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Expert Opinion

1. Introduction
2. Tissue engineering
3. Drug discovery
4. Conclusions
5. Expert opinion

Micro- and nanoscale technologies for tissue engineering and drug discovery applications

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Micro- and nanoscale technologies are emerging as powerful enabling tools for tissue engineering and drug discovery. In tissue engineering, micro- and nanotechnologies can be used to fabricate biomimetic scaffolds with increased complexity and vascularization. Furthermore, these technologies can be used to control the cellular microenvironment (i.e., cell–cell, cell–matrix and cell–soluble factor interactions) in a reproducible manner and with high temporal and spatial resolution. In drug discovery, miniaturized platforms based on micro- and nanotechnology can be used to precisely control the fluid flow, enable high-throughput screening, and minimize sample or reagent volumes. In addition, these systems enhance reproducibility and significantly reduce reaction times. This paper reviews the recent developments in the field of micro- and nanoscale technology and gives examples of their tissue engineering and drug discovery applications.

Keywords: drug discovery, microfluidic, nanotechnology, surface pattering, tissue engineering

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1. Introduction

Tissue and organ failure are serious and common medical conditions for which treatment options include organ transplantation, surgical repair, artificial prostheses, and drug therapy [1-3]. Transplantation is frequently hindered by the lack of tissue donors. To address this challenge, tissue engineering approaches are being developed to generate functional three-dimensional (3D) tissues. In the field of drug therapy, a significant effort has been made by pharmaceutical companies to find new therapeutic agents. However, despite increasing investments in the drug discovery process, only a few drugs are approved annually. Both tissue engineering and drug discovery have been hindered by a number of scientific and technical challenges including the inability to precisely control the spatial and temporal features of the cellular microenvironment, the lack of materials with desired functional properties, the requirement for large sample volumes, low throughput and slow reaction times.

Micro- and nanotechnologies can be used to fabricate materials with specified structures and functional properties to address these limitations [1,4-7]. **Figure 1** shows selected micro- and nanoscale approaches to tissue engineering and drug discovery. Microfluidic platforms, surface micropatterning and 3D nanofibrous scaffolds can be used to control the extracellular microenvironment, such as cell–cell, cell–extracellular matrix (ECM), and cell–soluble factor interactions, for basic biology and tissue engineering studies. By using engineered tissue platforms, complex human normal and disease models may be built up for

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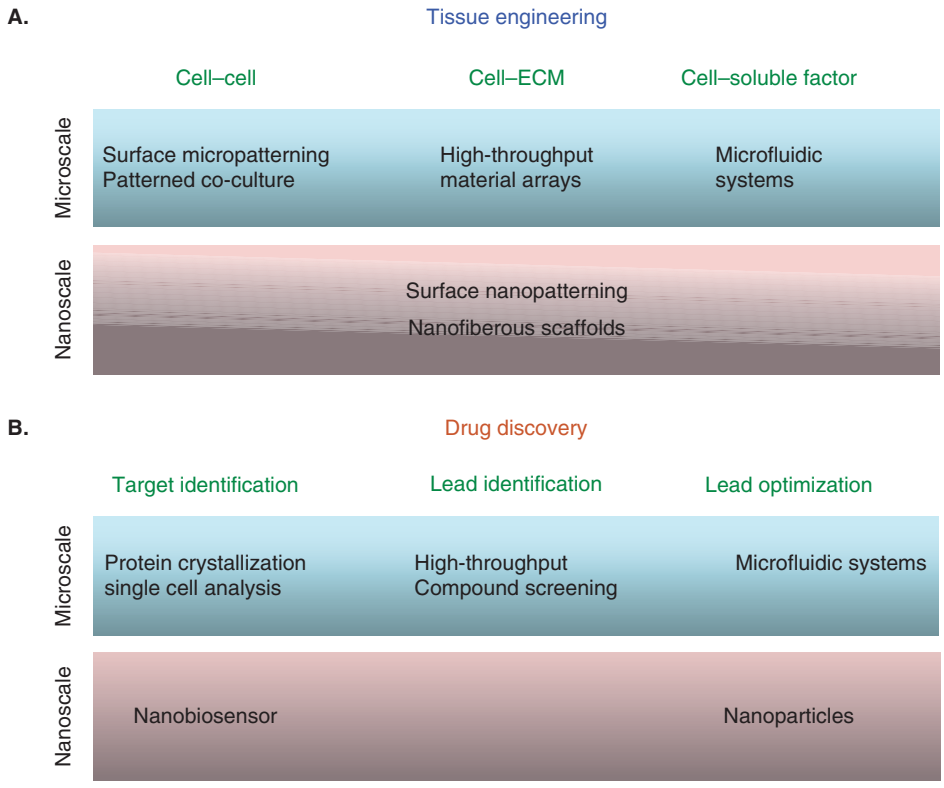

Figure 1. Micro- and nanoscale approaches used in tissue engineering and drug discovery. A. A variety of micro- and nanoscale technologies can be used for tissue engineering studies for generating 3D tissues as well as for controlling different cell–microenvironment interactions. **B.** Micro- and nanoscale methods can also be used for drug discovery applications to identify potential drug targets as well as for lead optimization.

drug discovery process. Target validation and preclinical toxicology are two potential application areas within drug discovery. Moreover, micro- and nanoscale tools can be used to perform cell sorting, high-throughput screening, protein crystallization and biosensing for drug discovery studies.

Microtechnologies that have been adapted from the microelectronic industry typically involve top-down fabrication approaches, such as photolithography, microcontact printing and micromolding. Due to their wide range of fabrication length scales, which span from features that are much smaller than cells to as large as tissues, microscale technologies have been increasingly used for cell biology and biochemical analysis as well as chemical synthesis [4,7,8]. For example, integrated microfluidic devices present several advantages to biological studies by precisely manipulating extracellular microenvironments and enabling high-throughput experiments [9,10]. Microscale technologies can also be used to fabricate 3D hydrogel scaffolds with controlled cell–cell interactions [11]. In contrast, nanotechnology involves features that are 1 – 100 nm in dimensions. Nanotechnology can be used to synthesize nanomaterials with properties that are often different from their bulk materials [12]. Examples of nanoscale technologies for tissue engineering and drug discovery applications include nanofibrous scaffolds, nanopatterned substrates, controlled release nanoparticles and quantum dots [13]. This review provides a broad overview of the recent developments in the application of micro- and nanotechnologies to tissue engineering and drug discovery. Due to space limitations, a comprehensive review of this area is beyond the scope of this manuscript. Interested readers are directed to additional resources for further readings on this topic [1,13,14].

2. Tissue engineering

Tissue engineering is an interdisciplinary field at the interface of engineering, materials science, medicine and biology [1,15,16]. In typical tissue engineering approaches, cells are seeded onto a 3D biodegradable scaffold. As cells deposit their own matrix, the scaffold degrades, resulting in the formation of a biological tissue construct. Critical limitations with present tissue engineering techniques include the inability to create vascularized tissue constructs, the insufficient mechanical strength of engineered tissues and the lack of a suitable source of functional cells that are immunologically compatible with the host. To address these challenges, micro- and nanoscale-based platforms can be used to generate scaffolds to control tissue formation.

