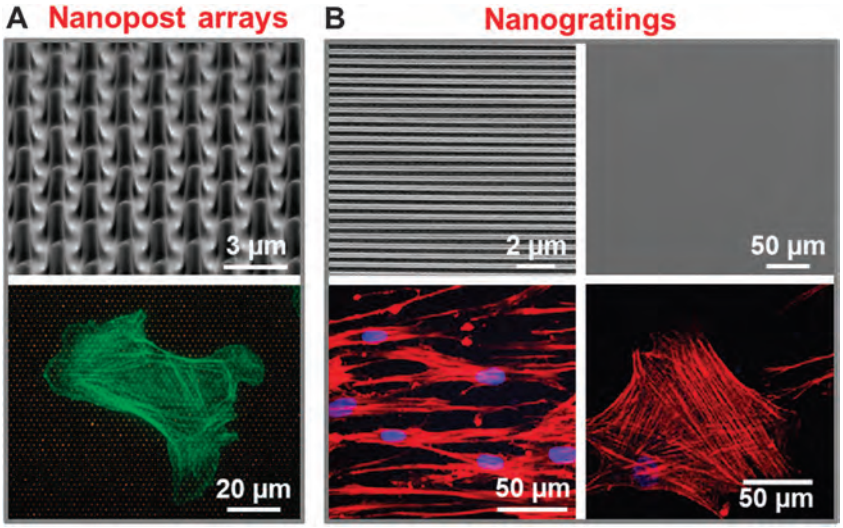

Fig. 8 Three-dimensional nano-biointerfaces for mechanotransduction studies of cells. (A) Elastic nanopost arrays can measure the traction force of cells. (B) PDMS nanogratings can regulate mechanotransduction and differentiation of cells through FA kinase. (A) Adapted from ref. 34 Copyright 2007, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Adapted from ref. 35 Copyright 2013, American Chemical Society.

nanogratings with 250 nm line width, it was revealed that this nano-topography could modulate cell mechanotransduction through integrin activated FA kinase and actomyosin cytoskeleton contractility to induce hMSC differentiation and gene expression (Fig. 8B).[35] Moreover, this research also demonstrated the size-dependent FA regulations of cells on smooth-, micro-, and nano-topographies. Compared to microgratings and flat PDMS control, cellular FAs were significantly smaller and more elongated on the nanogratings. These results may provide important information for the nanoscaled mechanotransduction mechanism as well as the crucial factors regulating stem-cell fate.

3.3.2 Electrophysiological recording. Electrophysiological techniques are important tools for understanding and manipulating the bioelectrical signals and activities of body, especially the information processing of brain neurons. The ideal device for electrophysiological recording is aiming to have high spatial resolution for single neurons, and also the capacity to simultaneously observe large neural networks. However, the existing techniques including patch-clamp pipettes and metallic microelectrode arrays (MEAs) could not satisfy both of these two factors at the same time, therefore providing insufficient information to understand and control neural activities. With the development of nanomaterials and nanofabrication techniques, many new platforms are developing to address some of the current problems in electrophysiology. The exploration of three-dimensional nanostructures for neuronal interfacing and accurate bioelectrical recording has shown great advantages for fundamental studies of cell electrophysiology. They will also provide valuable tools for biomedical applications at the brain interface such as neural prosthesis and implants.

CNTs with advanced conductivity, chemical-stability, and biocompatibility have been used for coatings of traditional flat metal electrodes and sharpened wire electrodes for novel extracellular recording devices. CNT coatings enhanced the effective area of electrode surfaces, reduced microelectrode impendence and increased charge transfer capability, therefore promoting both recording and electrical stimulation of neurons (Fig. 9A).[36] The electrochemical deposition method for forming CNT coatings provides a convenient way for fabricating nanocomposited electrodes with variable shapes and electrochemical properties. Moreover, CNTs can also affect neural electrophysiological activities. It was hypothesized that CNTs could form tight contacts with neuronal membranes, favoring electrical shortcuts and enhancing the signal transmission of neurons cultured on CNT surfaces.[37] This could explain the mechanism of how CNTs improve neuronal performances, and help design smart neural interfaces and devices.

