2-2019

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Minireview

Biomaterial substrate modifications that influence cell-material interactions to prime cellular responses to nonviral gene delivery

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Impact statement

This review summarizes how biomaterial substrate modifications (e.g. chemical modifications like natural coatings, ligands, or functional side groups, and/or physical modifications such as topography or stiffness) can prime the cellular response to nonviral gene delivery (e.g. affecting integrin binding and focal adhesion formation, cytoskeletal remodeling, endocytic mechanisms, and intracellular trafficking), to aid in improving gene delivery for applications where a cell-material interface might exist (e.g. tissue engineering scaffolds, medical implants and devices, sensors and diagnostics, wound dressings).

Abstract

Gene delivery is the transfer of exogenous genetic material into somatic cells to modify their gene expression, with applications including tissue engineering, regenerative medicine, sensors and diagnostics, and gene therapy. Viral vectors are considered the most effective system to deliver nucleic acids, yet safety concerns and many other disadvantages have resulted in investigations into an alternative option, i.e. nonviral gene delivery. Chemical nonviral gene delivery is typically accomplished by electrostatically complexing cationic lipids or polymers with negatively charged nucleic acids. Unfortunately, nonviral gene delivery suffers from low efficiency due to barriers that impede transfection success, including intracellular processes such as internalization, endosomal escape, cytosolic trafficking, and nuclear entry. Efforts to improve nonviral gene delivery have focused on modifying nonviral vectors, yet a novel solution that may prove more effective than vector modifications is stimulating or "priming" cells before transfection to modulate and mitigate the cellular response to nonviral gene delivery. In applications where a cell-material interface exists, cell priming can come from cues from the substrate, through chemical modifications such as the addition of natural coatings, ligands, or functional side groups, and/or physical modifications such as topography or stiffness, to mimic extracellular matrix cues and modulate cellular behaviors that influence transfection efficiency. This review summarizes how biomaterial substrate modifications can prime the cellular response to nonviral gene delivery (e.g. integrin binding and focal adhesion formation, cytoskeletal remodeling, endocytic mechanisms, intracellular trafficking) to aid in improving gene delivery for future therapeutic applications.

Keywords: Biomaterials, transfection, priming, extracellular matrix, gene delivery, biotechnology

Experimental Biology and Medicine **2019; 244: 100–113. DOI: 10.1177/1535370218821060**

Introduction

Gene delivery is the transfer of exogenous genetic material into somatic cells to modify their gene expression, with applications including tissue engineering, regenerative medicine, sensors and diagnostics, and gene therapy. Viral vectors such as lentivirus or adenovirus are considered the most effective systems to deliver nucleic acids due to high efficiency, innate endosomal release mechanisms, and stable transgene expression, which is applicable for long-term therapy.[1] Although viral gene delivery systems have had clinical success,[2] the use of viral vectors has safety concerns and many other disadvantages, including gene size limitations, insertional mutagenesis, and immunogenicity.[3] As an alternative to viral gene delivery, nonviral gene delivery has emerged as a more cost-effective option with the ability to deliver larger genetic cargoes, improved scalability, lower immune response, and flexible delivery methods (i.e. physical and chemical).[4,5] Physical delivery methods allow for movement of nucleic acids across the cellular membrane by creating transient openings through the use of electroporation,[6] ultrasound,[7] gene guns,[8] and magnetofection.[9] Although the use of physical delivery methods is feasible, disadvantages are still apparent;

nucleic acid degradation can occur in the extracellular and cytosolic environments, physical delivery methods themselves can cause cellular damage, and logistical concerns arise regarding *in vivo* treatments (e.g. electrode placement for electroporation).[1] Given the challenges with physical delivery, both *in vitro* and *in vivo*, chemical delivery methods for nonviral gene delivery are more commonly used.[10,11] Chemical nonviral gene delivery is typically accomplished by electrostatically complexing cationic lipids or polymers with negatively charged nucleic acids. Forming a complex condenses the DNA, which can protect the nucleic acid from degradation, and the formed complexes elicit a lower immune response and show a lower toxicity compared to viral vectors.[11]