In addition, such technologies can be used to manipulate the cellular microenvironment and, in turn, influence cellular behavior.

2.1 Microtechnologies for tissue engineering

2.1.1 Cell patterning for controlling cell shape

Cell microarrays in which cells are selectively localized to specific regions of a substrate are useful tools for performing high-throughput experiments or for controlling cells shape as well as cell–matrix and cell–cell interactions [1]. Using micropatterned substrates, it was demonstrated that the differentiation of human mesenchymal stem cells is a function of cell shape [17]. Adipogenesis was induced on small islands, resulting in round cells. On the other hand, osteogenesis was induced on fully spread cells that attached to larger adhesive islands.

Microarrays can also be used to track cell behavior on a microfabricated platform. Chin and colleagues reported using a microwell array for the clonal tracking of adult hippocampal progenitor cells infected with a retrovirus to express green fluorescent protein (GFP) upon differentiation to neural lineage [18]. A large number of GFP-positive cells could be tracked in each microfabricated well to analyze the progeny of stem cells in a high-throughput manner. Similarly, a microwell device made by photopatterning poly(ethylene glycol)-diacrylate hydrogel was used to generate microarrays for hepatocyte culture [19]. Hepatocytes were micropatterned and allowed to interact with collagen-modified regions inside the hydrogel microwells to study hepatocellular behavior. Recently, microfabricated poly(ethylene glycol) (PEG) wells have been used to initiate the formation of embryoid bodies (EBs) in a controllable manner for stem cell differentiation [20]. Embryonic stem-cell (ESC) aggregates were formed with desired sizes and shapes as defined by the geometry of the microwells. EBs generated in this manner remained viable and resulted in a more homogenous differentiation response than EBs formed in suspension. The micropatterned cell substrates can also be incorporated into microfluidic channels to enable high-throughput testing of soluble microenvironmental parameters on cell behavior [21]. Many cell types exhibit improved function in native tissues in comparison with two-dimensional tissue culture substrates because the 3D environment favorably alters the interactions of cellular receptors as well as the resulting cell shape and polarity [1]. To generate 3D cell microarrays, cells can be encapsulated within micropatterned hydrogels by using photolithography [11] and dielectrophoretic forces [22,23].

2.1.2 Patterned co-cultures for studying cell–cell interactions

Cell–cell contact is important for a variety of biological processes, such as cell proliferation and differentiation. A number of surface patterning techniques [24], such as layer-by-layer deposition [25-28], stencil micropatterning [29,30], and topological patterning [31], were used to enable the control of cell–cell contact *in vitro*. For example, Chen and co-workers developed a micropatterned substrate in which cells were grown on adhesive islands to control the degree of cell–cell contact (**Figure 2A**) [32]. The study showed that cells grown in pairs proliferated more than single cells.

Micropatterning approaches can also be used to control cell–cell interactions for a larger number of cells. Bhatia and colleagues used photolithography to co-culture hepatocytes and fibroblasts on micropatterned substrates in a controlled manner, to study the effects of non-parenchymal cell contact on hepatocyte phenotype maintenance [33]. These studies revealed a number of critical interactions for maintaining hepatocyte phenotype in culture. In addition to photolithography, patterned co-cultures were created using layer-by-layer deposition of ionic polymers [25]. For example, Khademhosseini and colleagues have generated co-cultures of ESCs and NIH-3T3 fibroblasts on fibronectin islands using layer-by-layer deposition of hyaluronic acid (HA) with poly-L-lysine and HA with collagen [25,34]. In these examples, the addition of each layer could be used to switch the surface adhesiveness, thus enabling the formation of a monolayer of cells around an original pattern. Layer-by-layer assembly [26-28], an emerging tool for functional thin film fabrication, was developed by alternating deposition of poly(ethylene oxide) and poly(acrylic acid) layers [26]. The total film thickness of this hydrogen bonded poly(ethylene oxide)/poly(acrylic acid) film was decreased with an increasing pH of the assembly solution, and layer-by-layer assembly was modulated by adjusting the ionic strength of the deposition solution. Microscale topographies can also be used to generate patterned co-cultures by enabling the sequential docking of cells on a substrate (**Figure 2B**) [31]. For example, patterned co-cultures of human ESCs and murine feeder cells could be generated on a microwell patterned substrate. In this approach, human ESCs were seeded within microwells and co-cultured with mouse embryonic fibroblast cells.

The dynamics of cell–cell contact is also important for a number of biological applications, such as wound healing and morphogenesis. To fabricate patterned co-cultures with temporal control, a micromachined silicon platform consisting of two interdigitating pieces was developed to dynamically manipulate cell–cell interactions [35]. In this system, the distance between the interdigitating plates can be set to control the proximity between different cell types. Using this device, the dynamics of intercellular communication between hepatocytes and stromal cells in co-cultures was analyzed to demonstrate that the maintenance of the hepatocytes required small distances (< 400 μm) from stromal cells. Patterned cells in a co-culture system can also be formed by reversible sealing of microfabricated stencils. For example, parylene-C stencils were used to generate micropatterns of proteins and cells including NIH-3T3 fibroblasts, hepatocytes and ESCs [30]. These studies