As discussed above, nanostructures such as SiNWs can penetrate cell membrane to directly access the interior parts of cells. Utilizing this capability of SiNWs, MEAs integrated with vertical Ti/Au coated SiNWs were fabricated for intracellular electrophysiological recording (Fig. 9B).[38] In most cases, the nanowires penetrated directly membranes of neurons cultured on them. If not, a short voltage pulse was used to electropermeabilize cell membranes to promote the penetration of nanowires. Intracellular recording improved the signal-to-noise ratio and facilitated the measurement of postsynaptic potentials. This platform combined the high scalability of MEAs with the intracellular penetration capability of SiNWs, realizing simultaneously electrical interfacing to multiple neurons and high single-cell resolution.

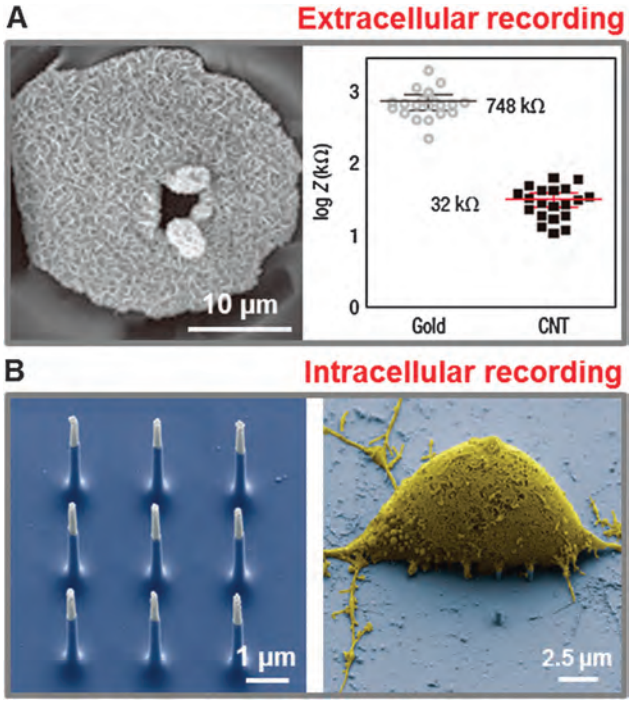

Fig. 9 Three-dimensional nano-biointerfaces for electrophysiology recording. (A) CNT coatings on flat metal electrodes enhance effective surface area, reduce impedance and increase charge transfer, therefore improving extracellular neuronal recording. (B) Vertical Ti/Au coated SiNWs integrated with MEAs for simultaneously intracellular recording of multiple neurons with high single-cell resolution. (A, B) Adapted from ref. 36 Copyright 2008 and ref. 38 Copyright 2012, Nature Publishing Group.

4. Three-dimensional nano-biointerfaces for biomedical applications

4.1 Cancer cell detection

4.1.1 Rare cell capture and isolation. By combining the specific molecule recognition with the topographical interactions between cell surface structures and substrate structures, three-dimensional nano-biointerfaces have been designed for rare cell capture and isolation from whole blood. The rapid and controllable cell adhesion/detachment capability makes three-dimensional nano-biointerface a new platform for high-quality rare cell detection and cancer diagnosis.

The detection of rare cells such as circulating tumor cells (CTCs) is very important for early diagnosis of metastasis and disease treatment of malignant epithelial tumors. However, it remains a great technical challenge as the number of target tumor cells in peripheral blood is very rare. Traditional isolation methods are mainly based on cell size, density, and specific molecule, lacking of specificity and efficiency. After the first exploration of SiNWs,[13] a series of three-dimensional nano-biointerfaces have been developed for highly-efficient capture of CTCs. For example, Au nanoclusters embedded silicon nanowires (Au–SiNWs) were fabricated through rapid thermal chemical vapor deposition, and then modified with anti-EpCAM antibodies for capturing target tumor cells (MCF7, breast cancer cell line) (Fig. 10A, the top part).[39] EpCAM (epithelial cell adhesion molecule) is an overexpressed protein on the membrane of most epithelial cancer cells. The related EpCAM antibody is therefore produced and widely used for recognizing CTCs. Compared with smooth Au substrate, the Au–SiNW substrate showed increased cell-capture efficiency due to the enhanced topographical interactions. After the cell capture, the Au–SiNW substrate was further used for photothermal killing of cancer cells through the irradiation of NIR light.[39] Utilizing candle soot as the template, silica coatings with dendritic nanostructures were formed by chemical vapor deposition of silicon tetrachloride and then calcination. The nanodendritic silica coatings achieved higher capture efficiency of target cancer cells (*i.e.*, MCF7) compared with flat quartz substrates after anti-EpCAM modification.[40] They also showed the potential for clinical diagnosis with the capability to capture MCF7 cells of rare number from artificial blood samples. The underwater transparent character of nanodendritic coatings facilitates the direct monitoring and detection of cancer cells (Fig. 10A, the bottom part). These studies provide novel ideas for realizing multifunctional three-dimensional nano-biointerfaces for highly-efficient rare cell detection to benefit cancer treatment as well as cancer biological study.