While nonviral gene delivery circumvents many disadvantages associated with viral systems, nonviral gene delivery suffers from low efficiency due to extracellular and intracellular barriers that impede transfection.[12] Some of the extracellular barriers that prevent the complexes from entering the cell include mass transport limitations, complex degradation, and aggregation.[13] After complexes overcome extracellular barriers, the positively charged complexes can interact with the cellular membrane, facilitating internalization into the cell.[11] Internalization of the complexes is typically accomplished through one of three different endocytic pathways, including macropinocytosis, clathrin-mediated, and caveolae-mediated endocytosis.[14] Internalization of complexes by macropinocytosis occurs when an actin-formed membrane ruffle fuses with the plasma membrane and complexes are engulfed into a large invagination (>0.2 μm)[15] called a macropinosome. After complexes are engulfed, the macropinosomes mature from an early macropinosome to a late macropinosome, which is a leaky vesicle that may facilitate escape of the complexes into the cytosol,[16] possibly allowing complexes to avoid lysosomal degradation.[17] Although macropinocytosis has been shown to be responsible for internalization of large complexes (>0.2 μm), most studies have identified receptor-mediated endocytosis (e.g. clathrin- or caveolae-mediated) as the responsible mechanism for internalization of DNA complexes.[18] Clathrin-mediated endocytosis is modulated by cell division control protein (Cdc42), a protein involved in focal adhesion formation,[19] which is from the Rho family of GTPases that can modulate other cellular processes like adhesion, migration, cytoskeletal remodeling, and vesicle trafficking.[20] In clathrin-mediated endocytosis, complexes are internalized into vesicles coated with clathrin that bud from the plasma membrane of the cell.[21] After DNA complexes are engulfed within a clathrin-coated vesicle, the invaginations form into early endosomes, mature into late endosomes, and ultimately fuse with lysosomes where complexes may be degraded[17] or released into the cytosol through an endosomal escape mechanism, often mediated by the vector by causing rupturing of the endosome with acidification[22] or destabilizing the membrane.[23] Like clathrin-mediated endocytosis, in caveolae-mediated endocytosis, complexes are engulfed within a vesicle and the process is modulated by focal adhesions.[24] Studies have shown that smaller particles (50–100 nm)[25] are typically internalized through caveolae-mediated endocytosis as compared to clathrin-mediated endocytosis,[26] which has been shown to be used for larger complexes (100–200 nm). In caveolae-mediated endocytosis, after an invagination forms around the complexes, the vesicles form into early endosomes coated with caveolin, and then the vesicles around the complexes are transformed into caveosomes.[22] Similar to the macropinosome in macropinocytosis, the environment of the caveosome has been shown to not degrade DNA complexes[22]; however, for successful transfection, the complex must still escape from the caveosome to continue to be trafficked through the cell.

Once the DNA escapes from a macropinosome, endosome or caveosome into the cytosol, the DNA must then be trafficked through the cytosol to the nucleus. Trafficking can be facilitated by elements of the cytoskeleton including microtubules,[13] a cytoskeletal component formed by protein filaments, and actin stress fibers,[27] bundles of actin that anchor to focal adhesions and extend throughout the cell. Microtubules and stress fibers are critical cytoskeletal elements that regulate cellular adhesion and shape.[19] The family of Rho GTPases has been shown to mediate the assembly and disassembly of microtubules and stress fibers as well as transmit mechanical signals that affect the trafficking of vesicles (which may contain complexes[16]) along microtubules or stress fibers to the nucleus.[20] Once complexes reach the nucleus, nuclear entry of complexes can be facilitated by the nuclear pore complex or transport may occur when the nuclear envelope is disrupted during cell division[28]; thus, proliferation is often shown to enhance gene delivery.[29] Once the DNA transfers into the nucleus, the final barriers to transfection include transcription of the transgene in the nucleus and translation of the transcript to a therapeutic protein.

Efforts to improve nonviral gene delivery have focused on overcoming the aforementioned barriers by modifying nonviral vectors through methods such as PEGylation to improve circulation of the formed complexes,[5] conjugating moieties that disrupt the endosome through charge interactions,[30] or immobilizing nuclear localization signaling peptides to traffic complexes to the nucleus,[31] yet vector modification has had limited success in improving nonviral gene delivery efficiency *in vitro* or *in vivo*.[5] A novel solution that may prove more effective than vector modifications to improve nonviral transfection efficiency is stimulating or "priming" cells before transfection to modulate and mitigate the cellular response to nonviral gene delivery. For example, the addition of dexamethasone (a glucocorticoid) before the delivery of DNA complexes has been shown to enhance transfection efficiency and transgene expression in human mesenchymal stem cells (hMSCS), possibly by promoting normal cellular metabolism during transfection, as demonstrated by improved proliferation that was observed in cells treated with dexamethasone before transfection in comparison to untreated, but transfected cells.[32] Further, several other clinically approved drug groups (e.g. steroids, GABAA modulators, antioxidants) have been shown to promote transfection efficiency by modulating the cellular

oxidative stress[33] in response to branched polyethylenimine (bPEI), a nonviral vector.

