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Engineering Biomaterials with Micro/Nanotechnologies for Cell Reprogramming

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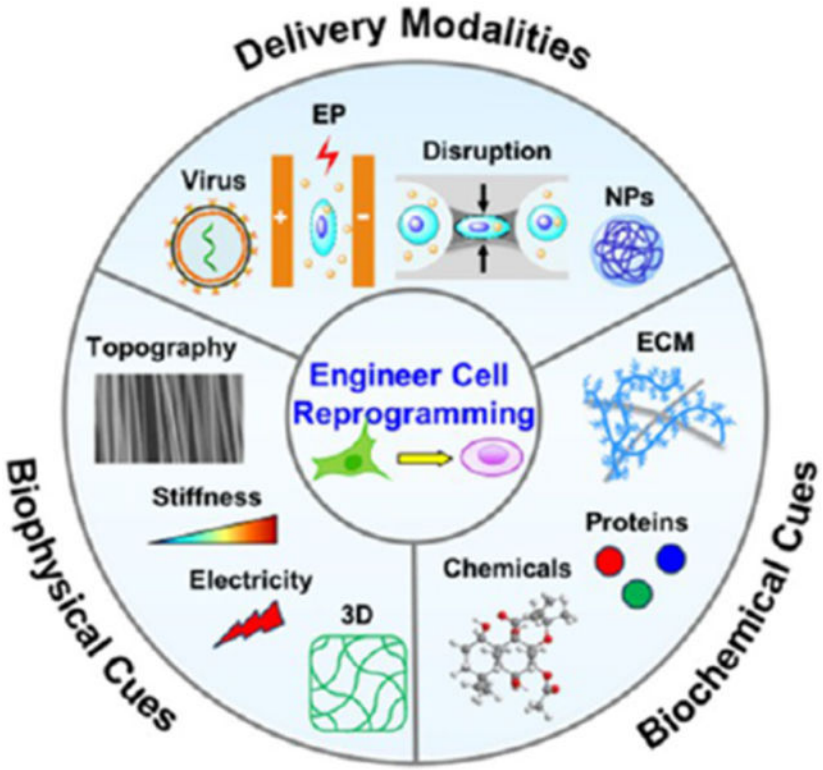
Abstract

Cell reprogramming is a revolutionized biotechnology that offers a powerful tool to engineer cell fate and function for regenerative medicine, disease modeling, drug discovery, and beyond. Leveraging advances in biomaterials and micro/nanotechnologies can enhance the reprogramming performance *in vitro* and *in vivo* through the development of delivery strategies and the control of biophysical and biochemical cues. In this review, we present an overview of the state-of-the-art technologies for cell reprogramming and highlight the recent breakthroughs in engineering biomaterials with micro/nanotechnologies to improve reprogramming efficiency and quality. Finally, we discuss future directions and challenges for reprogramming technologies and clinical translation.

Graphical Abstrac



Keywords

cell reprogramming; biomaterials; nanotechnology; biophysical cues; biochemical cues; three-dimensional niches; tissue engineering; drug delivery

During mammalian development, cells progressively differentiate from a totipotent zygote into a terminally differentiated state with functionally and phenotypically distinct fates to form organized tissues and organs. Such cell fate specifications are precisely controlled and mediated by definitive transcriptional regulatory networks.[1,2] Terminally differentiated somatic cells normally maintain their phenotype throughout the lifespan. Adult cells,

however, may lose the capability to heal certain damaged tissues, such as the central nervous system,[3,4] heart,[5,6] and cartilage.[7] Cellular reprogramming technology provides a revolutionizing approach that bypasses the rules of cell and developmental biology in cell fate determination, enabling the conversion of mature somatic cells to pluripotent cells or to other different cell lineages.[8] Since the landmark work of reprogramming somatic cells into induced pluripotent stem cells (iPSCs) with a subset of specific transcription factors by Yamanaka in 2006,[9] tremendous advances have been achieved not only in iPSC reprogramming and differentiation but also in other cell-reprogramming approaches such as direct or indirect cell reprogramming *in vitro* and *in vivo*.[10-14] Such significant developments of reprogramming technologies have offered multiple powerful tools and strategies for regenerative medicine, disease modeling, and drug discovery.[15-17]

Biomaterials play imperative roles in treating disease and improving the quality of life for patients. Many natural and synthetic biomaterials have been engineered for diverse biomedical applications, including drug delivery,[18,19] cell transplantation,[20,21] tissue engineering,[22,23] cancer therapy,[24-26] device-based therapies, and medical diagnostics and imaging.[27,28] The development of various biomaterials-based platforms have offered robust approaches to modulating cell reprogramming *in vitro* and *in vivo*.[29-34] In particular, the physicochemical properties of biomaterials can be tailored by sophisticated chemistry and micro/nanoprocessing, not only for the delivery of reprogramming factors but also as tunable physicochemical cues on two-dimensional (2D) substrates or in three-dimensional (3D) microenvironments.[32,35-38]

Here, we review the development and current state of cell reprogramming, with a focus on engineered biomaterials-based drug-delivery strategies and biophysical and biochemical cues for modulating cell reprogramming, followed by a discussion on the future directions of this fast-growing field.

CELL REPROGRAMMING: DEVELOPMENT AND CURRENT STATE

Although Spemann initially proposed the concept which would later be coined as somatic cell nuclear transfer (SCNT) in 1938, it was not until 1952 that Briggs and King successfully developed the technique and demonstrated that introducing the nuclei of a blastula cell into an enucleated egg could give rise to a cleaved blastula.[8] Ten years later, Gurdon transferred the nuclei from differentiated *Xenopus* intestinal epithelial cells into enucleated eggs, and this resulted in the formation of fully functional tadpoles.[39] These findings revealed that the reversal of cell differentiation, now known as reprogramming, was feasible, which initiated mammalian cloning experiment using SCNT in 1996 and the derivation of SCNT-derived human embryonic stem cell lines in 2013.[40,41]

In comparison to SCNT and cell fusion, cell reprogramming offers a more robust approach through the ectopic expression of defined transcription factors (Figure 1). The concept of reprogramming regained wide interest in 2006 when Takahashi and Yamanaka demonstrated that they were able to generate iPSCs from mouse somatic cells *via* the combined expression of four transcriptions factors, *Oct4, Sox2, Klf-4,* and *c-Myc* (OSKM).[9] It has been demonstrated that these iPSCs closely resemble embryonic stem cells (ESCs) in terms of

karyotype, stemness phenotype, and differentiation capacity. This significant breakthrough highlighted the possibility of generating any desired cell type from a somatic cell through a combination of iPSC reprogramming and directed differentiation, and numerous pioneering studies have unraveled the mechanisms for cell reprogramming.[42-44] Although human ESC-derived cells have been primarily used in clinical trials, iPSCs are considered superior to ESCs without the ethical concerns involving the destruction of embryos. It is encouraging that some preliminary clinical trials have recently launched using human iPSC cells (hiPSCs)-derived cells to treat diverse diseases, including macular degeneration, aplastic anemia, heart disease, endometriosis, Parkinson's disease, and spinal cord injury.[45-49] However, iPSCs can be associated with genetic and epigenetic abnormalities that may arise during the reprogramming process.[50] As a result, there is an increased risk of tumorigenicity and immunogenicity when using iPSC-derived cells. Moreover, some additional hurdles, including unstable differentiation, low conversion efficiency, and lengthy differentiation still exist, thereby limiting the clinical translation of iPSC-based cell therapies.

To circumvent these issues, scientists have explored other methods of manipulating cell fate (Figure 1). Direct reprogramming is an alternative strategy that directly converts fully differentiated somatic cells into distinct cell types using transcription factors, microRNAs, or chemical compounds.[10] In comparison to iPSC reprogramming, this method is considered faster and safer as cells do not proceed through an intermediate pluripotent state. The direct reprogramming was reported in the late 1980s that the forced expression of MyoD, a transcription factor in the skeletal muscle lineage, could induce mouse embryonic fibroblasts (MEFs) into stable myoblasts.[51] Since then, numerous studies in the past two decades have shown that this method can be applied to obtain a wide variety of target cell types, including mouse or human hepatocytes,[52,53] neurons,[54-56] pancreatic β cells,[57,58] renal tubular epithelial cells,[59] immune T cells,[60] embryonic Sertoli-like cells,[61] endothelial cells,[62] and cardiomyocytes.[63-66] Direct reprogramming is a promising approach for personalized medicine as it has been shown that patient-derived cells recapitulate the disease phenotype, which offers great potential for disease modeling, drug screening, and personalized treatments. For example, direct neuron cell reprogramming has been used to study human disease progression *in vitro,* such as Alzheimer's disease.[67] Although direct reprogramming can be utilized to generate cells with a mature phenotype, this may not be ideal for clinical translation. Thus, more recently, direct reprogramming has been applied to generate various types of expandable stem cells or progenitors, such as neural stem cells,[68] oligodendrocyte precursor cells,[69] hepatic stem cells,[70] hematopoietic stem cells,[71,72] nephron progenitor-like cells,[73] intestinal progenitor cells,[74] skeletal muscle progenitor cells,[75,76] and cardiac progenitor cells.[77,78] By providing extensive proliferative capacities and restricted differentiation potentials, these directly reprogrammed progenitors have been shown to be more engraftable and desirable for transplantation in regenerative therapies.[71] In addition, it is worth noting that directly reprogrammed cell populations usually have cellular heterogeneity. Thus, cell purification is commonly required to isolate the target cells from the rest of the population using fluorescence-activated cell sorting (FACS),[71] immunopanning,[69] or other sorting techniques.

Indirect reprogramming has been demonstrated as another reprogramming approach. This process entails converting somatic cells through a transient induction of a partially

reprogrammed cell using the Yamanaka factors, followed by differentiation into the target cell type.[79] Similar to direct reprogramming, this indirect approach does not give rise to pluripotent cells and thus bypasses the teratoma-forming potential that is typically associated with iPSCs. Using this indirect reprogramming method, several types of lineage-specific cells and multipotent progenitors have been generated from mouse and human cells.[80-83]

To date, most reprogramming studies have been conducted *in vitro.* Alternatively, several studies have shown that resident cells can be directly reprogrammed into another desired cell type *in situ,* such as mouse pancreatic cells,[84-86] neurons,[87-93] oligodendrocytes,[94] cardiomyocytes,[95-97] hepatocytes,[98,99] retinal cells,[100] epithelial cells,[101] and lymphocytes,[102] through the overexpression of lineage-specific transcription factors, microRNAs, small molecules, and other reprogramming factors. Such studies have shed light on an emerging field in exploiting *in vivo* lineage reprogramming to mend the broken tissues by harnessing and converting internal cells into targeted functional cells without cell transplantation, risks of contamination, and tumorigenesis.[14] Notably, the *in vivo* microenvironment offers an optimal niche for enhancing direct cell reprogramming. Similarly, iPSC reprogramming has also been investigated *in vivo*.[103-107] It has been reported that iPSCs derived *in vivo* demonstrate totipotency and a closer resemblance to ESCs in comparison to iPSCs generated *in vitro*.[105] In contrast to the finding that DNA damaged and aged cells display lower reprogramming efficiencies *in vitro,* tissue injury and senescence promotes *in vivo* OSKM-mediated reprogramming.[106,107]

Although significant advances have been made for *in vitro* cell reprogramming during the past decade, *in vivo* reprogramming is still in the nascent stages. Moreover, numerous obstacles related to reprogramming efficiency, quality, safety, and controllability must be overcome before there is robust translation of reprogramming therapies. Promisingly, engineering proper delivery systems and microenvironmental cues *via* micro/nanotechnologies is expeditiously promoting vital breakthroughs in the field of cell reprogramming.