Three-dimensional nano-biointerfaces have also been applied for the diagnosis of other diseases such as leukemia and lymphoma by detecting the related rare cells. For example, DNA aptamer modified SiNW substrate was used for highly-efficient recognition and capturing of target T lymphocytes.[22] The DNA aptamer was synthesized to have specific affinity to target T cells (CCRF-CEM, a human acute lymphoblastic leukemia T cell line) while have low affinity with the control cells (Ramos, a human B lymphocyte line). The result showed that the DNA aptamer modified SiNWs achieved higher capture efficiency of CCRF-CEM compared with the related smooth Si substrate while having low non-specific Ramos adhesion (Fig. 10B, the top part), providing a probable way for efficient leukemia treatment. In another example, cholesteryl-succinyl silane based nanofibrous lipid membranes were fabricated using electrostatic spinning. The lipid membranes, with the ability of cholesterol, were functionally immobilized with membrane proteins murine anti-CD20 monoclonal antibodies (mAbs) just by immersing the membrane in mAb solutions. The mAb functionalized nanofibrous lipid membranes further realized specific recognition and capture of human mantle cell lymphoma Granta-22 cells (Fig. 10B, the bottom part).[41]

Three-dimensional nano-biointerfaces such as antibody modified SiNWs has also been integrated with a microfluidic system for highly-efficient capture of rare cells (*i.e.*, CTCs) from patient blood samples.[42] The three-dimensional nanostructure enhanced the topographical interactions between cells and substrate. Moreover, the serpentine chaotic mixing channel of microfluidic system increased the contacting frequency between cells and substrate. These two factors make the integrated microchip a powerful platform for CTCs capture.[42]