While these studies demonstrate that the addition of pharmacological agents can alter the cell response to transfection through the media environment, cell priming can also come from cues from the substrate. There are many nonviral gene delivery applications where a cell-material interface exists and the cellular response to transfection could be enhanced by substrate priming. For example, some applications include coating a vascular stent with poly(lactic-co-glycolic acid) (PLGA) bilayer nanoparticles and DNA encoding vascular endothelial growth factor to prevent restenosis,[34] loading a collagen patch with bPEI and DNA encoding platelet-derived growth factor-BB to increase wound healing,[35] or coating a titanium (Ti) bone implant with poly-(d,l-lactide) and polymer vectors complexed with DNA plasmids encoding bone morphogenetic protein-2 (BMP-2) to encourage osseointegration.[36] To use a cell-material interface to prime cells for more efficient transfection, substrate properties can be tuned through chemical modifications such as the addition of natural coatings, ligands, or functional side groups, and/or physical modifications such as topography or stiffness. The cell-material interface is known to influence cell behaviors that are innately controlled by the extracellular matrix (ECM) proteins[37] that adsorb onto a culture surface or exist natively in tissue. The cellular behaviors include morphology,[38–40] adhesion,[41,42] and migration[43,44]; all behaviors which have also been shown to influence transfection success.[45] For example, migration involves the production of cytoplasmic protrusions like filopodia, long filamentous actin protrusions from the cell that, in addition to propelling the cell forward, have been shown to "carry" complexes into the intracellular environment of the cell body.[46] Likewise, membrane ruffles are actin-based features used by the cell to guide motility but have also been shown to be associated with macropinocytosis,[47] suggesting these features could aid in internalization of DNA complexes. Furthermore, the processes of cell adhesion and migration require focal adhesion assembly and disassembly,[48] which may affect endocytosis through the activation of RhoGTPases such as Cdc42, which modulates clathrin-mediated endocytosis[19]; in addition, focal adhesions anchor actin stress fibers that may facilitate intracellular trafficking. Given these insights into ECM-induced cellular behaviors that are intricately related to transfection success, substrate modifications to biomaterials can be used to mimic the extracellular cues from the ECM to enhance nonviral gene delivery. This review highlights current research using chemical and physical substrate modification techniques that can modulate cell-material interactions to prime the cellular response to improve nonviral gene delivery.

Modifications to surface chemistry that influence cellular responsiveness to gene delivery

The surface chemistry of a substrate significantly impacts cell-material interactions,[49,50] typically translated to the cell through the ECM proteins that bind to the substrate.[51] The composition, conformation, and density of protein adsorption are controlled by surface chemistry, and in turn the cell response is mediated by interactions with the absorbed proteins, through integrin binding to the adsorbed proteins.[50] Cellular responses shown to be influenced by surface chemistry include adhesion,[52,53] morphology,[54] and migration,[55] which are all cellular behaviors shown to be important in transfection success.[45] Surface chemistry can be tuned through natural and synthetic material coatings, modifying or adding chemical side groups, or by immobilizing nucleic acids to substrates to affect the presentation of the genetic cargo to the cell.