DELIVERY SYSTEMS FOR CELL REPROGRAMMING

Various genetic and epigenetic regulators or reprogramming factors have been utilized for cell reprogramming (Figure 2A), including transcription factors in the form of proteins,[11,108] episomal plasmids,[109,110] messenger RNA (mRNA),[111] micro-RNA (miRNA),[112,113] small molecules,[67,114,115] mini-circle DNA,[116,117] synthetic transcription factors,[118] and CRISPR-Cas9 gene-editing tools.[119] It is important to note that although these reprogramming factors can yield differences in the reprogramming efficiency and reproducibility, this can potentially be improved upon using an optimal delivery system.[120,121] Thus, the choice of a safe and effective delivery system is paramount in achieving successful reprogramming with specific reprogramming factors.[122,123] Current delivery modalities for cell reprogramming can be classified into three groups: biological, physical, and chemical delivery systems (Figure 2B), and their comparisons are summarized in Table 1. Herein, we will focus on the recent advances of physical and chemical delivery systems, considered as biomaterials-based approaches in a broad sense, for cell reprogramming.

Biological Delivery Systems.

Viral delivery of reprogramming factors relies on genetically engineered viruses to transduce transcription-factor-encoding plasmids into cells, including integrative vectors based on retrovirus and lentivirus and nonintegrative vectors based on adenovirus, adenovirus-associated virus (AAV), and Sendai virus.[64,124] Recombinant viral vectors are often preferred for cell reprogramming because of their high transduction efficiency. However, viral gene delivery usually has limited DNA packaging capacity, unpredictable gene dysfunction, cytotoxicity, immunogenicity, and tumorigenicity. Detailed information on virus-based delivery for iPSC and direct cell reprogramming can be found in several excellent review papers.[11,14,120,125,126]

Physical Delivery Systems.

Physical delivery strategies involve directly delivering reprogramming factors into the cytoplasm or the nucleus *via* membrane-disruption methods, including electroporation and mechanical disruption (*e.g.,* cell squeezing and nanostructure penetration).

Electroporation.—Electroporation has become one of the most popular nonviral gene-delivery techniques since initially reported in 1982.[127] Bulk electroporation (BEP) is commonly conducted with millimeter- to centimeter-sized chambers, where the cell suspension and molecules to be delivered are mixed in a conducting buffer solution between two electrodes. Electrical pulses are applied to induce transient permeability of cell membrane and diffusion/endocytosis for cytosolic cargo delivery.[128] Electroporation-based methods can deliver a diverse range of cargos, including nucleic acids or genes (small interfering RNAs, miRNAs, mRNA, DNA plasmid), proteins or protein complexes, small molecules, and nanoparticles. This multifaceted capability provides the potential for a multitude of *in vitro* and less amenable to *in vivo* applications, such as gene editing,[129] adoptive immunotherapy,[130-132] regeneration medicine,[133-135] cell reprogramming,[136,137] and others.[128,137,138]

BEP-based delivery has been used to deliver episomal vectors to generate integration-free hiPSCs *in vitro*[109,139] and to induce direct reprogramming into neurons[140] and oligodendrocytes *in vivo*.[94] Nevertheless, despite its simplicity, BEP-mediated transfection has drawbacks, including excessive cell damage caused by high electric fields, highly stochastic transfection, and low efficiency of intracellular delivery (diffusion dominated).[141,142] To overcome these challenges, microfluidic-based electroporation technologies, such as microchannel electroporation (MEP), have been developed to operate at lower voltages, enabling the enhancement in efficiency and viability,[143] single-cell[143] or high-throughput molecular delivery,[144] and precise dose control.[138] Recently, another vortex-assisted microfluidic electroporation system, named microscale symmetrical electroporator array (μSEA), has been developed with integrated micropatterned planar electrodes in the vortex cell-trapping chamber and equipped with real-time visualization functionality (Figure 3). Such a system not only allows for sequential intracellular delivery of various molecules but also presents several other benefits, including dosage control, real-time visualization, multimolecular delivery, high transfection efficiency, and improved

cell viability.[123] However, the cargo size of the MEP approach is still limited because its cytosolic cargo delivery relies on diffusion and endocytosis.

With advances in nanofabrication and engineering techniques, recently, nanoelectroporation (NEP) systems have been developed for efficient cell transfection.[145] For example, a nanochannel electroporation device was designed with a microchannel–nanochannel–microchannel structure, and single cells were precisely positioned at the tip of the nanochannel *via* optical tweezers (Figure 4A).

In contrast to other electroporation methods, NEP can transfect cells faster and more efficiently at the single-cell level with a precise dosage of transfection agents (*e.g.*, oligodeoxynucleotide, siRNA, quantum dots nanoparticles, GFP plasmid), while not affecting cell viability.[141] However, it is hard to trap adherent cells by optical tweezers, and it is even less likely suitable for trapping large numbers of fast, adherent cells (*e.g.*, MEFs) for cell reprogramming. Therefore, a more advanced high-throughput NEP biochip has been further developed by the same lab whereby cells were loaded using a centrifugal spinning method (Figure 4B). This technique greatly increased the cell loading efficiency from 10 to 80% compared to optical tweezers and allowed for a large number (*i.e.*, 10 000 cells for a device) of MEF cells to be transfected with OSKM plasmid for mouse iPSC reprogramming.[146] After NEP transfection, more than 90% of cells were alive and the transfection efficiency was significantly higher than that with BEP. However, further systematic characterization of specific gene and protein expressions of transfected cells should be performed to determine the final iPSC reprogramming efficiency and elucidate additional advantages of using nanoelectroporation for *in vitro* cell reprogramming.

In addition, the NEP techniques also enable tissue nanotransfection (TNT) of nonviral cargos for rapid, highly effective, and deterministic *in vivo* reprogramming (Figure 5). The TNT platform could efficiently promote the reprogramming of mouse skin cells into neurons with the delivery of Ascl1/Brn2/Myt1l (ABM) plasmids. This approach elicited 50- to 250-fold greater gene expression *in vivo* compared with conventional bulk electroporation. In particular, *via* the delivery of Etv2/Foxc2/Fli1 (EFF) plasmids, skin cells could be reprogrammed into endothelial cells to increase vascularization and rescue skin tissue and whole limbs under necrotizing ischemia. Moreover, the TNT platform could also mediate RNA-based transfection of microRNA302/367 cluster to induce pluripotency of skin.[136] Another nanotransfection approach is nanostraw electroporation, which can achieve high-throughput cell transfection with dosage control and high uniformity.[147] Although the nanoscale electroporation platforms have displayed more efficient and enhanced transfection capabilities, some challenges remain to be addressed, including precisely targeted cell delivery *in vivo* and complex fabrication protocols that require special expertise for the manufacturing of miniaturized electroporation systems.

Mechanical Disruption.—Mechanical disruption is an alternative method to disrupt the cell membrane for instantaneous delivery.[122,148] Unlike electroporation methods, mechanically triggered cell disruption does not depend on electric fields, external materials, endocytosis, or cargo properties.[122] This approach is particularly applicable to difficult to transfect cell types. Recently, the advances of microfluidics and nanotechnology have

enabled mechanical-disruption approaches, such as cell squeezing and nanostructure-based penetration for cell reprogramming.

Cell squeezing permits the rapid deformation of cells flowing through a narrow microfluidic channel that is half to one-third of the cell's diameter in size, thus leading to mechanical disruption of the plasma membrane that allows for the diffusion of various materials of interest into the cytosol.[149-152] Particularly, microfluidic-mediated cell squeezing has been used to deliver OSKM recombinant proteins into human fibroblasts for iPSC reprogramming and showed a 10-fold improvement in colony formation compared to electroporation or cell-penetrating peptides (Figure 6A).[152] To disrupt both the plasma membrane and nuclear envelope, electric-field-driven transport was combined with cell squeezing in a microfluidic device for the enhanced delivery of plasmid DNA (Figure 6B).[144] A major advantage of the cell squeezing method is the use of simple devices to transfect diverse cargos into large numbers of cells within a short time period. However, current cell-squeezing methods have a critical limitation regarding inconsistent transfection efficiency for asynchronous cell populations with different sizes. Thus, more advanced squeezing devices need to be designed with a certain range of geometric constrictions to disrupt cells of different sizes.[122]

In addition, several nanostructure-based penetration methods have demonstrated the potential for effective high-throughput delivery of an assortment of biomolecules into living cells, including nanowires,[153] nanoneedles,[154] nanospears,[155] and nanostraws.[147,156] The nanoscale devices can penetrate the plasma membrane, thus enabling the straightforward delivery of different biomolecules, loaded onto the surface or within the bulk structure, into the cytosol and nucleus.[137] It has been demonstrated that porous silicon nanoneedles can efficiently deliver nucleic acids into the cytosol (greater than 90% efficiency), and in particular, *in vivo* neovascularization can also be achieved using the nanoneedle-mediated minimally invasive and localized delivery of angiogenic VEGF-165 gene (Figure 6C).[157] Nanostraw is another alternative cell penetration approach to mediate cytosolic delivery of different biomolecules. Unlike other nanostructure-mediated delivery methods, microfluidic systems can be integrated with nanostraw platforms to allow permanent fluidic access and spatial and temporal control in delivery.[158] Additionally, electroporation systems can be further combined to generate nanostraw electroporation devices (Figure 6D).[147] These devices have been shown to greatly enhance plasmid transfection (~81%) for Chinese hamster ovary cells, who display relatively low (5–10%) transfection rates using nanostraw-mediated plasmid delivery. As such, this nanostraw electroporation system offers a powerful, high-throughput transfection platform with excellent dose, spatial, and temporal control, as well as high efficiency and cell viability. Although nanostructure-mediated delivery techniques remain in the early stage of rapid development, they have provided some exciting delivery platforms for a wide range of delivery applications and show great promise for the delivery of different reprogramming factors for *in vitro* and *in vivo* cell reprogramming. However, all the nanostructure-mediated penetrations usually lack a cell-specific targeting mechanism and cannot easily navigate 3D tissues, thus nanodevices are optimally suited for transfection into easily accessible 2D tissues such as the epidermis and retina.[101]

Chemical Delivery Systems.