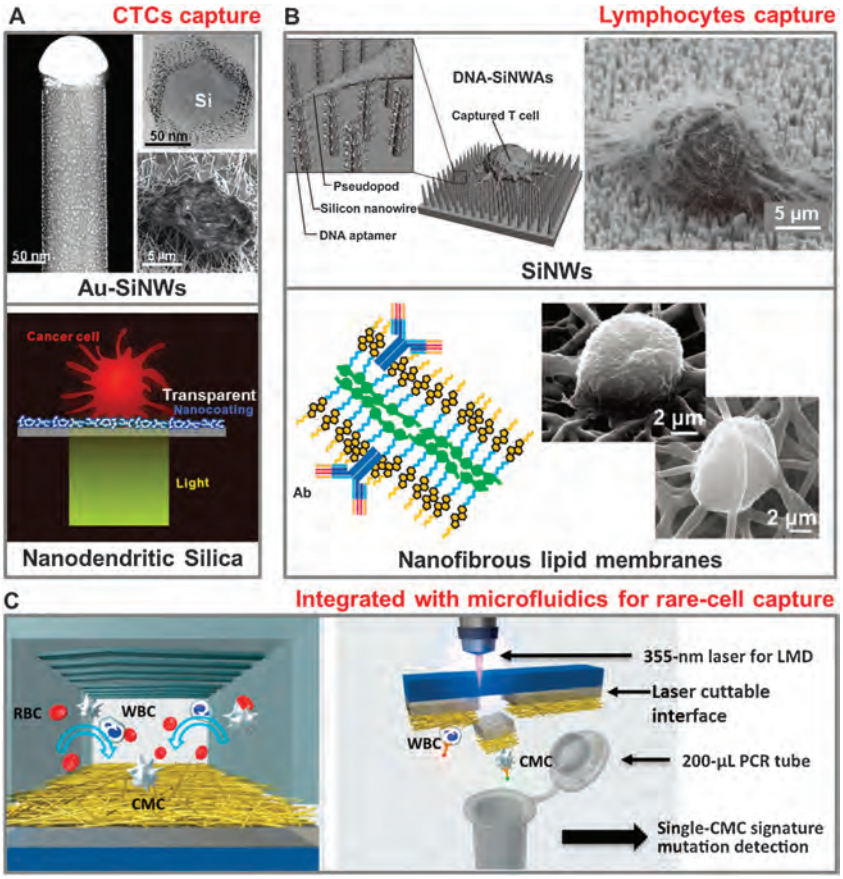

Fig. 10 Three-dimensional nano-biointerfaces for rare cell capture and detection: (A) for capturing CTCs; (B) for capturing lymphocytes; (C) integrated with microfluidics for rare-cell capture. (A) Au nanoclusters embedded SiNWs modified by anti-EpCAM for CTCs capture and photothermal therapy (the top part); and transparent silica nanodendritic coatings modified by anti-EpCAM for CTCs capture and direct monitoring (the bottom part). (B) Aptamer modified SiNWs for T lymphocytes capture (the top part); and electrospun nanofibrous lipid membranes with immobilized antibodies for capturing B lymphoma cells (the bottom part). (C) PLGA nanofibers integrated with microfluidic chaotic mixing chip for highly-efficient CTCs capture. By adding a layer of LMD film into the nano-biointerface, pure and single CTC captured on the substrate was collected through laser cutting for further single-cell molecule analysis. The top part of (A): adapted from ref. 39 Copyright 2012, American Chemical Society. The bottom part of (A), the top and bottom parts of (B), and (C): adapted from ref. 40 Copyright 2013, ref. 22 Copyright 2011, ref. 41 Copyright 2011, and ref. 43 Copyright 2013, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Compared with commercial CTCs detection devices, *e.g.*, the Cell Search system, the microchip is more feasible, convenient, and economical. In another example, poly(lactic-*co*-glycolic) acid (PLGA) nanofibers were electrospun onto laser microdissection (LMD) film, and then integrated with PDMS chaotic mixer. The integration of LMD film within the nano-biointerface endows this multifunctional microchip with the capability to release single CTC accurately and conduct molecular analysis of pure single cells conveniently (Fig. 10C).[43]

4.1.2 Cancer-cell electrochemical detection. By detecting the abnormal and overexpressed components such as proteins, glycans, and receptors on cancer-cell surfaces, cancer cells can be detected, monitored, and analyzed. Electrochemical detection provides a promising avenue because of its convenient and nondestructive characteristics although many other intelligent methods have also been used. In recent years, three-dimensional nano-biointerfaces have also been gradually explored as electrochemical cytosensor for highly-efficient detection and analysis of cancer cells and cancer-cell surface molecules. RGD–SWNTs nanocomposited electrodes were used for cancer-cell capture and the following glycomics studies.[20] In brief, screen-printed carbon electrodes (SPCE) were coated with RGD functionalized SWNTs, and cancer cells (K562 leukemia cells) were then captured on the nanocomposited electrodes through the affinity interaction between cell–surface integrins and RGD peptides. Horseradish peroxidase (HRP) labeled lectins were then used to recognize and conjugate the specific glycans on cancer-cell surfaces. The amount of immobilized HRP was further detected and analyzed through the characteristic electrochemical signals of enzyme catalysis in H_2O_2/*o*-phenylenediamine solution, reflecting the quantity of glycans on cancer-cell surfaces (Fig. 11A).[20] By using four different lectins, the expression degrees of four different glycans were simultaneously analyzed on electrochemical cytosensor arrays containing four nanocomposited electrodes. Compared with flat graphite electrode, the SWNT-composited electrode