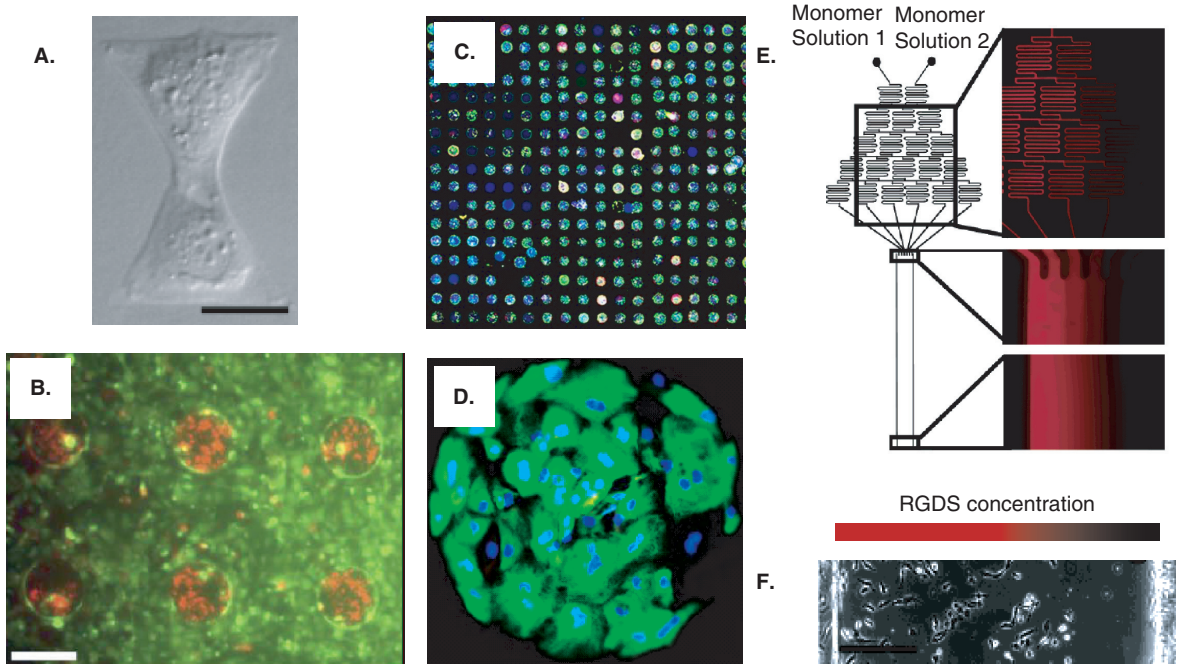

Figure 2. Microscale approaches to control cell–cell, cell–ECM, and cell–soluble factor interactions A. Effect of cell–cell contact on proliferation. A differential interference constrast image of two cells patterned in an agarose microwell. Scale bar is 25 µm. (Reprinted with permission from NELSON *et al.*: Copyright (2003), The Company of Biologists) [32]. **B.** A fluorescence image of human ESCs and mouse embryonic fibroblasts in a patterned co-culture after 1 day. Scale bar is 200 µm. (Reprinted with permission from KHADEMHOSSEINI *et al.*: Copyright (2006), Elsevier) [31]. **C.** High-throughput testing of biomaterial arrays on proliferation and differentiation of human ESCs. The image shows fluorescently labeled cells on polymer microarrays containing a library of polymers. **D.** A close-up image of a single polymer pattern that was seeded with human ESCs. Cells were stained for cytokeratin 7 (green) and DNA/nucleus marker SYTO24 (blue). (Reprinted by permission from Macmillan Publishers Ltd: ANDERSON *et al.*: *Nat. Biotechnol.* (2004) **22**:863-866, Copyright (2004)) [36]. **E.** Hydrogels can be fabricated with gradients of various properties imbedded in the bulk materials. Schematic diagram and the corresponding fluorescent image of a microfluidic gradient generator. **F.** Endothelial cells attached to the regions of the hydrogel that contained high RGDS concentrations. Scale bar is 200 µm. (Reprinted with permission from BURDICK *et al.*: Copyright (2004), American Chemical Society) [46].

ECM: Extracellular matrix; ESC: Embryonic stem cell; RGDS: .

generated techniques to finely control the degree of cell–cell contact with applications ranging from fundamental cell biology to tissue engineering.

2.1.3 *High-throughput arrays for tissue engineering*

Microtechnologies can be used to miniaturize experiments to facilitate high-throughput analysis [1]. High-throughput arrays are emerging as important tools to test the effect of large combinatorial libraries of biomaterials, environmental stimuli, and chemicals on cell behavior. For example, high-throughput arrays of materials were fabricated by using robotic spotters [36,37]. Langer and colleagues developed a high-throughput polymer microarray made from combinations of multiple macromers to study the growth and differentiation of human ESCs [36]. **Figure 2C** shows an example of a high-throughput polymer chip consisting of different combinations of acrylated macromers. This miniaturized microarray was used to screen a wide range of cell–ECM and cell–biomaterial interactions (**Figure 2D**). Similarly, an ECM microarray was generated for the analysis of mouse ESC differentiation into hepatic fates in response to various combinations of ECM molecules [37].

Microfluidic systems can also be used to perform high-throughput experiments. The high-throughput capability of microfluidic systems has been greatly improved by the increased sophistication of microfluidic pumping and valving systems. A large number of on-chip valves can be integrated into a single microfluidic device to precisely manipulate nanoliter fluids and enable multiple functions on a single platform [38]. Although such systems can integrate a number of functions, they need to be improved to analyze various biological phenomena in a rapid and reproducible manner. For example, cell lysate, DNA, and mRNA purifications from bacterial cells were studied by using these microfluidic systems [39]. Purification and recovery of mRNA were performed on a single microfluidic chip which was used to analyze different samples in parallel. Also, a real-time dynamic gene-expression chip with embedded microvalve arrays and chambers was used to quantify fluorescent protein transcriptional reporters [40]. Thus, these

high-throughput microscale technologies could be of great promise for studying cell–microenvironment interactions and biological systems.

2.1.4 *Microscale scaffolds*

Biodegradable scaffolds provide encapsulated cells with a 3D geometry to induce tissue formation [2]. Biodegradable polymers have shown great promise as 3D scaffolds for regenerative medicine. There are two types of biodegradable polymers: natural and synthetic. Natural polymers include alginate, chitosan, HA derivatives, collagen, fibrin; synthetic biodegradable polymers include poly(glycolic acid), poly(lactic acid), poly(lactic-*co*-glycolide) (PLGA) and poly(ε-caprolactone) [41,42]. Synthetic biodegradable polymers have been widely used because their mechanical and physical properties, such as degradation rate and stiffness, can be controlled. For example, poly (D,L-lactic acid) is biocompatible and is used as an implant material [43]. PCL can be useful for bone tissue engineering and drug delivery systems because it can entrap antibiotic drugs [44]. In addition, hydrogels using PEG conjugated with the arginine-glycine-aspartic acid (RGD) peptide facilitated the adhesion of osteoblast cells and could be useful for studying bone regeneration [45]. Although these synthetic biodegradable polymers are useful to study tissue engineering, many challenges, such as the lack of vascularization in engineered tissue constructs and precise control, must be addressed before medically-relevant 3D tissue scaffolds can be realized.

Microfluidic systems can be used to synthesize microengineered scaffolds to address these challenges [15]. For example, a microfluidic gradient generator can be used to create hydrogel scaffolds with gradients of signaling molecules (**Figure 2E, F**) [46]. By generating gradients of monomers conjugated with RGDS within the hydrogels, the attachment of endothelial cells along the adhesive peptide gradient can be controlled and characterized. In addition, to overcome the limitations associated with the lack of vascularization, microfabrication technology can be used to fabricate prevascularized scaffolds [47]. For example, poly(glycerol sebacate) (PGS), a biodegradable and biocompatible polymer, was used to create capillary networks by molding the polymer from prefabricated masters with features resembling branching vasculature [47]. Other biodegradable elastomers, such as PLGA, were also used to fabricate capillary networks [1]. These systems may lead to the formation of *in vitro* microvasculatures for use in engineered tissues and organs. In addition, layer-by-layer microfluidic patterning was used to generate biomimetic 3D scaffolds [48,49]. In this approach, sequential deposition of cells and matrix that was molded by a microchannel, were used to generate controllable 3D microstructures of multiple cell types and matrices. Hydrogel microfluidic devices that contained cells in the hydrogels were also fabricated to generate synthetic prefabricated microvasculature [50]. Using this cell-laden hydrogel microfluidic device, cell viability throughout the volume of the construct was optimized and analyzed.

Another microscale application for generating tissue structures is the use of the assembly approach. In this method, building blocks of individual tissue components are generated and subsequently assembled to generate larger structures. Sefton and colleagues used rod shaped microgels that were seeded with hepatocytes and coated with a monolayer of endothelial cells as building blocks. These modular pieces were stacked in a packed bed to generate a tissue-like structure [51]. Alternatively, the shape of the individual pieces can be controlled to enable their assembly by using directed or self-assembly [11]. The modular design and assembly of these approaches can affect many areas of tissue engineering and 3D cell culture.