Chemical delivery approaches, often involving nanoparticles or nanocarriers, are attractive in the drug- and gene-delivery field due to their suitable size for cell penetration, large surface area for loading molecular cargo, and protection of loaded substances.[159] As a carrier for nonviral gene delivery, nanoparticles are usually regarded a advantageous to viral transduction in terms of larger transgene delivery, low immunogenicity, ease of transfection, low genetic integration, and minimal insertional mutagenesis risks,[160] although the efficiency might be lower than that with virus-based methods. Nowadays, chemical-based nanocarriers, including lipids, polymers, and hybrid systems, have been widely applied to deliver plasmid DNA, miRNA, mRNA, recombinant proteins, and small molecules for diverse disease treatments[161-164] and have been utilized for the delivery of reprogramming factors for cell reprogramming.

Lipid Nanoparticles.—Lipids are amphiphilic molecules, consisting of hydrophobic tails and hydrophilic heads. As one of the most widely used nonviral gene carriers, lipid-based vectors/nanoparticles can deliver diverse cargos, including drugs, DNA, RNA, and genome-editing proteins into cells in an endocytosis-dependent manner.[165-168] Cationic lipids (*e.g.*, DOTAP and DOSPA) contain three structural components: a cationic headgroup, one or two hydrophobic tails, and a linking group between these domains, which are the main components that bind with negatively charged nucleic acids or molecules to form the lipid-based lipoplexes. In addition, neutral lipids (*e.g.,* DOPE and DOPC) within the formulations can function as “helper lipids” to enhance nanoparticle stability and transfection activity.[161]

Currently, several lipid-based vectors have been developed and applied to achieve cell reprogramming (Figure 7A).[111,113,169,170] Lipid-mediated repeated transfection was used to deliver expression plasmids containing the Yamanaka factors to reprogram MEFs into iPSCs, without evidence of plasmid integration, although the conversion efficiency was low (*i.e.*, less than 0.0029%).[171] iPSCs also can be reprogrammed from human fibroblasts with enhanced efficiency through the delivery of a modified RNA cocktail using cationic lipid carriers.[111] In addition, lipid-based vectors can also be used to achieve *in vivo* cell reprogramming. It has been reported that the delivery of a single miRNA (miR-21) using DOPC/M-DOPE/Tween-80/DiO lipid nanoparticles can convert wound site macrophages to fibroblast-like cells in diabetic mice.[172] Similarly, mice cardiac fibroblasts can be directly reprogrammed into cardiomyocytes *in vitro* and *in vivo via* lipid-based delivery of modified miRNAs.[97] The miRNA-mediated reprogramming efficiency was significantly increased (*i.e.*, by more than 10-fold) when combined with JAK inhibitor I. Therefore, further optimization of delivery methods that combine miRNA with small molecules for *in vivo* reprogramming may amplify their therapeutic implications.

Polymer Nanoparticles.—Polymer-based drug- and gene-delivery systems have been widely used to treat broad pathological conditions *via* less invasive or noninvasive routes.[166,173] The most commonly used polymeric carriers are nanoparticles because they offer advantages such as chemical flexibility, stable formulation, excellent biocompatibility, facile preparation, and controlled release capability. Recently, delivery strategies based on natural and synthetic polymers have been adopted for cell reprogramming (Figure 7B).

Natural biopolymers like polysaccharides have been generally used as polymeric backbones for the formation of nanoparticles or as valuable drug- and gene-delivery carriers.[174,175] For example, a positively charged polysaccharide was employed to deliver plasmid mixtures (encoding *Oct4, Sox2*, miR302/367) to generate hiPSCs from human umbilical cord mesenchymal stem cells.[176] Similarly, the direct reprogramming of mouse fibroblasts into neural cells can be achieved by co-delivery of a group of plasmids (*Ascl1, Brn4, Tcf3*) with ethanediamine-modified *Porphyra yezoensis* polysaccharide (Ed-PYP) nanoparticles.[177]

Besides natural polymers, cationic synthetic polymers have immense chemical diversity and unlimited potential for functionalization.[161] Several synthetic polymer nanoparticles have been used for cell reprogramming, such as those based on polyethylenimine,[178] poly(β-amino ester),[179] poly- (amidoamine) dendrimer,[180] and cationic bolaamphiphile.[181] Nontoxic cationic polyurethane–short-branch polyethylenimine (PU–PEI) nanoparticles have been developed to deliver *Oct4/SirT1* plasmids into aged-related retinal pigment epithelium cells or light-injured rat retinas.[178] For example, the PU–PEI-mediated *Oct4/SirT1* gene transfer converted the retinal pigment epithelium cells into progenitor-like cells, which rescued retinal cell loss and improved electroretino-graphic responses in light-injured rat retinas. On the other hand, biodegradable polyurethanes with functional groups can provide a more robust platform for subsequent diverse modifications and thus can be potentially utilized for drug and gene delivery for cell reprogramming.[182] Poly(β-amino ester) is another polymer that has been widely applied as a gene-delivery system. For example, it has been employed to deliver genes encoding disease-specific chimeric antigen receptors (CARs) in order to reprogram T cells for therapeutic purposes.[102] The poly(β-amino ester) carriers also exhibited higher transfection efficiency of delivering a single polycistronic plasmid (pCAG-OSKMG) for human iPSC reprogramming, when compared with commercial transfection reagents.[179] In addition, dendrimers such as poly(amidoamine) are promising nanocarriers due to their high loading capability, excellent biocompatibility, and functionalization capability. Positive charges on the terminal amine groups of poly-(amidoamine) dendrimers can promote gene loading, cell penetration, and endosomal escape during transfection, resulting in functional gene transfection.[180] Furthermore, mouse iPSCs can be generated from MEFs using a single plasmid (pOSKM) delivered through arginine-terminated polyamidoamine dendrimer (G4Arg) nanoparticles, which exhibited a transfection efficiency (~15%) higher than that of commercially available carriers, such as Lipofectamine 2000 (~5%) or FuGENE HD (~8%).[183]

Taken together, synthetic materials not only enable the delivery of small molecules, proteins, and a variety of other compounds but also offer the potential for controlled nucleic acid transfection *in vitro* and *in vivo*, providing flexibility and possibility to manipulate cell fate. Next-generation drug- and gene-delivery systems with optimized kinetics will further improve targeted delivery and the efficiency of cell reprogramming.

Inorganic/Hybrid Nanoparticles.—Inorganic and hybrid materials can serve as nonviral drug and gene carriers that generally possess versatile properties suitable for intracellular delivery, including diversity, multiple functionality, excellent biocompatibility, targeted delivery, and controlled release.[184,185] Recently, several reports have used inorganic and hybrid particles for cell reprogramming (Figure 8), including mesoporous silica,[186] calcium

phosphate,[187,188] graphene oxide,[189,191] and gold-based nanoparticles.[190] Mesoporous silica nanoparticles (MSNs) have been widely used as nanocarriers for gene and drug delivery, mainly due to their tailored mesoporous structure, high surface area ratio, biocompatibility, and functionalization capability.[192] One study reported that avidin-capped MSNs adsorbed with three transcription factors (*Ascl1/Brn2/Myt1l*) could directly convert mouse fibroblasts into functional dopaminergic neuronlike cells. Interestingly, the co-delivery of a neurogenesis inducer, ISX-9, in the MSNs further enhanced the reprogramming efficiency.[186] Therefore, this strategy of co-delivering small molecules and plasmids can also be applied to other delivery carriers to achieve enhanced reprogramming performance. In addition, calcium phosphate nanoparticles exhibited the efficient delivery of four plasmids encoding OSKM to generate iPSCs from human umbilical cord mesenchymal stem cells.[187] The delivered nucleic acids formed ionic complexes with cationic calcium ions through their electrostatic interactions, which were easily transported into cells *via* ion-channel-mediated endocytosis. Interestingly, the hiPSC reprogramming can be accelerated upon incorporating calcium-phosphate-based hybrid nanoparticles into 3D collagen scaffolds, benefiting from the controlled release of OSKM genes and the biophysical regulation of the 3D niche.[193] Additionally, gold nanoparticles have emerged as another promising platform for gene and drug delivery due to their high surface area, inertness, and easy fabrication.[194] Cationic gold nanoparticles (AuNPs) are efficient carriers because they can interact with nucleic acids as well as the negatively charged plasma membrane.[190,195] For example, it has been reported that *in vitro* and *in vivo* cardiac reprogramming can be achieved using hybrid AuNP-based nanoparticles. The AuNPs modified with arginine-rich peptide (RRRGYC) were mixed with GMT (*Gata4, Mef2c,* and *Tbx5*) plasmids and polyethylenimine (PEI) to make the AuNP/GMT/PEI hybrid nanocomplexes, which promoted the cardiac reprogramming efficiency of human and mouse fibroblasts. More importantly, upon injection into infarcted mouse models, the nanocomplexes enhanced the *in vivo* cardiac reprogramming outcome and cardiac function.[190] Furthermore, hybrid materials such as graphene oxide (GO)–PEI complexes have been engineered to deliver mRNAs encoding OSKM for hiPSC generation.[191] Intriguingly, GO–Fe_3O_4–PEI complexes were designed to achieve stimuli-sensitive gene delivery for the reprogramming of peripheral blood mononuclear cells into hiPSCs. Notably, the cell transfection and reprogramming efficiency were significantly improved by the combined treatment of magnetic stirring and near-infrared photothermal stimulation.[189]

Despite the fact that inorganic or hybrid nanoparticles are promising as reprogramming delivery carriers, there are still some critical issues that need to be addressed. First, the loading capability and stability of genes or drugs are limited to a certain extent by surface adsorption. The second shortcoming is the possible aggregation of the inorganic nanoparticles loaded with nucleic acids, which may reduce the transfection efficiency. Another problem is the excretion of inorganic nanoparticles, and their accumulation in cells may be harmful; thus the *in vivo* safety of inorganic particles should be rigorously demonstrated before clinical translation.

Altogether, in addition to the reprogramming factors, the selection of an appropriate carrier among various biological, physical, or chemical delivery systems is critical to enable and enhance cell reprogramming. Undoubtedly, diverse drug- and gene-delivery strategies that

have been developed for genome editing, tissue engineering, and disease therapies can also be tested and applied for cell reprogramming.[19,122,123,196-199] From the perspective of clinical application, using non-gene-integrating platforms to generate reprogrammed cells is preferable to those that integrate into the genome. Moreover, considering the advantages and disadvantages summarized in Table 1 and translation potential, chemical carrier-mediated delivery systems, especially multifunctional, stimuli-sensitive drug-delivery systems,[200,201] may be more promising for *in vivo* reprogramming therapies *via* the controlled local release of reprogramming factors and targeted delivery.

BIOPHYSICAL AND BIOCHEMICAL REGULATION OF CELL REPROGRAMMING

In addition to biochemical signals, biophysical factors are broadly involved in orchestrating morphogenesis, development, homeostasis, and tissue remodeling by modulating cell proliferation, migration, differentiation, lineage commitment, or apoptosis.[202-204] Cells can convert extracellular biophysical cues into intracellular biochemical signals *via* mechanosensing and mechanotransduction, which in turn can regulate chromatin organization, gene expression, protein translation, and cell function.[202,205,206] In the past decade, biomaterials and devices have been used to manipulate external biophysical and biochemical cues for cell reprogramming.[33,34] By using advanced polymerization chemistries and micro- or nano-manufacturing techniques, biomaterials with desirable physical and chemical properties can be designed and fabricated.[207] For elucidative purposes, we focus on the effects of microenvironmental factors, including micro/nanotopography, stiffness, extracellular force stimulation, 3D microenvironments, and biochemical modifications, on cell reprogramming (Figure 9).