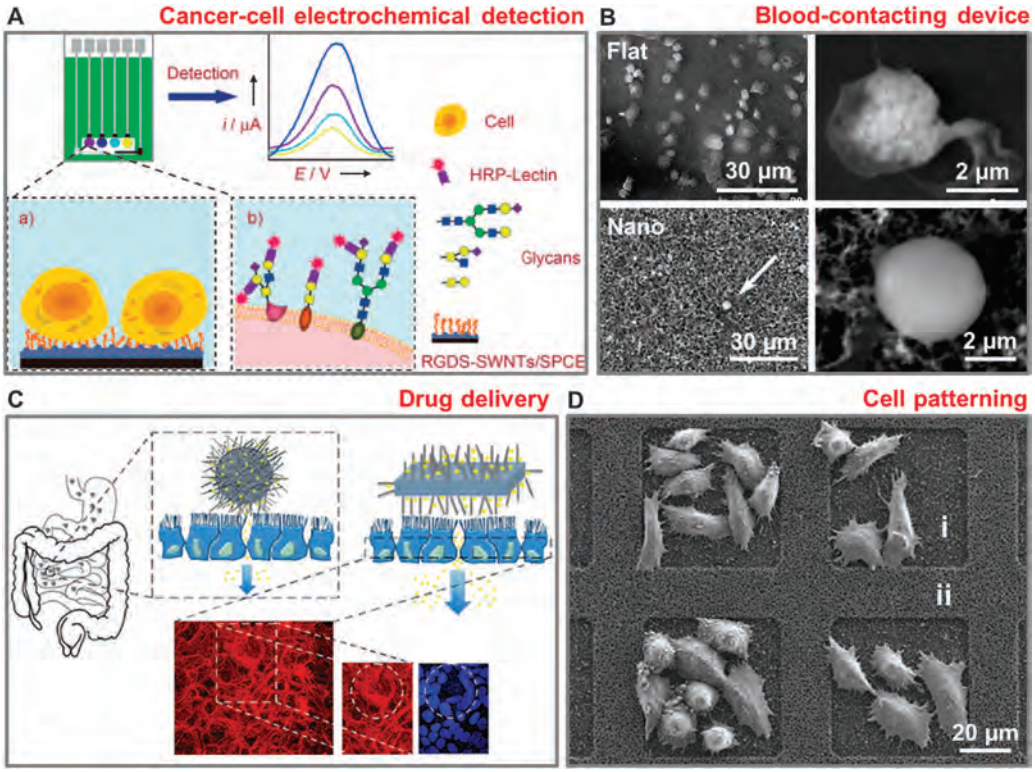

Fig. 11 Three-dimensional nano-biointerfaces for biomedical applications: cancer-cell electrochemical detection; blood-contacting device; drug/gene delivery; and cell patterning. (A) HRP-lectin modified SWNTs–SPCE for dynamic electrochemical detection of glycan on cancer-cell surfaces. (B) Fluorinated PCU–CNT nanocomposited surfaces for effective reduction of platelet adhesion. (C) Shape effect of SiNW coated microparticles for adhesion based drug delivery to intestinal mucosa. (D) Superhydrophilic (i) superhydrophobic (ii) SiNW micropatterns for cell patterning. (A, B) Adapted from ref. 20 Copyright 2009 and ref. 45 Copyright 2005, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Adapted from ref. 48 Copyright 2012, American Chemical Society. (D) Adapted from ref. 50.

produces a three-dimensional contact model for increased glycan recognition and enhanced signal, providing a sensitive, facile, and high-throughput platform for dynamic analysis of cell–surface glycans during drug induction as well as the discovery of novel therapeutic targets.

Artificial lectin 3-aminophenylboronic acid (APBA) functionalized multiwalled carbon nanotube (MWNT) film coated glassy carbon electrode was used as the electrochemical cytosensor for the capture and impedance detection of K562 cells.[44] Through the affinity interactions between cell–surface glycans and boronic acid group of APBA, cells were captured on the nanocomposited electrode. Using fructose as a competitor and mediator, captured cells were then released as a result of the reversible binding of APBA with 1,2- or 1,3-diols, realizing the reusable detection of the electrode.[44] Compared to direct electropolymerization of APBA on electrode, the nanocomposited APBA–MWNT coated electrode provides more boronic acid sites for highly-efficient cell capture. The high conductivity of carbon nanotubes and the porous structures also make the nanocomposited electrode more sensitive for electrochemical impedance cytosensing using $[Fe(CN)_6]^{3-/4-}$ as a redox probe. In brief, these nanocomposited cytosensors based on three-dimensional nano-biointerfaces will provide a novel platform for the effective and nondestructive cell sensing and detection, as well as the direct monitoring of cell activities such as proliferation and apoptosis.

4.2 Blood-contacting device

The blood compatibility of implants and other medical devices is one of the most important factors influencing their clinical applications. One essential subject is to avoid the adhesion and activation of platelets on these blood-contacting devices. Besides traditional chemical and biological surface treatments of material surfaces, efforts have also been gradually devoted to create surface topography on blood-contacting materials for efficient anti-platelet adhesion. Microtextures and multiscaled microstructures have been designed to reduce platelet adhesion and enhance blood compatibility of materials. Recently, three-dimensional nano-biointerfaces have also been explored for the potential usage in blood-contacting devices.