In addition, surface topography can also affect cell behaviors, such as cell adhesion, proliferation and differentiation in 3D microenvironments. Hemispherical cavities in hexagonal patterns of titanium substrates were used to study the role of microtopography on cellular behavior [52]. It was demonstrated that cells preferentially adhered to cavities of 30 μm and 100 μm diameter, whereas they did not recognize the cavities of 10 μm diameter. Cells attached within 30-μm diameter cavities adopted a 3D shape. Actin cytoskeletal condensation was observed at the cavity edges. Besides titanium substrates, microfabricated quartz substrates were used to study fibroblast attachment and motility [53]. Photolithographic fabrication generated quartz that was similar to the structure of a 3D fibrous gel. It was revealed that the proliferation and motility of fibroblasts were sensitive to the micropit topography. The smaller pit diameter (7 μm) increased fibroblast proliferation rates. Furthermore, pit-patterned surfaces of polystyrene film were used to investigate osteoblast adhesion and proliferation [54]. The hemispherical island-structured poly(L-lactic acid) PLLA surfaces were created by using a polystyrene template with hemispherical pits. It was demonstrated that cell adhesion on PLLA surfaces was enhanced with microscale roughness in comparison to the smooth surfaces. These surface topography techniques could be useful tools for controlling cellular behavior and 3D tissue construct formation.

2.1.5 *Microfluidic systems for spatial control of cell–soluble factor interactions*

Microfluidic systems are powerful tools for controlling the spatial and temporal aspects of cell–soluble factor interactions. The low Reynold's number regimes within microfluidics can be used to limit convective mixing to enable the formation of soluble gradients. Gradient-generating microfluidic devices have been used for real-time monitoring of cell migration, proliferation, differentiation, and apoptosis [55-59]. For example, Jeon and colleagues developed the serpentine gradient generator to study the neutrophil chemotaxic response to IL-8 [60]. Chemotaxis of breast

cancer cells was also investigated in a microfluidic gradient device [61]. It was demonstrated that cancer cells migrated toward high concentrations within epidermal growth factor gradients. Moreover, proliferation and differentiation of human neural stem cells exposed to gradients of growth factor mixtures was also studied [55]. Free diffusion-based gradients were created in a microfluidic device. These microfluidic platforms are useful to study cell–soluble factor interactions and are increasingly used by biologists and tissue engineers to study cell behavior and to generate improved tissues.

Controlling oxygenation and shear stress resulting from the flow of soluble factor are important in tissue engineering. A microfluidic device containing peristaltic oxygenating mixers and injectors was developed to provide high oxygen transfer rates without bubbles for the control of the growth rate of microbial cells [62]. Besides microfluidic devices, bioreactors also have the potential to control oxygenation and shear stress. An oxygen-permeable membrane bioreactor was used to investigate temporal cell morphology and metabolic functions of human hepatocytes [63]. This bioreactor was connected to a media perfusion system to mimic the *in vivo* sinusoidal organization and enable oxygenation of cells on 25-µm thick membranes. Using this system, liver-specific functions, such as protein synthetis and detoxification activities were analyzed. A bioreactor containing microfabricated groove substrates also allowed oxygen delivery and controlled shear stress [64]. Hepatocytes were cultured within microgroove substrates that minimized shear stress. Oxygenation and shear stress increased with increasing media perfusion rate. These microfluidic devices and bioreactors could be useful for manipulating the oxygenation and shear stress in well-defined microenvironments.

2.2 Nanotechnologies for tissue engineering

Nanotechnology can be used to create nanofibers, nanopatterns and controlled-release nanoparticles with applications in tissue engineering. These techniques are particularly useful for mimicking native tissues because many biological structures, such as ECM fibers are in the range of tens of nanometers [65]. For example, polymeric nanofibers that mimic collagen fibers can be fabricated by electrospinning [65] and self-assembly [66]. In general, the synthesis of nano-structured materials can be generated by using one of two approaches. In one approach, nanomaterials are synthesized by miniaturizing existing materials with nanoscale resolution. These techniques include nanopatterning and electrospinning. In the other approach, molecular build-up, such as self-assembly [66] and layer-by-layer deposition [67], can be used to generate nanomaterials [68].

2.2.1 *Electrospun nanofibers*

Electrospun nanofibers are versatile tools to fabricate tissue engineering scaffolds with biomimetic mechanical, chemical and biological properties (**Figure 3A – C**) [65,69,70]. Typically, electrospun scaffolds are highly porous and can be engineered with controlled sizes, shapes, and fiber alignments. Electrospinning has been widely used for the fabrication of a variety of tissues (e.g., bone, cardiac muscle) due to its inexpensive and simple setup (**Figure 3A**) [65,70,71]. A number of synthetic polymers, such as PLGA and PLLA and natural materials, such as collagen, have been studied using electrospinning. Moreover, aligned poly(L-lactid-*co*-ε-caprolactone) nanofibers were used to guide cell orientation and form blood vessel-like structures [72,73]. The differentiation of neural stem cells was also investigated using electrospun PLLA scaffolds [74]. Interestingly, the shape of nanofibers can be controlled to enhance the scaffold function. Nanofibers with a core-shell structure were made for the controlled release of molecules encapsulated within the hollow cores [75].

2.2.2 *Nanotextured substrates for tissue engineering*

In the body, the cellular microenvironment comprises a variety of nanostructured surfaces [76]. The basement membranes of various tissues are composed of complex mixtures of nanostructures (5 – 200 nm), which influence cellular behavior [77,78]. Nanotechnology can be used to modify the surface topography to regulate cell adhesion, morphology and migration. For example, the immobilization of carbon nanofibers was used to generate a topology similar to the epithelial basement membrane, to increase the osteoblast proliferation compared with flat glass surfaces [79]. Furthermore, electrospun fibers on a glass substrate were used to change the surface nanotopography [80]. Chemical treatment is another way to generate nanoscale surface features. The roughness of a PLGA surface was modified by treating the substrate with various concentrations of NaOH [81]. This study demonstrated that endothelial and smooth muscle cell density increased on the nano-structured PLGA surfaces. Lithographic techniques were also used to modify the topography of nanoscale surfaces. For example, electron-beam lithography was used to fabricate nanostructures at 50 nm length scales [82]. Human mononuclear blood cells, platelets, fibroblasts and endothelial cells were seeded on nanopatterned surfaces for cellular behavior study. These studies have demonstrated that nanostructured surfaces can be used to manipulate the cellular microenvironment *in vitro* in a controlled manner.

2.2.3 *Self-assembled nanomaterials*

Self-assembled nanostructures can be generated from different materials, such as peptide amphiphile (PA), hyaluronan, chitosan, and apatite/amelogenin. Several methods, such as pH induction, layer-by-layer deposition, electrolytic deposition (ELD) and biomimetic coating, can be used to induce self-assembly.