Micro/Nanotopography.

Manipulation of micro/nano-scale surface topography has a profound influence on cell functions, such as cell morphology, adhesion, proliferation, migration, differentiation, and stemness maintenance.[208-210] Although extensive research has demonstrated that topography can direct cell differentiation toward specific cell lineages,[211,212] more recent studies have provided some insight into the role of topography in cell reprogramming.

Biomaterial topography, in the form of parallel microgrooves and aligned nanofibers, significantly improved iPSC generation through alterations in cell shape that resulted in an increase in histone acetylation and methylation and mesenchymal-to-epithelial transition (MET) promotion.[213] Consistent with this observation, another study further confirmed that the MET process mediated topography-induced epigenetic changes during iPSC reprogramming from mouse somatic fibroblasts using graphene-coated substrates.[214] These substrates, which have two-dimensional honeycomb structures, yielded almost 4 times the number of positive mouse iPSC colonies compared to glass surfaces. Apart from iPSC reprogramming, micro-topography can also influence the direct reprogramming process. For example, microgratings promoted the direct reprogramming of fibroblast into induced neurons by modulating neurite branching, neurite outgrowth, and gene expression.[215,216] Similarly, the efficiency of direct cardiac reprogramming can be enhanced with topography

modulation.[217,218] Parallel microgrooves were found to be capable of not only modifying the epigenetic state (*e.g.,* increased histone acetylation) but also regulating myocardin transcription factor A signaling to promote direct reprogramming into cardiomyocytes. Notably, microgrooved topography generated more mature cardiomyocyte-like cells, which may enhance functional performance after cell transplantation into an infarcted heart.[217]

In contrast to microtopography, nanotopography has demonstrated a more influential role in inducing differentiation of hiPSCs to a neuronal lineage,[219] possibly due to the fact that these nanoscale features may more closely resemble the *in vivo* microenvironment. Upon examining how micro- and nanotopographical cues could influence direct reprogramming into induced dopaminergic neurons, it was determined that nanogrooved substrates further enhanced neuronal gene expression, maturation, and reprogramming efficiency through modulation of histone mark expression and MET.[220] However, whether nanotopography can regulate direct reprogramming into other cell lineages remains to be further explored. The underlying mechanisms of topographical influence on cell reprogramming are not fully understood. However, the mechanisms that have been elucidated for topography-mediated cell differentiation may also be applicable in reprogramming. For example, the aforementioned studies suggest that both micro- and nanotopographical cues can influence cell and nuclear shape to alter the epigenetic state, gene expression, and thus cell fate.[208-210] In addition, topography is strongly correlated to focal adhesion dynamics, leading to intracellular signaling transduction pathways triggered by topographic parameters of substrates. Moreover, topography-induced cytoskeletal changes can induce nuclear deformation that leads to altered nuclear mechanics and chromatin state, both of which can affect cell phenotype and function.[221]

Stiffness.

Substrate stiffness is one of the most widely applied physical cues to modulate stem cell differentiation.[222-224] For example, soft substrates promote the differentiation of human MSCs into neurons or adipocytes, whereas osteogenesis is favored on stiffer substrates.[225] Matrix stiffness can also modulate the cell-reprogramming process. Recently, it has been demonstrated that soft polyacrylamide hydrogels with a stiffness of 100 Pa enhanced mouse iPSC reprogramming from MEFs on the surface of the hydrogel,[226] whereas another study reported that the optimal stiffness for mouse and human iPSC generation was between 300 and 600 Pa in 3D polyethylene glycol (PEG) hydrogels.[227]

Mechanistic studies have revealed that substrate stiffness can alter cell adhesion and cytoskeletal reorganization, *via* mechanotransductive mechanisms, to activate signaling pathways that modulate gene expression and, thus, cell behavior.[222,228] Although several intracellular mechanosensitive signaling cascades are regulated by matrix stiffness, such as AKT/YAP/RUNX2,[229] p190RhoGAP,[230] and YAP/TAZ mechanotransduction,[29,231] the underlying molecular mechanisms of how stiffness regulates cell reprogramming remain unknown and require further investigation.

Extracellular Force Stimulation.

Extracellular mechanical forces can influence organ development and function in health and disease by triggering a series of cytoskeletal-mediated mechanotransduction and intracellular signaling cascades.[202,232,233] Recently, extracellular forces including shear stress, mechanical stretching, and electromagnetic forces have been used as effective biophysical cues to modulate cell reprogramming.

Extracellular fluids, including blood plasma, interstitial fluid, and lymph, are crucial in the exchange of substances and the maintenance of the microenvironment. Fluid shear stress is an essential mechanical factor in flowing liquid that can induce cytoskeletal organization remodeling, trigger mechanosensitive gene expression, and alter cell behavior, demonstrating an important role in vascular homeostasis, vascular remodeling, cardiac development, atherogenesis,[234,235] and cancer metastasis.[145,236] In addition, fluid movement enhances convective mixing and transport. Compared to conventional adherent culture, faster and more efficient mouse iPSC reprogramming can be achieved in a stirred suspension bioreactor with retroviral transduction of transcription factors (*i.e., Oct4, Sox2*, and *Klf4,* with or without *c-Myc*), displaying more than a 10-fold increase in efficiency compared to that with the adherent culture.[237] Fluid shear stress present within a dynamic culture suppressed differentiation and provided the additional benefit of selective aggregate formation, enabling reprogrammed cells to flourish. As a result, dynamic suspension-induced reprogramming is a promising and scalable approach for basic research and clinical uses.[238] Interestingly, 2D culture with dynamic mixing at the mid to late phase of reprogramming also demonstrates significant improvement of mouse iPSC reprogramming, which was found to be attributed to the enhancement of cell proliferation.[239]

Mechanical stress and strain *in vivo* play fundamental roles in guiding cell behavior and tissue homeostasis.[240] At the cellular level, the mechanical stretch has demonstrated crucial regulatory effects on cell spreading, growth, lineage commitment, and stem cell differentiation on 2D substrates.[241,242] In addition, externally applied stretch (uniaxial, biaxial, and equiaxial) has been utilized to engineer functional and mature tissues in 3D scaffolds, such as in heart tissue,[243,244] skeletal muscles,[245] and blood vessels.[246,247] Mechanical stretch can also be used for *in vivo* tissue regeneration, for example, by activating hair stem cells and eliciting macrophage recruitment and M2 phenotype polarization to facilitate hair regeneration in a strain/duration-dependent manner.[248] Recent studies have shown that mechanical stretch has a positive effect on reprogramming. When dermal fibroblasts transduced with OSKM were seeded onto flexible membranes that were mechanically stretched, 3 and 5 times more human iPSC colonies were obtained upon equiaxial stretching for 4 days at 3 and 8% strain, respectively, than with the unstretched groups.[249] However, cyclic mechanical stretch did not improve the yield of direct cardiac reprogramming.[218] Therefore, the effects of mechanical stretch may be dependent on specific signaling pathways involved in the reprogramming process. In addition, various parameters of stretching such as mode, magnitude, rate, frequency, duration, continuity, and relaxing period may have profound effects on cell signaling and reprogramming, which is worth further studies.

Moreover, the mechanical forces driven by electromagnetic or electric fields can improve cellular reprogramming. For example, extremely low-frequency electromagnetic fields (EL-EMF) significantly enhanced the reprogramming efficiency from mouse fibroblasts to iPSCs. EL-EMF induced dynamic regulation of epigenetic changes through the activation of a histone lysine methyltransferase.[250] In addition, electro-magnetized AuNPs provided an excellent interface to deliver EMF for cell reprogramming. Application of EMF to cells cultured on AuNPs directly reprogrammed fibroblasts into induced dopaminergic neurons both *in vitro* and *in vivo.* EMF stimulation specifically activated the histone acetyltransferase Brd2, leading to histone H3K27 acetylation and neuron-specific gene activation.[195] Furthermore, a triboelectrical stimulation system can be combined with nonviral gene delivery to directly convert fibroblasts into functional neuronal cells with higher efficiency.[251] The triboelectrical stimulation promoted dynamic changes of intracellular calcium levels in reprogrammed cells, including increased calcium influx and ERK1/2 pathway activation. These electromagnetic or electric stimulation systems can potentially serve as electroceuticals to provide less invasive and non-chemical-based methods to facilitate *in vivo* reprogramming or *in situ* direct cell conversion.

3D Microenvironment.

3D microenvironments can provide biomimetic systems to direct cell fate, including stemness maintenance, organogenesis, and tissue development.[252-254] Of particular interest are biomaterials-mediated and self-assembled cellular 3D microenvironments that can serve as powerful platforms to modulate cell reprogramming and highlight the clinical potential of reprogramming technologies.

3D biomaterial-based microenvironments enable robust spatial and temporal control of biophysical and biochemical cues to modulate cell fate both *in vitro* and *in vivo*. It has been demonstrated that mouse and human iPSC reprogramming can be enhanced in 3D PEG-based hydrogels by optimizing parameters such as stiffness, degradability, and biochemical composition.[227] It was determined that cell conversion could be vastly improved using highly degradable gels with a stiffness of 600 Pa. This 3D approach resulted in a more than 3-fold increase in the reprogramming efficiency compared to 2D environments, as a result of accelerating the MET process and increasing epigenetic remodeling. In addition, electrospun fibrous scaffolds, which have been widely applied as biomimetic microenvironments for biomedical applications, can also promote *in situ* stem cell neuronal reprogramming, resulting in neural network formation and brain engraftment.[216]

Furthermore, several studies have shown that reprogramming cells in 3D microenvironments can enhance cell phenotypic functions. Chitosan substrate-mediated gene delivery of a naked *Foxd3* plasmid can reprogram human fibroblasts into neural crest stem-like cells that self-assemble into 3D cellular spheroids. Notably, the Foxd3-transfected spheroids demonstrated significantly greater functional rescue of the CNS in impaired zebrafish models compared to that with dispersed cells.[255] In addition, under Matrigel-embedded 3D culture conditions, the combined expression of *Gata6, Cdx2*, *Hnf4a,* and *Foxa3* directly converted mouse fibroblasts into fetal intestine-derived progenitor cells (FIPCs) and allowed for the formation of spherical organoids, which further developed into budding organoids.[74] Particularly, the

derived intestinal organoids regenerated colonic tissue upon transplantation, demonstrating great promise for the treatment of intestinal diseases. Therefore, these organoids, which consist of 3D structures with a heterogeneous cell composition, may serve as biomimetic systems that can recapitulate specific gene and protein expression, tissue morphology, and physiological functions. In the same study, it was determined that the defined transcription factors also induced human FIPCs from human dermal fibroblasts and human umbilical vein endothelial cells.[74] However, in contrast to mouse iFIPCs, human iFIPCs did not generate budding organoids, suggesting additional factors or functional 3D niches may be needed.