Natural material coatings and chemical side groups to prime bolus gene delivery

Natural material coatings are an attractive option for substrate modifications due to their innate biocompatibility, sustainable production, and ability to integrate with cells and tissues.[56] For example, chitosan is a cationic polysaccharide derived from deacetylated chitin from crustaceans. While chitosan is well known for its use in nonviral gene delivery to form complexes with DNA plasmids,[57,58] there is also promising evidence that surface coatings formed with chitosan can alter cell-material interactions resulting in enhanced transfection success. Hsu *et al*.[59] investigated the transfection of murine adipose-derived adult stem cells (ADAS) with naked DNA plasmid encoding TRE-Tight-DsRED2 when the cells were cultured on glass coverslips coated with chitosan or chitosan modified with hyaluronan (HA). Transfection in cells cultured on chitosan (or chitosan-HA) coating was compared to transfection in cells cultured on tissue culture polystyrene (TCPS) controls. Transfection efficiency was significantly increased by up to 7-fold higher in cells cultured on chitosan and chitosan-HA coatings (10% and 21% of cells transfected, respectively), compared to cells cultured on TCPS (~3%). This difference was attributed to enhanced spheroid formation (3D cell colonies) seen in cells cultured on chitosan and chitosan-HA-coated substrate compared to those cultured on TCPS. Spheroid formation on chitosan and chitosan-HA coatings has been previously shown to be caused by enhanced migration rate over time,[60] which suggests focal adhesion formation (a regulator of cell migration[61]) may have been promoted in cells on the chitosan and chitosan-HA coatings. To further test whether focal adhesion formation was influenced by chitosan or chitosan-HA coatings, the authors used Western blot to investigate the activation of Ras homolog gene family, member A (RhoA), which modulates focal adhesion assembly[61] and is associated with spheroid formation. They showed that RhoA activation was upregulated in ADAS cells cultured on chitosan and chitosan-HA coatings. The upregulation of RhoA activity further supports the hypothesis that chitosan and chitosan-HA coatings may promote focal adhesion turnover in adhered cells and given that stress fibers that facilitate intracellular trafficking attach to focal adhesions, improved intracellular trafficking of complexes on these stress fibers may have improved transfection seen on chitosan and chitosan-HA coatings in this study. While transfection

was shown to be higher on the chitosan and chitosan-HA coatings compared to controls, the authors also reported that transfection efficiency was significantly higher in cells cultured on chitosan-HA coatings compared to cells cultured on chitosan coatings. Therefore, the ability of chitosan and chitosan-HA coatings to differentially stimulate endocytic pathways (i.e. clathrin-mediated and caveolae-mediated endocytosis) in cells was investigated. ADAS cells cultured on the chitosan coatings were shown to exhibit more clathrin-mediated endocytosis and ADAS cells cultured on the chitosan-HA coatings were shown to exhibit more caveolae-mediated endocytosis, suggesting that the increased transfection seen in cells cultured on chitosan-HA compared to those on chitosan coatings may be due to the bias towards caveolae-mediated endocytosis, which allows for bypassing of the lysosomal compartment and thus avoiding DNA degradation in that compartment.[62] Together, these data demonstrate that coating substrates with chitosan or chitosan-HA prime cells for transfection, potentially through RhoA activation, may in turn promote focal adhesion assembly, and thus upregulate endocytosis, and facilitate intracellular trafficking. Moreover, the addition of HA, an essential component of native ECM,[52] further primes the cells potentially through upregulating caveolae-mediated endocytosis, a more advantageous pathway for transfection that may avoid lysosomal degradation.

Other studies have demonstrated that transfection can be influenced by coating substrates with natural ECM proteins such as collagen I/IV, vitronectin, laminin, and fibronectin.[45] Investigations with murine mesenchymal stem cells (mMSCs) transfected with linear PEI-DNA complexes encoding enhanced green fluorescent protein (EGFP) and luciferase (LUC), showed that cells cultured on fibronectin had increased transgene expression compared to cells cultured on all other coatings (collagen I/IV, vitronectin, laminin) and thus fibronectin has often been investigated as a protein coating to enhance nonviral gene delivery. For example, Dhaliwal *et al.*[63] compared the effect of culturing mMSCs on fibronectin or collagen I coated onto TCPS, and then transfected with linear PEI-DNA complexes encoding EGFP and LUC. Cells cultured on collagen coating demonstrated lower transfection efficiency compared to cells on uncoated control surfaces, while transfection efficiency was enhanced by up to 10-fold in cells cultured on fibronectin compared to uncoated controls. While cell proliferation could be correlated to transfection as a function of protein coating, the influence on cell proliferation could not sufficiently explain the difference in transfection between cells cultured on collagen and fibronectin-coated substrates. Given that integrins can bind to the arginyl-glycyl-aspartic acid (RGD) ligand on fibronectin[64] and such binding can modulate endocytic pathway activation, investigations were performed to see if the protein coatings could influence the internalization pathways used by cells. A significant decrease in transfection and internalization after inhibiting clathrin-mediated endocytosis in cells cultured on fibronectin compared to cells with no treatment was observed (i.e. an order of magnitude of decrease in transgene expression and 92% reduction of internalization), suggesting that fibronectin coatings (presumably through integrin binding to fibronectin[64]) affected transfection to adhered cells through endocytic pathway activation, which could possibly be mediated by cytoskeletal organization. Therefore, the authors investigated the role of the cytoskeleton in transfection outcomes, which showed that disrupting actin stress fibers and actin–myosin activity led to increased transgene expression in cells cultured on fibronectin but not collagen I, suggesting there was an increased actin network and higher cellular contractility (from actin–myosin interactions[65]) in cells cultured on fibronectin compared to cells cultured on collagen I.