Molecular self-assembly of peptides and proteins can be used to make hydrogels for tissue engineering applications (**Figure 3D**) [66]. Self-assembled peptides typically contain

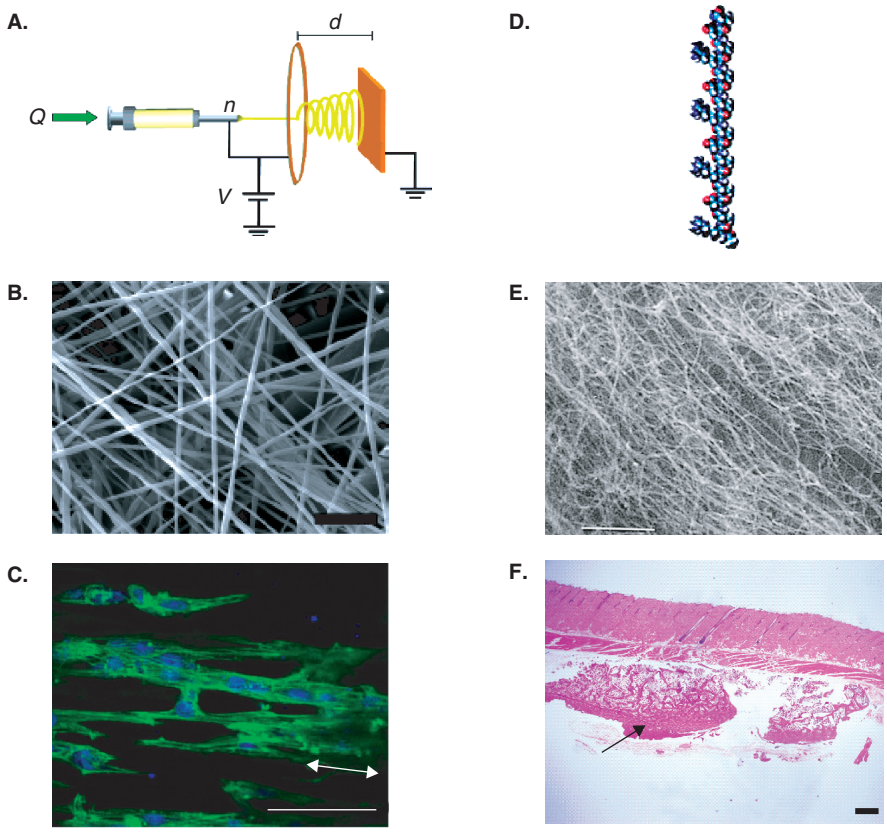

Figure 3. Polymeric nanofibers for tissue-engineering applications. To synthesize polymeric nanofibers, electrospinning and self-assembly can be used. **A.** Scheme of a typical electrospinning setup. The experimental parameters, such as flow rate (Q), needle gauge (n), voltage (V), distance (d), can control the properties of the fibers. **B.** Scanning electron micrographs of electrospun nanofibers. Scale bar is 10 μm. (Reprinted with permission from PHAM *et al.*: Copyright (2006), American Chemical Society) [69]. **C.** A confocal laser scanning microscope image of cardiac myocytes on predefined oriented fibers of PLGA + PEG-Poly(D,L-lactide) (PLA) diblock copolymer. Scale bar is 20 μm. (Reprinted with permission from ZHONG *et al.*: Copyright (2005), Elsevier) [70]. **D.** Schematic image of a peptide that can self assemble to form 3D hydrogels. (Reprinted with permission from Macmillan Publishers Ltd., ZHANG *et al.*: *Nat. Biotechnol.* (2003) **21**:1171-1178, Copyright (2003)) [66]. **E.** Scanning electron micrograph of self-assembled PA nanofiber networks containing BMP-2. **F.** 3D nanofiber scaffolds with BMP-2 significantly induced ectopic bone formation around the injected site. Arrows indicate the newly generated ectopic bone. Scale bar is 1 mm. (Reprinted with permission from HOSSEINKHANI *et al.*: Copyright (2007) Elsevier) [85].
BMP-2: Bone morphogenic protein-2; PA: Peptide amphiphile; PEG; Poly(ethylene glycol); PLA: Poly(lactic acid); PLGA: Poly(lactic-*co*-glycolide).

hydrophobic and hydrophilic regions that assemble into sheets or fibers, which can be further assembled into hydrogels by charge shielding. PA molecules were designed to self-assemble into nanofibers, which resulted in the generation of aqueous gels from pH changes [83]. By modifying the alkyl tail length and peptide amino acid composition, self-assembling behavior was studied. Oxidized supramolecular fibers were not self-assembled at acidic pH due to the distorted conformation from intramolecular disulfide bonds. A class of PA molecules were also allowed to self-assemble into 3D nanofiber networks with high aspect ratio for tissue engineering scaffolds [84]. PA self-assembly entrapped cells in the nanofibrillar matrix. In addition, bone regeneration was induced by the controlled release of bone morphogenetic protein-2 from 3D PA nanofiber scaffolds (**Figure 3E, F**) [85].

Although PA molecules make self-assembled nanofibers, they exhibit limited cell attachment. To address this limitation, a branched PA (b-PA) conjugated with RGD peptides was developed. The b-PA containing the RGD sequence was used as a self-assembling coating for fiber-bonded PGA scaffolds [86]. The RGD sequence on a b-PA nanofiber improved its accessibility and flexibility. The smooth muscle cells preferentially attached to b-PA coated scaffolds. PA-containing RGD peptides can be synthesized by standard solid-phase chemistry [87]. Osteogenic differentiation of mesenchymal stem cells was also studied using a 3D network of nanofibers generated by self-assembly of RGD-modified PA molecules. These nanofibers significantly induced proliferation and osteogenic differentiation of mesenchymal stem cells. Given past breakthroughs and future potential, these self-assembled nanofibers could

be useful for 3D tissue constructs and regenerative medicine. Self-assembled peptides are at present under investigation for other tissue engineering systems and are likely to become a powerful method for engineering scaffold materials.

Nanomaterials can be made by layer-by-layer deposition of ionic molecules. For example, a nanoscale self-assembled multilayer can be fabricated by alternating depositions of anions (i.e., hyaluronan) and cations (i.e., poly-L-lysine, chitosan) [67]. These nanoengineered films can be used for various applications, such as the coating of biomaterials and tissues. Self-assembled nanocoatings of HA and chitosan were deposited on arteries for protection and healing [67]. Moreover, titanium oxide nanoparticles fabricated by this method induced attachment and growth of mesenchymal stem cells, thus demonstrating that this technique can be used to modify surface adhesiveness [88].

Nano-biocomposite coatings have been also developed by ELD. For example, a uniform collagen fibril/octacalcium phosphate composite coating was developed by using ELD carried out in a three-electrode electrochemistry system [89]. Using this process, collagen fibrils were self-assembled at the cathode and, simultaneously, used as a substrate for octacalcium phosphate crystal growth. This composite coating that consisted of a porous collagen fibril network showed higher elastic modulus. To generate dental restorative biomaterials, an enamel-inspired nanocomposite with amelogenin supramolecular assembly was synthesized by ELD [90]. ELD was used to create the composite coatings through co-precipitation of self-assembled amelogenin and calcium phosphate. These synthesized composites of amelogenin and calcium phosphate are potential dental restorative biomaterials. During ELD, silicon wafers were used as a coating substrate due to their uniform and smooth surface. However, silicon wafers showed minimal cell attachment. To overcome this limitation, a titanium alloy can be used as a coating substrate. Calcium phosphate/chitosan coating on titanium alloy was fabricated by ELD [91]. The amorphous calcium phosphate was homogeneously distributed throughout the chitosan aggregates on the cathode. This system was used to study bone marrow stromal cell attachment.