Organ-on-a-chip is a burgeoning technique and powerful tool for disease modeling, drug discovery, toxicology research, and regenerative medicine.[256] Organ-on-a-chip systems are manufactured with advanced micro/nanofabrication techniques based on microfluidics, materials, and tissue engineering, enabling the design of customized cellular and tissue microenvironments with precise perfusion, mechanical and structural control, electrical stimulation, less probability of contamination, and high-throughput analysis.[257] The engineered architectures can recreate partial or all functions of native tissues, such as biological tissue barriers, parenchymal tissue functions, and multiorgan interactions. In particular, diverse 3D organoids, including liver,[258] pancreatic islet,[259] lung,[260] and brain,[261] that have been developed within organon-a-chip systems through *in situ* iPSC differentiation have demonstrated complex tissue and organ-specific functions. Most recently, organ-on-a-chip has shown great potential and superiority in cell reprogramming through the confinement of the microenvironment. Through the manipulation of the concentration and the sequential delivery of reprogramming factors (mixed mRNAs encoding transcription factors) and small molecules, an automated microfluidic platform dramatically improved human iPSC reprogramming with a 50-fold increase in efficiency (Figure 10).[262,263] In the same platform, the generated hiPSCs could be precisely differentiated into functional cardiomyocytes and hepatocytes. The ability to perform both cell reprogramming and differentiation of patient-specific cells within these devices hold great promise for personalized drug screening, bringing us closer to more personalized medicine.

Biochemical Modifications.

Biochemical cues such as extracellular matrix (ECM) components, small molecules, growth factors, and other signaling molecules regulate not only stem cell fate but also cell reprogramming.[254] ECM and its derivatives are commonly used as hydrogels or bioactive molecules immobilized onto 2D substrates and 3D scaffolds. One study demonstrated that fibrin and a fibrin–collagen composite were superior to Matrigel and collagen I in supporting indirect cardiac reprogramming.[264] It was determined that matrix identity and tractional forces played major roles in cell reprogramming, whereas matrix mechanics, matrix microstructure, and cell proliferation influenced the process to a lesser extent.[264] To interrogate the effect of ligand identity, ligand density, and substrate modulus on cell reprogramming, ECM-coated polyacrylamide substrates can be developed to decouple physicochemical features.[265] In addition, PEG hydrogels can be modified with specific bioactive molecules to modulate specific cellular responses while preventing unwanted adsorption of proteins. PEG hydrogels modified with high concentrations of laminin and RGD peptide nearly doubled the efficiency of induced cardiomyocyte-like cells

from fibroblasts.[265] Therefore, cellular reprogramming can be enhanced using customized engineered materials with immobilized biosignals.

Small molecules and growth factors have been widely explored to enhance or induce either iPSC reprogramming[114,266] or direct reprogramming into various cell types, including neurons,[67] cardiomyocytes,[66] pancreatic β-like cells,[267] and hepatocytes.[268] Indeed, these small molecules and proteins can be mixed or conjugated, as ECM molecules, onto 2D cell culture substrates and 3D scaffolds to influence cell fate. Of note, such biochemical stimuli can be endowed and controlled by the defined delivery systems and can also be integrated with biophysical cues to maximize cell reprogramming, especially for *in situ* reprogramming.

FUTURE PERSPECTIVES

Cell reprogramming is a highly valuable method for engineering cell fate. This comprehensive review highlights the recent progress in engineering biomaterials with advanced micro/nanofabrication techniques to provide delivery platforms and biophysical and biochemical cues for enhancing *in vitro* and *in vivo* cell reprogramming. However, many challenges remain to be addressed before the clinical application of reprogramming technologies, including efficiency, quality, safety issues, *in situ* targeting, and the underlying signaling mechanisms. To address these challenges, multidisciplinary collaborative research from the fields of materials science, bioengineering, biology, and medicine would be required.

Optimized delivery systems need to be developed or selected from a diverse group of drug- and gene-delivery strategies to achieve the desired reprogramming outcome. First, reproducibility and higher efficiency will be the critical criteria in developing the optimal delivery methods for cell reprogramming. For *in vivo* reprogramming, if specific parts of the body, organs or tissues, or a subpopulation of cells are targeted for cell type conversion, a drug-delivery system incorporated with a specific ligand, antibodies, peptides, or aptamers can be developed. Second, as small molecules offer powerful tools to manipulate cell fate by inducing or enhancing cell reprogramming, the co-delivery of nucleic acids with small molecules or the multidelivery of chemical cocktails can be developed to promote cell reprogramming *in vitro* and *in vivo*. Third, smart and stimuli-responsive delivery systems can be custom-designed to achieve more efficient *in situ* targeted delivery of reprogramming factors, which is an emerging area of biomaterials research. Fourth, biophysical and biochemical cues can also be incorporated into the drug-delivery system to enhance cell reprogramming, yet the optimal conditions for reprogramming remain to be clarified.

Biophysical manipulation provides a promising approach to regulate cell fate and improve the reprogramming process. First, the majority of biophysical cues (including topography, stiffness, and extracellular force) and biochemical cues (including ECM, soluble factors, and small molecules) have been employed for cell reprogramming individually, which demonstrates the feasibility of these approaches and helps elucidate the underlying mechanisms. Combining such cues may synergistically enhance reprogramming efficiency, but it is a challenging task to figure out the optimal conditions with so many possible

combinations, crosstalk, and potential side effects. In addition, temporal regulation of the reprogramming process at the early or late phase may require different microenvironmental factors. If sufficient experimental data can be collected and specific experiments can be designed to address the combinatory effects, a machine learning approach can be used to optimize the reprogramming conditions and protocol. Second, to make it relevant to *in vivo* conditions, reprogramming in 3D scaffolds with desired and integrated cues will be a key step to generate and apply reprogramming-mediated engineered tissues *in vitro* or in *situ.* Third, whether and how biophysical factors may influence *in vivo* reprogramming is not well understood and deserves further investigation in the future.

Advances in other cutting-edge technologies will continue to expand the frontiers of cell reprogramming. First, microfluidic platforms enable the delivery of reprogramming factors in a more controlled and efficient way from the perspectives of composition, dosage, and spatiotemporal features, which can also offer well-defined nanotopographical and mechanical cues, including fluid shear stress, cyclic strain, and compression. It is expected that the orchestrated interplay of the controlled delivery and combined physical and chemical cues can further enhance cell reprogramming. Meanwhile, the microfluidic environment allows directed generation of functional cells in the same platform, which creates a model of “tissue/organ reprogramming-on-a-chip”. Second, 3D printing holds great promise for engineering tissues and organs for disease modeling and regenerative medicine.[269] 3D printing enables the manipulation of the microenvironment with spatially defined mechanical, structural, and chemical properties, thus, making it suitable to generate 3D complex niches for cell reprogramming. Third, single-cell sequencing analysis will provide insight into the heterogeneous cellular responses and fate determination during the reprogramming process[270,271] and help elucidate the signal pathways, molecular identity, and underlying mechanisms by which biophysical and biochemical cues regulate cell reprogramming. Fourth, CRISPR/Cas9 genome-editing platforms provide powerful tools not only for genetic correcting of hereditary diseases[272-274] but also for somatic cell reprogramming, as demonstrated in recent studies for the derivation of iPSCs[275] and neurons.[276] Viral-free CRISPR/Cas9 delivery systems have shown promising results, which may enable more *in vivo* applications. Hence, simultaneous cell reprogramming and CRISPR/Cas9 gene editing, through the co-delivery of both gene-editing systems and reprogramming factors, may guide the design of next-generation cell-reprogramming technologies.

The advancement and integration of cell reprogramming and biotechnologies present exciting opportunities for research and technology translation in these interdisciplinary frontiers. We expect that the rational design and development of delivery systems and biomaterials that control microenvironmental cues will accelerate the translation of reprogramming technologies for cell engineering and therapies.

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VOCABULARY

embryonic stem cells
pluripotent stem cells derived from the undifferentiated inner cell mass of a blastocyst, an early stage embryo

somatic cell nuclear transfer
a technique involving the transfer of somatic cell nucleus into the cytoplasm of an enucleated egg

cell differentiation
a process whereby a cell changes from an immature to more specialized phenotype

iPSC reprogramming
induced conversion of somatic cells into pluripotent stem cells

direct reprogramming
induced conversion of a mature cell into another cell type without proceeding through an pluripotent state

indirect reprogramming
transient induction of a partially reprogrammed cell using the Yamanaka factors, followed by differentiation into the target cell type

transcription factor
a protein that can regulate gene transcription by binding to a specific DNA sequence

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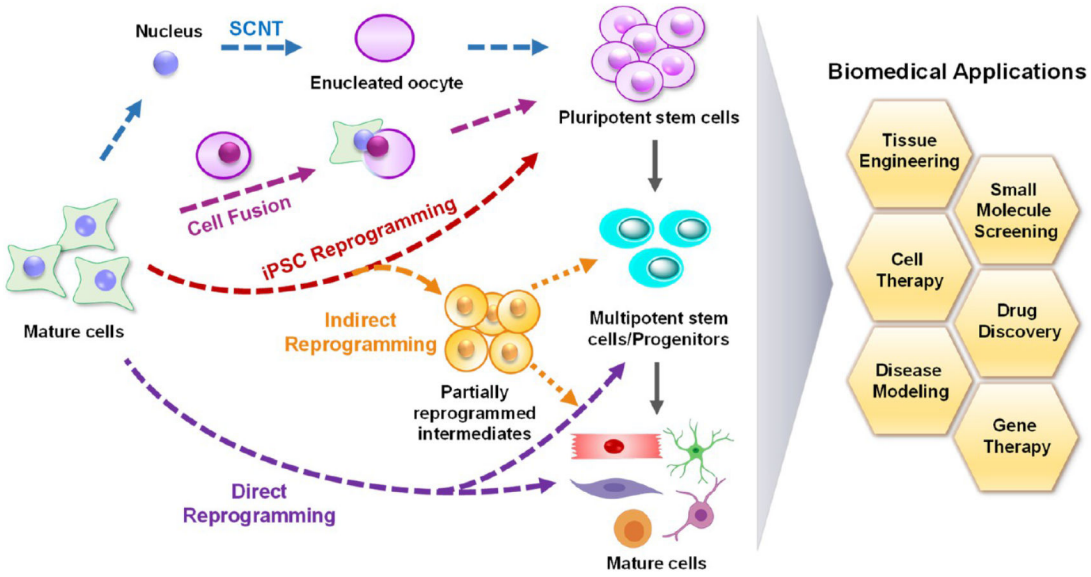

Figure 1.

Cell-reprogramming strategies for biomedical applications. Schematic illustrating the process of SCNT, cell fusion, iPSC reprogramming, indirect reprogramming, and direct reprogramming. Cell reprogramming holds great promise for various biomedical applications, as indicated in the figure. SCNT, somatic cell nuclear transfer; iPSC, induced pluripotent stem cell.