Using vertical CNT arrays as the template, a layer of fluorinated poly(carbonate urethane)s (PCUs) was coated on CNT arrays to form nanostructured PCU surfaces.[45] Superhydrophobicity was reached on the surfaces by the cooperation of fluorinated components and nanostructures of PCU, reducing the contacting area of water solution as well as proteins and platelets in the solution on the surfaces. Largely improved blood compatibility was observed on the fluorinated

nanostructured PCU surfaces compared to the corresponding smooth ones (Fig. 11B). This work demonstrated the novel nanostructure effect by introducing superhydrophobicity to reduce protein adsorption and therefore platelet adhesion.

Recently, by grafting pNIPAM on SiNWs, this three-dimensional nanostructured pNIPAM surface exhibited largely reduced platelet adhesion above the LCST than the smooth pNIPAM surface grafted on smooth Si.[46] This result is due to the capability of nanostructures to maintain a relatively high ratio of water content on surface. When the temperature was below LCST, both of the nanostructured and smooth pNIPAM surfaces showed reduced platelet adhesion due to the high water content of both surfaces. These studies provide new ideas and open a new avenue for successful blood-contacting medical devices besides traditional surface treatment methods of materials.

4.3 Drug/gene delivery

Nanotechnology has been contributing significantly to drug/gene delivery for disease therapy and drug discovery since 1960s. A variety of nanocarriers such as liposomes, polymersomes, and nanoparticles have been used for ''cargo'' delivery with improved efficiency and safety. Since 1998, microneedle arrays have also been applied for enhanced transdermal drug and vaccine delivery using silicon or hydrogel materials. In recent years, three-dimensional nano-biointerfaces have also been designed and explored for highly efficient drug/gene delivery by taking advantage of their enhanced topographical interactions with cells and their capability to penetrate into cell interior.

A tight adhesion of drug carriers to the epithelium of mucous membranes is essential for realizing long-term and efficient drug delivery to small intestines. Epithelial targeting agents have been used for adhesive drug carriers, however, with reduced efficiency of epithelium binding because the mucous layer also possesses binding sites. Recently, by coating SiNWs onto spherical glass microparticles, the nanowire-coated microparticles realized geometrically enhanced adhesion to Caco-2 cell monolayer (an *in vitro* model of the intestinal mucosa) than the corresponding uncoated ones.[47] This enhanced adhesion stems from the topographical interactions between nanowires and nanostructured microvilli on the cell surfaces (Fig. 11C). When exposed in physiological conditions such as shear flow, the nanowire-coated microparticles exhibited better adhesion performance with enhanced lift-off force than microparticles with target agents. The shape of microparticles also impacts on the drug delivery efficiency to mucous membranes.[48] Compared to the spherical microparticles, the planar microparticles increased the drug-loading efficiency due to their three-times larger surface area per unit weight. Moreover, the planar microparticles also increased the transepithelial permeation of insulin, a model drug with moderate size, owning to the enhanced contacting area with cell layer (Fig. 11C). Thus, the nanowire-coated microparticles can act as a novel inorganic bioadhesive platform for mucosal drug delivery and other clinical applications.

SiNWs are also able to directly access cell interiors by physically penetrating cell membrane. Utilizing this capability, SiNW arrays have been used as a generalized platform for drug/gene delivery into various types of cells. By pre-modifying SiNWs with DNAs, siRNAs, and peptides, high-throughput delivery and co-delivery were realized spatially localized or as a microarray form to realize the control of cell activities and functions.[19] For example, by culturing on apoptosis-inhibiting peptide modified SiNWs, HeLa cells in culture media with apoptosis-inducing agents did not undergo apoptosis. But on SiNWs without the peptide cells underwent apoptosis rapidly. The SiNW-based universal platform is anticipated to be applied in wide areas such as drug discovery, gene therapy, and cellular reprogramming.