In addition to ELD, biomimetic coating was used to study bone tissue engineering [92]. A biomimetic coating on titanium surfaces containing apatite and amelogenin was applied to evaluate cell adhesion, spreading patterns and mRNA expression. The apatite/amelogenin coating increased osteogenic gene expression. The co-precipitation of amelogenin into biomimetic coatings is a potential method for osteoblast differentiation and bone tissue engineering.

3. Drug discovery

Micro- and nanoscale approaches have been used in various stages of the drug discovery process. For example, microreactors and nanobiosensors were used for target selection as well as lead identification and optimization via high-throughput screenings (**Figure 1**) [93,94]. In fact, many methods were developed using tissue engineering platforms, such as animal-on-a-chip, which provided a useful model to evaluate the toxicological and pharmacological profiles of drug candidates [95].

3.1 Microtechnologies for drug discovery

Miniaturized lab-on-a-chip systems show great promise for a variety of drug discovery applications. Potential applications include the ability to manipulate cells and reagents in microfluidic devices as well as to purify and characterize drug targets by crystallization [93]. Moreover, these techniques can be used for single cell analysis and high-throughput compound screening.

3.1.1 Crystallization for drug discovery

The interactions between drug candidates and protein targets can be studied by *in silico* and experimental methods [94]. *In silico* screening can be validated and supplemented with nuclear magnetic resonance (NMR)-based or X-ray crystallographic experimental screening methods. Although the ability of NMR to measure proteins in their native state is an important distinction, X-ray crystallography has the advantage of defining ligand-binding sites with greater certainty [96,97].

For experimental methods, crystallization is the rate-limiting process in finding macromolecular structures. Conventional methods to crystallize many molecules are expensive and time consuming [98-100]. Although robotic systems have been developed for high-throughput automated crystallization, they can not be widely used due to high equipment costs and the need for large sample volumes [98]. To overcome these limitations, high-throughput microfluidic systems were developed to increase the efficiency of protein crystallization [101]. **Figure 4A** shows a droplet-based microfluidic system in which hundreds of trials were rapidly analyzed. Droplets were created within immiscible fluids to crystallize molecules such as thaumatin (**Figure 4B**) [101]. Plugs, aqueous droplets surrounded by an immiscible carrier fluid, flew out of the microchannels and subsequently generated crystals [98]. Crystals grown in plugs can be screened and analyzed by X-ray diffraction. A robust and scalable fluid metering in a microfluidic device was also developed for rapid screening of protein crystallization [102]. This chip contains multiple on-chip valves for parallel reactions. Using this system, diffraction-quality crystals were grown and harvested from 5 nl of protein solution. The studies mentioned here have demonstrated that the miniaturization of crystallization processes achievable within microfluidic devices can greatly increase the efficiency of the macromolecular structure characterization process and provide a useful set of tools to analyze the nucleation and growth of protein crystals.

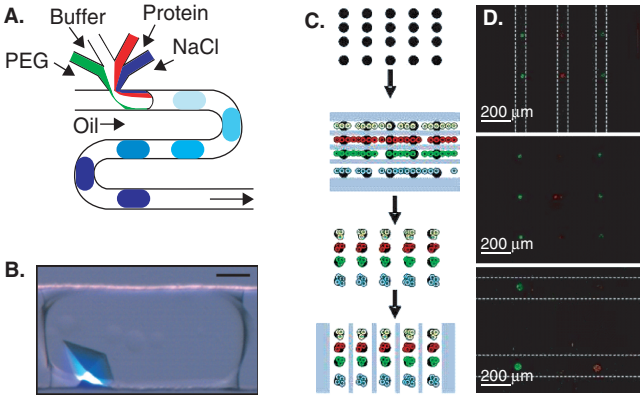

Figure 4. Microfluidic approaches for protein crystallization and cell-based screening. A. Multiphase fluids can be used to generate droplets inside microchannels comprised of proteins, precipitants and additives. **B.** A protein crystal (thaumatin) inside a droplet within a microfluidic device. Scale bar is 50 µm. (Reprinted with permission from ZHENG *et al.*: Copyright (2003), American Chemical Society) [101]. **C.** Schematic image of the formation of multiphenotype cell arrays within microchannels containing microwells. A reversibly-sealed microfluidic device was aligned on top of a microwell array to control the delivery of liquid to each well. **D.** Fluorescent images of scheme **C.** Cells were labeled with membrane dyes (CFSE, green) and SYTO (red). Different cell types (ESCs, AML12, and NIH 3T3 cells) are shown in the image (KHADEMHOSSEINI *et al.*: *Lab. Chip* **5**:1380-1386 [21]). Reproduced by permission of The Royal Society of Chemistry).
CFSE: Carboxyfluorescein diacetate succinimidyl ester;
ESC: Embryonic stem cell; SYTO: .

3.1.2 *Single cell analysis and separation*

The ability to manipulate and analyze single cells is important to study drug targets and understanding the underlying biology. A number of microfluidic approaches have been developed recently for individual cell analysis. For example, a microfluidic device integrated with microvalves and pumps was developed to study the intracellular calcium ion concentrations of single cells [103]. A single cell isolation chip with an incorporated polydimethylsiloxane trapping site was also fabricated to analyze cell-specific enzyme kinetics [104]. Furthermore, Rettig and co-workers developed single-cell arrays with high efficiency using microfabricated wells. The single cell occupancy as a function of settling time and microwell dimensions was characterized [105].

To isolate target cell types, a fluorescence-activated cell sorter (FACS) using a simple microfluidic T-shaped junction was created. This device was used to sort GFP expressing *Escherichia coli* cells by using electro-osmotic flows [106]. Dielectrophoresis was also used to sort cells [107]. In this process, dielectrophoresis-activated cell sorting achieved efficient separation between the dielectrophoretically labeled and unlabeled cells. When applying electric fields at the top and bottom walls of the microfluidic channel, only dielectrophoretically labeled cells were selectively deflected into the collection microchannel.

3.1.3 *High-throughput compound screening*

High-throughput microscale systems can significantly increase the efficiency of drug target selection, lead compound generation and identification by offering parallel experimentation and reduced reagent consumption. Such systems are mostly based on microfluidic and microarray technologies. A high-throughput microfluidic chip containing 1000 on-chip valves and 256 individual chambers was developed by Quake and his colleagues [108]. This device was used to test the presence of cytochrome c peroxidase-expressing *E. coli* cells. Similarly, a multi-layer microfluidic array was developed for high-throughput cell cytotoxicity screening [109]. Using this device, different cell types such as BALB/3T3, HeLa, and bovine endothelial cells were screened against a number of different toxins. Besides a multi-layer microfluidic device with multiple on-chip valves, a simple microfluidic device was used to screen compounds with a high-throughput. Khademhosseini and co-workers developed high-throughput screening devices in which cells were selectively docked in microwells within microfluidic channels (**Figure 4C, D**) [21]. Reversible sealing of PDMS molds was used to immobilize a series of microchannel patterns on the wells to enable sequential delivery of fluids to each microwell. This approach was used to seed various cell types, including hepatocytes and ESCs, inside different wells and subsequently expose each cell type to a unique series of chemicals. High-throughput studies can also be conducted by microfluidic systems within multi-well plates [110]. For example, a 96-well plate that incorporated multiple microfluidic networks and biosensors was used to detect multiple antibodies immobilized on ligands. The interactions of thousands of chemical compounds with target proteins could be simultaneously screened using these microfluidic systems.