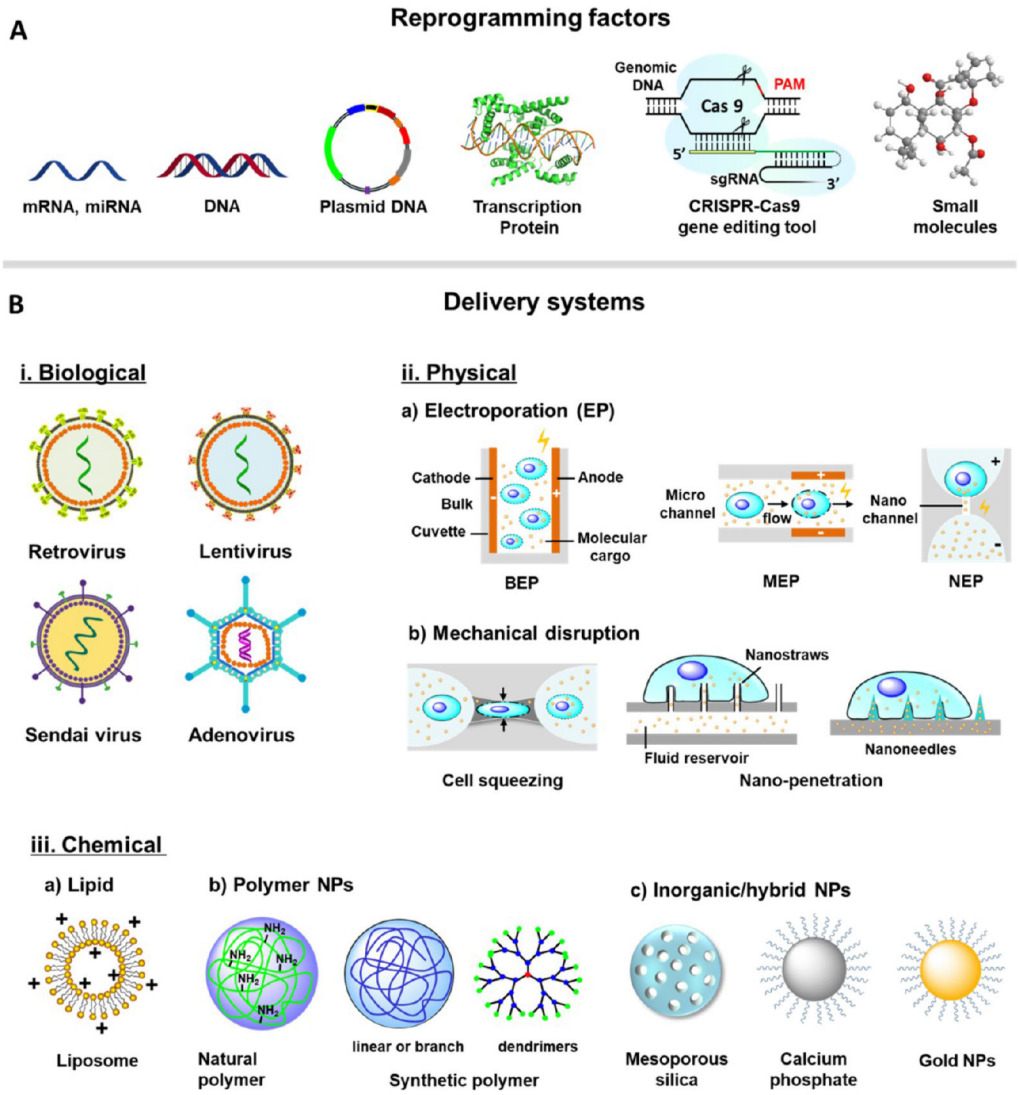

Figure 2.

Biomaterial- and micro/nanotechnology-based delivery approaches for cell reprogramming. (A) Cell reprogramming has been achieved using various reprogramming factors. (B) Three major delivery modalities that have been applied for cell reprogramming include biological, physical, and chemical methods. (i) Biological delivery relies on genetically engineered viruses to transfer DNA constructs into cells. (ii) Physical delivery methods include membrane-disruption approaches to deliver reprogramming factors into the cytoplasm or nucleus. (iii) Chemical delivery methods employ lipids, natural or synthetic polymers, and inorganic or hybrid carriers. sgRNA, single guide RNA; AAV, Adeno-associated virus; BEP, bulk electroporation; MEP, microchannel electroporation; NEP, nanochannel electroporation; nanopenetration, nanostructure-mediated membrane penetration; NPs, nanoparticles.

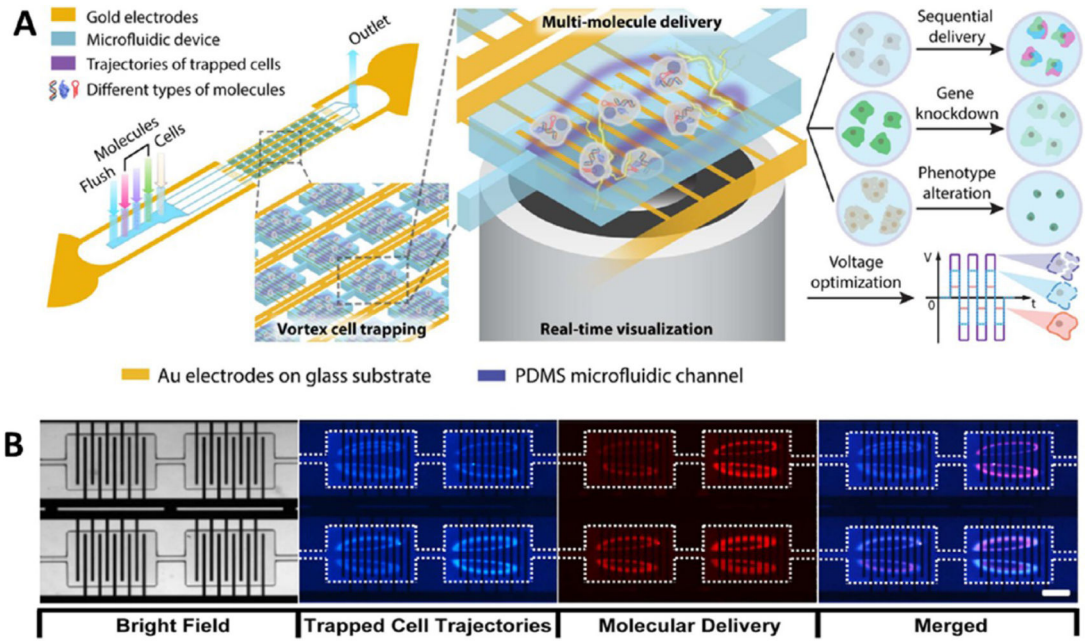

Figure 3.

Microchannel or microfluidic electroporation for cargo delivery. (A) Vortex-assisted microfluidic-based electroporation system integrated with micropatterned planar electrodes in the vortex cell-trapping chamber and equipped with real-time visualization functionality. The patterned electrodes were merged within a single cell-trapping chamber with the following parameters: chamber length = 720 μm; chamber width = 480 μm; electrode length = 450 μm; and electrode width = 20 μm. (B) Membrane-impermeable fluorescent molecule delivered into trapped cells *via* electroporation, HEK 293 cells (blue), propidium iodide (red). Reprinted with permission from ref 123. Copyright 2017 Springer Nature.

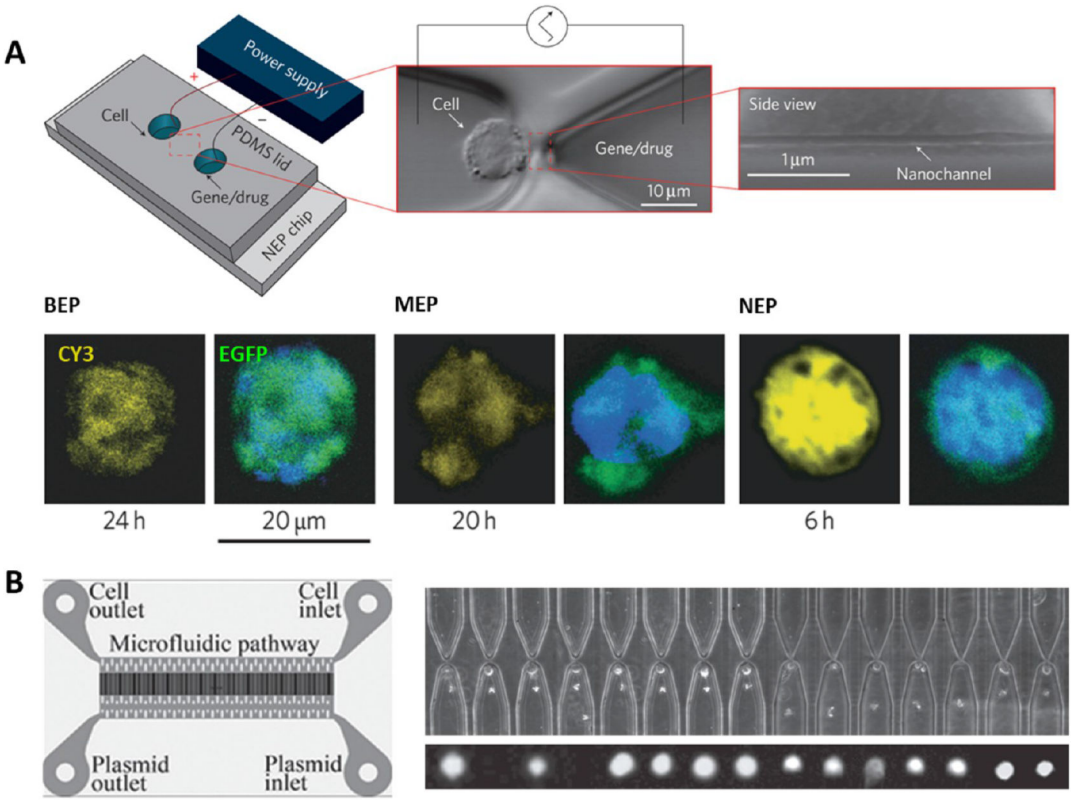

Figure 4.

NEP-mediated delivery. (A) NEP device comprises a microchannel–nanochannel–microchannel structure and electrodes in the reservoirs. Single cells are precisely positioned using optical tweezers. NEP (90 nm diameter, 3 μm length for nanochannel) transfection of mouse embryonic fibroblasts with Cy3-labeled GFP plasmid shows higher transfection and faster gene expression than using BEP and MEP (5 μm) delivery. Reprinted with permission from ref 141. Copyright 2011 Springer Nature. (B) High-throughput NEP transfection of mouse embryonic fibroblasts with green fluorescent liposomes containing the reprogramming factor (OSKM plasmid). A large number of cells are loaded through centrifugal spinning method. Reprinted with permission from ref 146. Copyright 2014 Wiley.