4.4 Cell patterning

Cell patterning at the biointerfaces has great implications for cell behavior studies, cell communication understanding, tissue engineering, genome/drug screening, *etc.* Traditional cell patterning is always achieved by chemical modification of adhesive/anti-adhesive molecules on smooth surfaces. These methods suffer from the cross contamination of cells/molecules and the migration of cells. Recently, by using three-dimensional nano-biointerfaces, the unique ''air-grid surface patterning'' strategy has been developed to achieve efficient cell patterns and microassays. This strategy generates cell pattern in confined areas without using biological or chemical agents, facilitating cell transfection efficiency for cell microassays, and multiple cell co-cultures with controlled spatial arrangement and geometry for studying cell–cell communications.

The air-grid surface patterning is achieved by creating micropatterned superhydrophilic spots separated by superhydrophobic gaps on three-dimensional nanostructures. The superhydrophilic spots efficiently support protein adsorption, cell adhesion, as well as cell survival. On the other hand, the ''Cassie–Baxter'' wetting state of water on superhydrophobic surfaces not only reduces water contacting area but also retains a layer of air, making the superhydrophobic gaps act as air grids to reach surprising protein and cell resistance. As a result, the attachment, adhesion, and migration of cells, and the cross contamination can be efficiently prevented by the superhydrophobic barriers. Several superhydrophilic/superhydrophobic micropatterns have been achieved on three-dimensional nanostructures with the help of micropatterning techniques. Using UV-initiated photopolymerization, superhydrophilic poly(2-hydroxyethyl methacrylateco-ethylene dimethacrylate) layer with nanoporous structures was coated on a glass plate. Then, using a photomask, superhydrophobic grid-like pattern was created on the superhydrophilic layer by photografting poly(2,2,3,3,3-pentafluoropropyl methacrylate-*co*-ethylene dimethacrylate) brushes. Finally, high-density cell microassays were created on the superhydrophilic/superhydrophobic micropatterns towards cell transfection studies.[49]

In addition to this kind of polymer-based micropatterns, inorganic three-dimensional nanostructures have also been utilized for creating superhydrophilic/superhydrophobic

micropatterns. By modifying SiNWs with octadecyltrichlorosilane, superhydrophobic surface was obtained. Using photolithography and oxygen plasma, superhydrophilic spots were produced by removing silane. The superhydrophilic/superhydrophobic micropatterns were further used for patterning and culturing Chinese Hamster Ovary K1 cells (Fig. 11D).[50] Different topographical interactions between cell surface structures and SiNWs were observed on the superhydrophilic/superhydrophobic areas. On the superhydrophilic surface, cell filopodia penetrated into the interspaces of SiNWs, leading to a strong adhesion. On the superhydrophobic surface, in contrast, cell filopodia just remained on the top of SiNWs, resulting in a weak adhesion. This weak adhesion also facilitated the formation of cell patterns as the occasionally adhered cells on the superhydrophobic barriers were easily removed away by simply PBS rinsing.

5. Conclusions and prospects

In this tutorial review, we have summarized the recent progress of three-dimensional nano-biointerfaces as an emerging platform for cell-related fundamental biological studies and advanced biomedical applications. The great value of three-dimensional nano-biointerfaces stems from its unique capabilities to guide cell fate in a controllable and accurate way, such as the enhancement of cell adhesion,[13,15,25] the control of cell adhesion/detachment[14,23,26] and stem-cell differentiation,[16,17,29] the help of cell interconnection to outside,[18,32] and the promotion of cell sensing to external mechno-/electrical signals.[34–38] During the exploration of three-dimensional nano-biointerfaces, the bio-inspired principle utilized for interface design is worth high approval and further research. Mimicking the interior disciplines of biological systems, such as the nanofibrous structures of ECM and the fractal morphology of cancer cells, some advanced three-dimensional nano-biointerfaces have been created for controlling cell behaviors and facilitating clinical detections.[12–15] In the human body, there are still many other three-dimensional nano-biointerfaces, such as the material-exchange interface of small intestine,[9] the air-exchange interface of lung, and the signal-exchange interface of eye. Learning from these interfaces will benefit the further design of three-dimensional nano-biointerfaces towards advanced biological sciences and technologies, to resolve tough problems faced by human health and progress. In spite of this, the exploration of three-dimensional nano-biointerfaces is still at an infant stage. There are crucial questions and challenges for the current and further directions of three-dimensional nano-biointerfaces that must be addressed.