3.1.4 *Microfluidic systems for the control of cell–soluble factor interactions*

Microfluidic systems can be used to analyze cell–drug interactions for lead optimization [111]. For example, using gradient-generating microchannels, it is possible to study the temporal and spatial effects of soluble factors on cell behaviour, such as chemotaxis [93]. Recently, pharmacological gradient profiling has been developed in a microfluidic device comprising a gradient generation component and an open-volume laminar flow [112]. Using this device, drug streams were held at different concentrations and voltage-gated K^+ ion channels were screened using scanning-probe patch-clamp measurements. Similarly, high-throughput microfluidic devices described in the tissue engineering section of this review may be applicable for a number of cell-based screening experiments.

3.1.5 *Drug delivery*

Drug delivery is an important part of the drug discovery and development process. A suitable delivery system can

enhance the therapeutic effect and decrease the drug toxicity by targeted delivery in a controlled manner. In the past few years, microfluidic systems have been increasingly used to synthesize drug delivery vehicles [113-115]. For example, Tan and co-workers reported the encapsulation of cells, proteins and microbeads in lipid vesicles using a microfluidic system [114]. An emulsified mixture of aqueous phase with the target in the liquid phase of the lipid, was injected into an aqueous mixture of ethanol and water to form lipid vesicles of controlled shapes. In addition to drugs, various cell types, such as Hela and yeast cells, were successfully encapsulated inside lipid vesicles. Monodisperse liquid droplets generated in microchannels were used to produce microspheres, polymeric rods and disks by trapping nonspherical monomer droplets in the solid state [115]. Furthermore, monodisperse particles can be generated by mimicking the double emulsion process inside a microchannel by a two-step method [113]. For a water-in-oil-in-water emulsion, aqueous drops were formed at the hydrophobic T junction and the organic droplets enclosing multiple aqueous droplets were generated at the hydrophilic T junction. The resulting precipitation of the polymer resulted in the formation of monodisperse particles that could be loaded with drugs for delivery applications.

3.2 Nanotechnologies for drug discovery

Nanotechnology is rapidly emerging in the field of the pharmaceutical drug discovery and development. It has been applied in two areas: nanosensors for detecting the biological signatures of certain diseases and nanoparticles that can be loaded with therapeutic agents for targeted delivery. Here, the use of nanotechnology in nanobiosensors and nanoparticles are briefly reviewed.

3.2.1 Nanobiosensors

Nanobiosensors are becoming increasingly important for the detection and analysis of drug molecules with high sensitivity and selectivity. Common nanoscale materials used to fabricate nanosensors include quantum dots and magnetic nanoparticles. Quantum dots are semiconductor nanostructures (2 – 10 nm) with size-dependant excitation and emission spectra. The range of excitation and emission wavelengths makes quantum dots useful for many imaging applications (**Figure 5A**) [116]. Quantum dots have several advantages over conventional fluorescent dyes, such as tighter emission band gaps and lower photo bleaching levels [117]. Because of these advantages, quantum dots have been widely used to track single molecules and individual cells *in vivo* and *in vitro*. Dahan and colleagues tracked drug receptors in the neuronal membrane using quantum dots (**Figure 5B**) [118]. Quantum dots can also be conjugated with various ligands to study signal transduction pathways [119]. Additionally, quantum dots can be designed to bond with individual biological targets, such as genes, nucleic acids, proteins and cells. For example, an ultrasensitive nanosensor capable of detecting low concentrations of DNA was reported, in which quantum dots were linked to DNA probes to capture DNA targets [120]. The target strand binds to a dye-labeled reporter strand, thereby forming a donor–acceptor ensemble. Unbound nanosensors produce near-zero background fluorescence. However, on binding to even a small amount of target DNA (≤ 50 copies), they generate a distinct signal.

Like quantum dots, magnetic particles can be used for imaging applications. For magnetic particles, magnetic nanosensors were used for rapid analysis of telomerase activity [121]. In this work, magnetic nanoparticles were with incorporated telomerase synthesized TTAGGG repeats, which used to switch the nanoparticles' magnet state. High-throughput adaptation of this technique using magnetic resonance imaging allowed the processing of hundreds of samples within a few minutes at ultrahigh sensitivities.

3.2.2 Nanoparticles

Nanoparticles play an important role in drug delivery systems [122]. Drugs can be encapsulated within nanoparticles and released in a controllable manner. Furthermore, drug delivery vehicles can be induced to target specific tissues, such as tumors, by coating polymeric nanoparticles with targeting molecules that specifically bind to receptors on the target cells. These nanoparticles can be coated with PEG to aid in their safe passage through the bloodstream [123]. The use of these delivery vehicles has significant promises for therapeutic applications. For example, a novel quantum dot-aptamer-doxorubicin (Dox) conjugate was developed for cancer therapy. The conjugate was capable of differential uptake and imaging of prostate cancer cells. This simple multifunctional nanoparticle system delivered Dox to the targeted prostate cancer cells and sensed the delivery of Dox by activating the fluorescence of quantum dots, which concurrently imaged the cancer cells [124].

For biologic therapeutic agents, such as proteins and DNA, delivery systems become the major concern in the drug discovery process. The biological properties of the molecules must be protected during transport to the target sites. After a safe passage to the target, the molecules must be released from the delivery systems. Biomimetic nanoparticles, such as artificial cells and viruses, have provided new possibilities to create such complicated delivery systems. Biodegradable polymer membranes were used to fabricate nanoscale artificial red blood cells [125]. These nanoscale artificial red blood cells (80 – 150 nm in diameter) were used to carry proteins, such as red-blood-cell enzymes. With a PEG–polylactide copolymer membrane, it was possible to increase the circulation time of these artificial cells [126]. For more complicated gene delivery systems, artificial viruses provided a useful model (**Figure 6**) [127]. Artificial viruses consist of a cationic core and an anionic shell. The cationic core is composed of plasmidic DNA to which functional peptides have been bound. The shell serves as a scaffold to