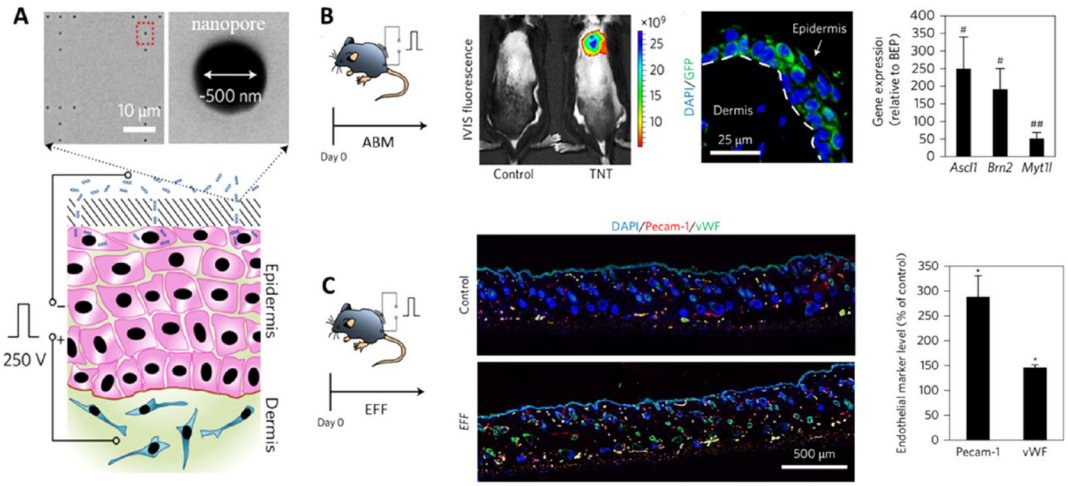

Figure 5.

Nanochannel electroporation mediates *in vivo* reprogramming. (A) Skin tissue nanotransfection through a nanochanneled device and a pulsed electric field to deliver reprogramming factors into skin cells. (B) Reprogramming skin cells into induced neurons with nanotransfection of Ascl1/Brn2/Myt1l (ABM) plasmids. Representative *in vivo* imaging system (IVIS) fluorescence of mouse skin treated with fluorescein-amidite-labeled DNA. GFP is a reporter of Ascl1 gene. (C) Reprogramming skin cells into induced endothelial cells *via* nanotransfection of Etv2/Foxc2/Fli1 (EFF) plasmids. Fluorescent staining of Pecam-1 and vWF show increased vascularization, and high-resolution laser speckle imaging shows enhanced perfusion in the EFF-treated area. Reprinted with permission from ref 136. Copyright 2017 Springer Nature.

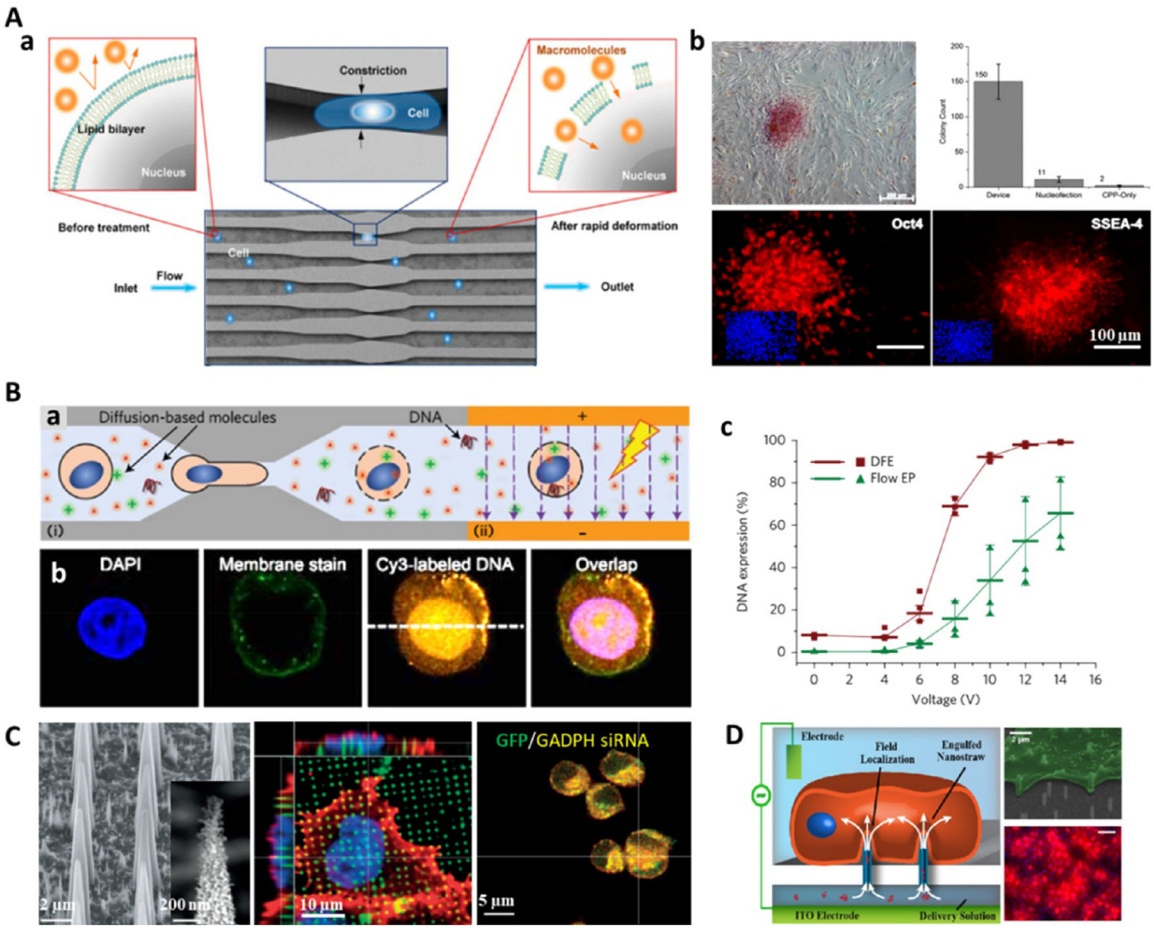

Figure 6.

Mechanical disruption mediates cell reprogramming. (A) (a) Cell squeezing device for intracellular delivery of macromolecules. (b) Human iPSC reprogramming in a cell squeezing device through the delivery of recombinant OSKM transcription factors into fibroblasts. Alkaline phosphatase staining showed the colony formation in a sample plate, and significantly higher numbers of colonies were present when compared to controls treated with nucleofection or cell-penetrating peptides (CPPs). Reprinted with permission from ref 152. Copyright 2013 National Academy of Sciences. (B) (a) Disruption and field-enhanced (DFE) delivery approach combines (i) cell squeezing and (ii) electroporation processes to disrupt both the plasma membrane and nuclear envelope for enhanced DNA delivery. (b) Immunofluorescent images show a CY3 fluorescence-labeled DNA plasmid was transferred into HeLa cells using DFE, and the fluorescent signal was present in both the cell nucleus and the cytoplasm. (c) DNA transfection efficiency at applied electric voltages. Reprinted with permission from ref 144. Copyright 2017 Springer Nature. (C) Porous silicon nanoneedles for intracellular co-delivery of GFP plasmid (green) and GAPDH-siRNA (yellow). Reprinted with permission from ref 157. Copyright 2015 Springer Nature. (D) Nanostraw electroporation mediated highly efficient intracellular delivery and transfection. Reprinted from ref 147. Copyright 2013 American Chemical Society.

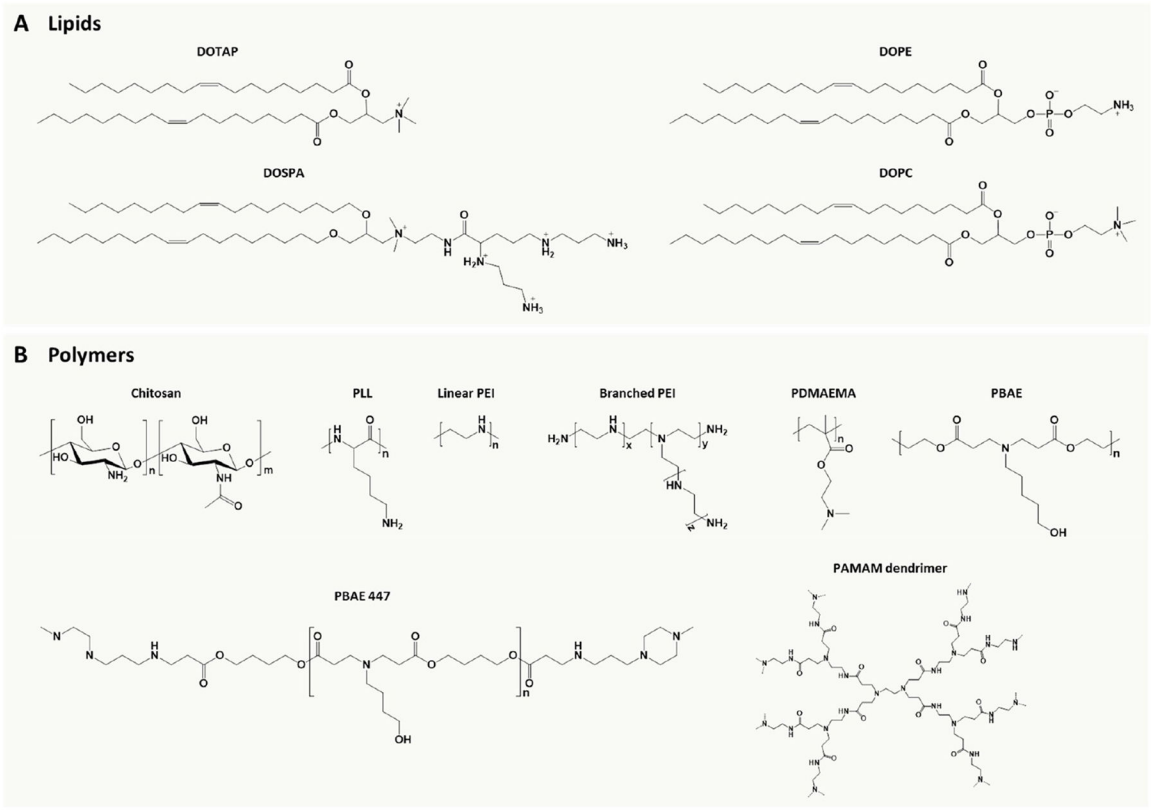

Figure 7.

Chemical structures of representative nonviral vectors for cell reprogramming. (A) Cationic and neutral lipids. (B) Natural and synthetic cationic polymers. Abbreviations: DOTAP, 1,2-dioleoyl-3-trimethylammonium propane; DOSPA, 2,3-dioleyloxy-*N*-(2-(sperminecarboxamido)ethyl)-*N,N*-dimethyl-1-propanaminium; DOPE, dioleoylphosphatidylethanolamine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; PLL, polylysine; PEI, polyethylenimine; PDMAEMA, poly((2-dimethylamino)ethyl methacrylate); PBAE, poly(*β*-amino ester); PAMAM, polyamidoamine.