From the aspect of fundamental studies, the design principle of three-dimensional nano-biointerfaces remains uncertain. The current design is mainly focused on the chemical and topographical factors inspired by human biological systems. There are actually many other factors in cell microenvironments that regulate cell activities and functions.[2] A proper regularity for designing three-dimensional nano-biointerfaces needs to be further explored and concluded. The integration of chemical (*e.g.*, specific molecule recognition), physical (*e.g.*, nanoscaled topographical interaction), mechanical (*e.g.*, tissue-like stiffness), soluble (*e.g.*, growth factors), as well as fluidic (*e.g.*, suitable fluid velocity that close to that of biological systems) factors together on the three-dimensional nano-biointerfaces is meaningful for mimicking *in vivo* cell microenvironments more precisely. Programmable biointerfaces with controlled surface topography will be a suitable avenue for systematically investigating the interactions between cells and materials, and guiding the design principle of three-dimensional nano-biointerfaces. Chiral recognition is also an important process of live activities.[8] The combination of chiral molecules with three-dimensional nanostructures may bring a new platform for understanding cell behaviors and biological phenomena. In addition, it is necessary to build smart and multifunctional three-dimensional nano-biointerfaces with multiple stimuli responsiveness, as it is a spatiotemporally changing microenvironment of biological system. From this perspective, multiple-responsive design of molecules, structures, and stiffness needs to be carried out on the nano-biointerfaces, for realizing a composited platform for comprehensive cell control. This will be a great challenge for current methods and technologies of material selection, structure fabrication, and chemical modification.

Additionally, suitable and accurate characterization methods of three-dimensional nano-biointerfaces are still under development. Although traditional environmental scanning electron microscopy has provided plenty of information on cell–substrate interactions at three-dimensional nanoscales, it is still hard to characterize live cells. In this respect, bioscope atomic force microscopy is a potential direction for further dynamic and *in situ* observing and detecting live cells without losing cell activities. Fluorescence microscopy is another main characterization method used. Currently fluorescence imaging at two dimensions is easy, however at three dimensions it remains difficult. To further realize the high resolution and high speed simultaneously for three-dimensional nanoscaled imaging will benefit finely and intimately investigating live cells to get more information. Besides, it is also hard to characterize the surface chemistry of three-dimensional nano-biointerfaces, which is very important for analyzing the modification quality of the interfaces, such as the configuration, density, and thickness of grafted molecules. A further integration of multiple characterization methods for three-dimensional nano-biointerfaces will be the final goal of biological studies and applications.

From the aspect of biomedical applications, some special capabilities of three-dimensional nano-biointerfaces offer great opportunities for developing advanced biological technologies to realize high-quality *in vitro* and *in vivo* biological detection and diagnosis. For example, the penetration of three-dimensional nano-biointerfaces into cell interiors not only helps the material exchanges between cells and outside to facilitate drug screening and gene therapy,[18,19] but also

provides a platform for controlling cell internal clocks and cycles. This will help stem-cell studies achieve the ultimate control of cell fate and realizing stem-cell based treatment and regenerative medicine. On the other hand, three-dimensional nano-biointerfaces have shown to improve the electrophysiological recording of neurons.[36–38] In the future, the designability and integratability of three-dimensional nano-biointerfaces will promote the appearance of new commercial technologies better than current devices such as patch clamps and traditional neural prosthesis. From this view, biocompatibility and bio-stability are also two important factors of three-dimensional nano-biointerfaces that need consideration. A proper interface with optimal mechanical properties, bio-stability, and surface chemical properties needs to be verified in detail.

Furthermore, three-dimensional nano-biointerfaces can play a function like the nano-villi and podia of cell surface due to its capabilities to promote cell/microenvironment material/information exchanges. This similarity will be helpful for designing artificial cells with biological functions. For example, by fabricating microspheres with surface nanostructures and then coating these microspheres with cellular membrane proteins or functional molecules, the composited microspheres can act as primary artificial cells to interact with other cells. If hollow porous microspheres are used in the above case, the microspheres can be further loaded with functional materials to impact the outside. These kinds of artificial cells based on three-dimensional nano-biointerfaces may provide another choice in addition to the traditional molecule-scaled construction strategy, to realize the better understanding of biological activities and processes.

Therefore, in the future, three-dimensional nano-biointerface is destined to play a huge impact on the arena of surface chemistry, nanotechnology, and biomedical applications. New phenomena and developments are expected to be achieved by the cooperation and endeavor from different communities of chemists, physicists, biologist, nano-scientists, engineers, and clinicians, *etc.*

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