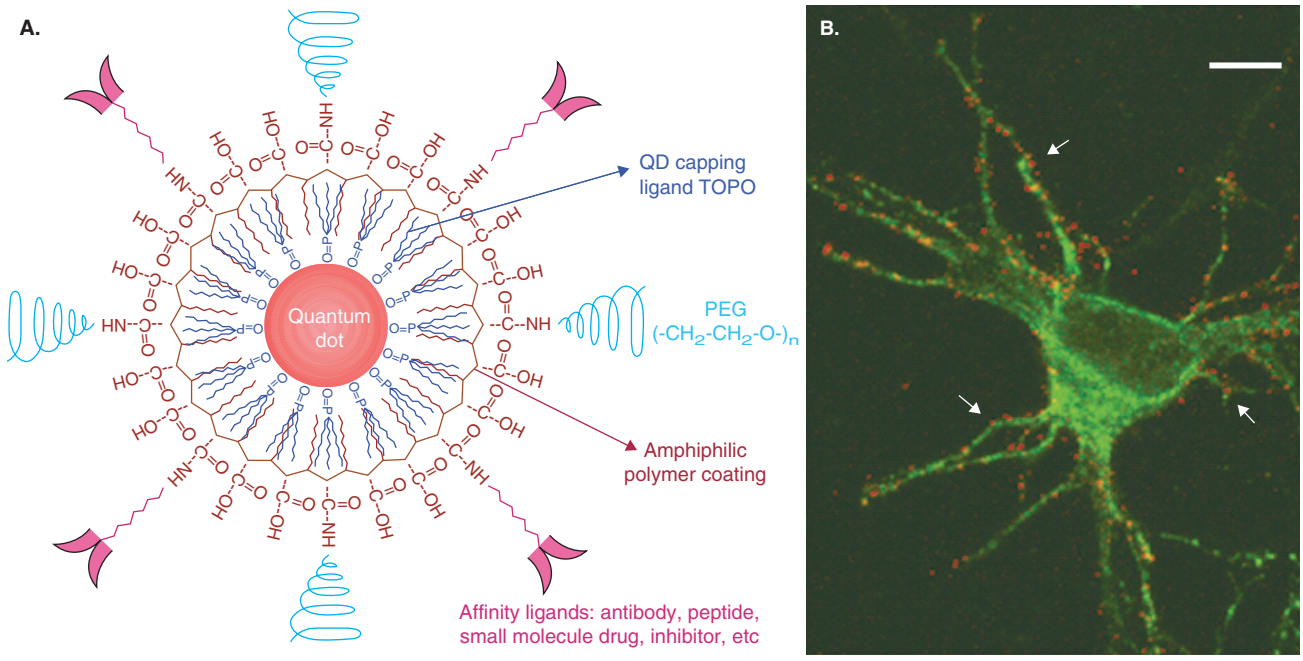

Figure 5. Quantum dots for cellular imaging and tracking. A. Schematic structure of the multifunctional quantum dot. (Reprinted with permission from GAO *et al.*: Copyright (2005), Elsevier) [116]. **B.** Quantum dots as a marker of GlyR localization in a neuron. The neuron is stained by microtubule-associated protein-2 (green) and arrows show quantum dot-GlyRs (red) located on dendrites. Scale bar is 10 µm. (Reprinted with permission from DAHAN *et al.* Copyright (2003), Science) [118].

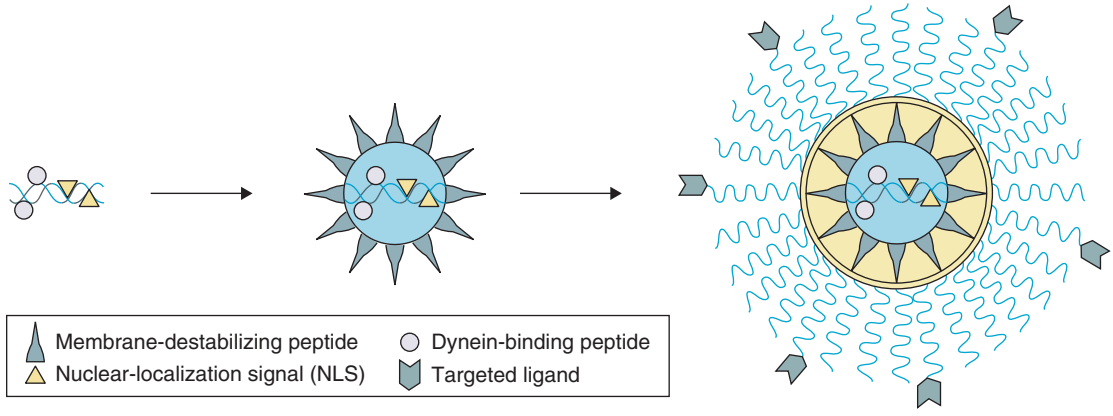

Figure 6. An artificial virus. A conceptual model of the assembly of a multi-layered artificial virus.
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which targeting ligands can be attached. Surface-exposed ligands mediate cell-specific attachment that induces the internalization of the artificial virus by receptor-mediated endocytosis. Such a complicated design is a result of the nature of gene delivery. The artificial virus should remain stable during its transport through the body and disassemble in a controlled fashion once taken up by target cells. The controlled intracellular disassembly can lead to the delivery of associated plasmidic DNA into the nucleus, where the transgene can be expressed. Although the artificial-virus technology is in its infancy, it is expected that such delivery systems will have a great impact on genetic therapies.

4. Conclusions

This paper reviewed the recent developments in the use of micro- and nanotechnologies for tissue engineering and drug discovery applications. Micro- and nanotechnologies are powerful tools for the manipulation of the cellular microenvironment (e.g., cell–cell, cell–ECM, and cell–soluble

factor interactions) on a two-dimensional surface and within 3D hydrogel scaffolds, for tissue engineering and cell-based assays. Furthermore, they are useful for drug discovery processes, such as target selection and lead identification/optimization. At the microscale, technologies such as lab-on-a-chip enable the development of high-throughput platforms that can be useful for screening applications. Moreover, at the nanoscale, electrospun or self-assembled polymeric nanofibers significantly enhance tissue repair and regeneration processes. Nanotechnology also has significant promise for drug discovery because of the potential for generating nanobiosensors and nanoparticles. Future developments in these technologies will successfully achieve a wide variety of applications in biomedicine.

5. Expert opinion

Micro- and nanotechnologies are versatile experimental tools for the study of tissue engineering and drug discovery. These approaches can be used to address a number of limitations (e.g., large volume of reagents, low throughput and the inability to precisely control the cellular microenvironment) imposed by macroscale methods. Despite their significance, challenges remain and need to be addressed.

First, for tissue engineering applications, improved biodegradable scaffolds are needed to provide cells with the proper signals to induce tissue formation. These scaffolds must exhibit the desired degradation rates, signaling cues, pore sizes as well as mechanical, chemical and biological properties that mimic native tissues. Second, automated microscale systems that can perform reaction, manipulation and analysis processes need to be developed for drug discovery applications. Using these systems, all processes ranging from target identification to lead optimization can be performed on a single chip. Third, the various technologies that have been independently developed must be merged to generate more powerful platforms. For example, a high-throughput microfluidic platform integrated with multiple nanoscale functions, such as nanopatterned substrates, 3D nanofibrous scaffolds, nanobiosensors and nanoparticles could potentially be created. These devices could be used to precisely regulate *in vitro* extracellular microenvironments (i.e., cell–cell, cell–ECM, and cell–soluble factor interactions) to direct cellular fates and manipulate high-throughput drug screening. Overall, the efforts in this field may lead to the development of novel microscale platforms and nanomaterials that can help to solve today's problems of tissue engineering constructs and drug discovery.

Declaration of interest

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