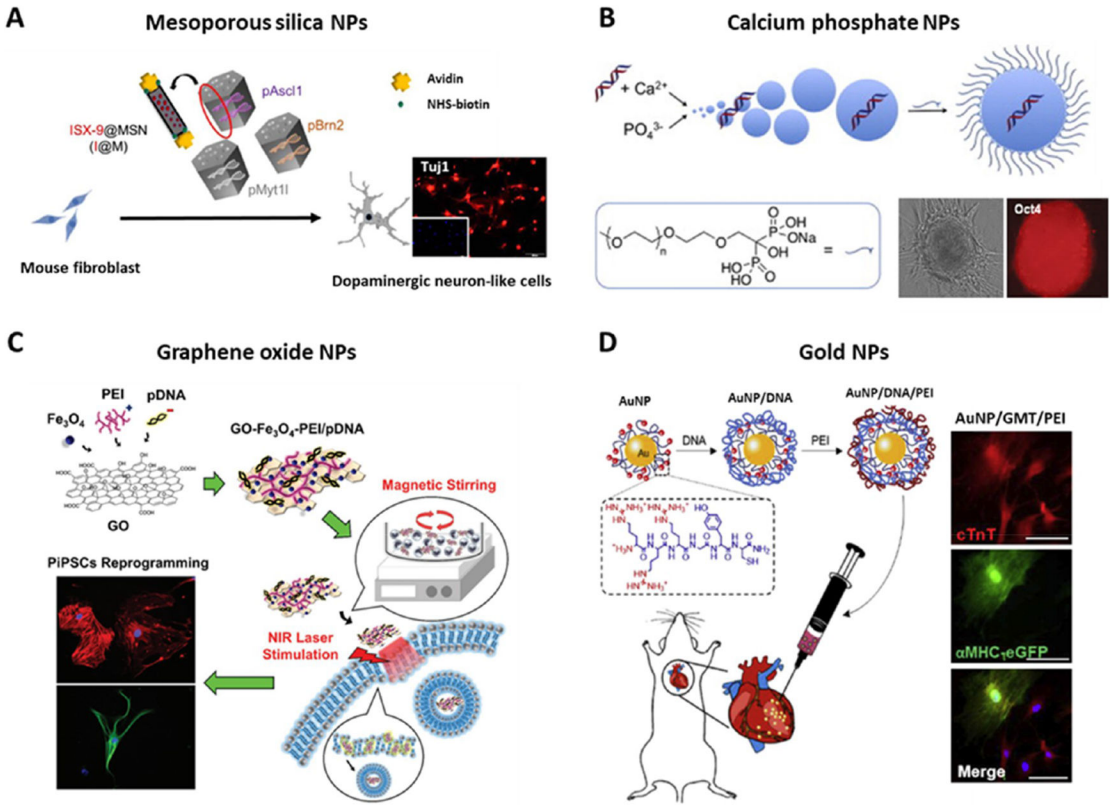

Figure 8.

Inorganic/hybrid nanoparticle-mediated nonviral delivery for cell reprogramming. (A) Mesoporous silica nanoparticle mediated neuron reprogramming from mouse fibroblasts through the delivery of Ascl1, Brn2, and Myt1l plasmids. Reprinted with permission from ref 186. Copyright 2018 Springer Nature. (B) Calcium phosphate nanoparticle mediated iPSC reprogramming from human umbilical cord mesenchymal stem cells (HUMSCs), *via* co-delivering Oct4, Sox2, Klf4, and c-Myc plasmids. Reprinted with permission from refs 187 and 188. Copyright 2013 Wiley and 2011 Elsevier. (C) Graphene oxide nanoparticle mediated iPSC reprogramming from human peripheral blood mononuclear cells through the delivery of episomal plasmids (pCXLEhOCT3/4-shp53, pCXLE-hSK, and pCXLE-hUL). Iron oxide nanoparticle (Fe_3O_4)-decorated with graphene oxide (GO) were mixed with DNA and PEI to make GO–Fe_3O_4–PEI complexes. Reprinted with permission from ref 189. Copyright 2017 Wiley. (D) Gold nanoparticles delivering Gata4, Mef2c, and Tbx5 (GMT) plasmids mediated *in vivo* cardiac reprogramming in an infarcted heart. Modified AuNPs (arginine-rich AuNPs, RRRGYC-AuNPs) were mixed with DNA and then PEI to make a AuNP/GMT/PEI nanocomplex. Reprinted with permission from ref 190. Copyright 2019 Elsevier.

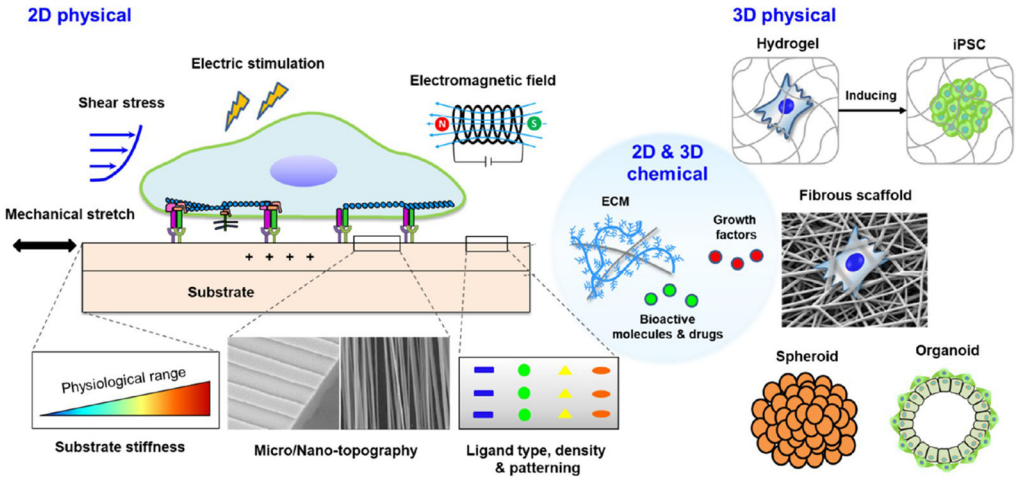

Figure 9.

Various biophysical and biochemical cues for cell reprogramming. Biophysical factors consist of topographical cues, substrate stiffness, extracellular forces (including mechanical stretching, shear stress, electrical and electromagnetic forces), ligand density/patterning, and 3D microenvironments. Biochemical cues, such as extracellular matrix, growth factors, and small molecules, can be coated or immobilized onto 2D substrates and 3D scaffolds to modulate cell reprogramming.

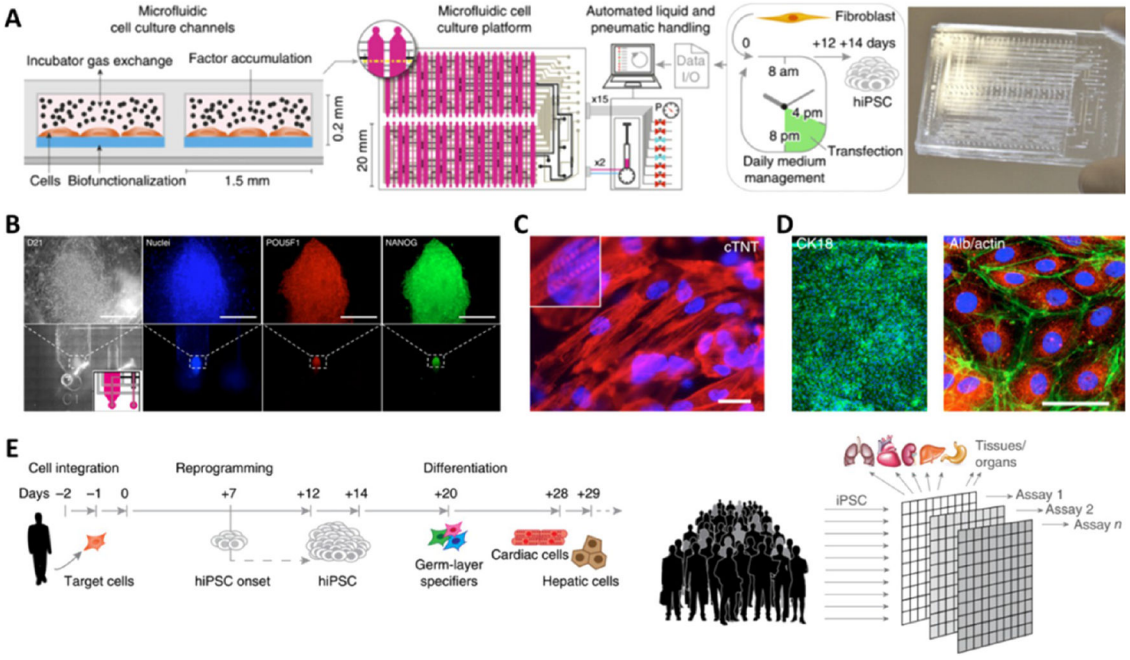

Figure 10.

Cellular reprogramming and differentiation on microfluidic chips. (A) Automated microfluidic platform for highly efficient hiPSC reprogramming by sequential delivery of mRNAs of POU5F1, SOX2, KLF4, c-MYC, NANOG, LIN28. (B) hiPSC colony formation within a microfluidic channel and the expression of pluripotency markers. (C) Differentiation of primary μ-hiPSCs into cardiomyocytes. (D) Differentiation of primary μ-hiPSCs into hepatic cells. (E) Vision of high-throughput biological assays *via* integrated cell reprogramming and differentiation in a microfluidic chip platform. Reprinted with permission from ref 262. Copyright 2016 Springer Nature.

Table 1.

Comparison of Delivery Methods of Reprogramming Factors for Cell Reprogramming

methods	biological		physical		chemical	
advantages	•	applicable both *in vitro* and *in vivo*	•	applicable *in vitro*	•	applicable both *in vitro* and *in vivo*, ease of administration
	•	high transfection efficiency	•	nonimmunogenic and no mutagenesis, low or no genetic integration	•	nonimmunogenic and no mutagenesis, no genetic integration
	•	works well for difficult-to-transfect cells	•	deliver diverse cargos without size limitations	•	deliver diverse cargos without size limitations
			•	rapid, instantaneous transfection	•	diverse biomaterials are available or can be specifically designed
					•	targeted delivery and controlled release
limitations	•	limited to nucleic acid delivery	•	less amenable to *in vivo* translation	•	low efficiency and slow delivery
	•	limited packaging capacity	•	loss of cytoplasmic content	•	carrier materials may perturb cell function and/or cause toxicity in unpredictable ways
	•	nonspecific target delivery *in vivo*	•	some approaches (*e.g.*, bulk electroporation) can lead to excessive cell damage, highly stochastic transfection, and low efficiency	•	complex, laborious, and expensive material synthesis is required for advanced smart carriers
	•	time-consuming, laborious, expensive and technically challenging	•	requires special expertise and complex fabrication protocols to achieve dose control		
	•	biosafety issues related to cytotoxicity, mutagenesis for integrating viruses, and adverse host immune response				