Cytoskeletal dynamics such as cellular contractility and stress fiber formation are modulated by RhoGTPases, which mediate adhesion signaling between cell surface receptors (i.e. integrins) and fibronectin.[66] Therefore, in a separate paper, the authors measured the endogenous activation of several RhoGTPases including RhoA, Ras-related C3 botulinum toxin substrate 1 (Rac1), and Cdc42 levels, which showed that the cells cultured on fibronectin had significant RhoGTPase activation, but the activity of the RhoGTPases was low or inhibited in cells cultured on collagen I, correlating to their transfection profiles as well. To further confirm the relationship between transfection success in cells cultured on fibronectin and RhoGTPase activity, the authors investigated the inhibition of RhoGTPase activity and the resulting effect on the cytoskeleton (i.e. stress fibers) and transfection in cells cultured on fibronectin, which showed a significant decrease in the formation of actin stress fibers and transfection success, suggesting that transfection enhancement in cells cultured on fibronectin may be due to upregulation of RhoGTPase activity that in turn increased formation of stress fibers that may mediate intracellular trafficking. Together, these studies provide compelling evidence that transfection success in cells cultured on fibronectin may be attributed to integrin binding, cytoskeletal dynamics (i.e. stress fibers, cellular contractility), and the activation of RhoGTPases, especially Cdc42 that modulates clathrin-mediated endocytosis.[19]

In a separate investigation, conjugation of the RGD ligand on fibronectin (and other ECM proteins) to which integrins bind and form the base of focal adhesions was conjugated to a substrate and analyzed for its ability to regulate nonviral gene delivery to cells adhered to the substrate. In Kong *et al.*,[67] this RGD peptide ((Gly)4-Arg-Gly-Asp-Ser-Pro) was bound to alginate hydrogels using carbodiimide chemistry, with varied density ($3–60 \times 10^9$ peptides/mm^2) and spacing between clustered islands of RGD (36–120 nm). Cellular proliferation and stress fiber formation (presumably mediated by focal adhesion formation[68]) were analyzed, which showed increasing RGD density increased the actin stress fiber formation and proliferation in MC3T3-E1 preosteoblasts cultured on hydrogels, while culturing cells on increased spacing of RGD islands showed the inverse. Next, cellular uptake and transfection experiments were performed using linear PEI complexed with rhodamine-labeled plasmid DNA encoding for LUC, which showed, similar to proliferation and actin stress formation, that DNA internalization and transfection increased with increasing RGD density.

These results suggest that RGD density may improve transfection through upregulating proliferation, possibly by disruption of the nuclear envelope during proliferation, and increasing actin stress fiber formation, which may aid endocytosis and intracellular trafficking. The results of these studies suggest that proteins of the ECM (especially fibronectin) are pivotal in affecting gene transfection success through cell-material interactions and that integrin binding may be the initiator to this type of priming, with downstream effects that can influence focal adhesion formation, stress fiber formation, and subsequent endocytic pathways and intracellular trafficking.

Natural coatings on biomaterials may enhance transfection efficiency by presenting factors similar to those found in the ECM that may stimulate endocytic pathways, but natural materials also show high batch variability based on their source. Therefore, modifying the substrate synthetically with functional groups that resemble those found in natural materials has also been investigated to enhance gene delivery. Synthetic additions to the substrate can be specifically manufactured with homogeneity, such as self-assembled monolayers (SAMs) that have highly defined chemistries that can present surface functional groups that may affect protein and cell attachment.[69] Kasputis and Pannier[70] investigated NIH/3T3 fibroblasts cultured on SAMs on gold substrates modified with $-CH_3$ and $-COO^-$ terminal functional groups, which are hydrophobic and hydrophilic, respectively. Transfection was performed using bolus delivery of complexes formed with DNA plasmids encoding EGFP and Lipofectamine 2000 (LF2000) or bPEI. Delivery of both types of complexes resulted in increased transfection (by ~2-fold) in cells cultured on hydrophilic substrates (i.e. SAMs with carboxyl terminal functional groups) compared to hydrophobic substrates (i.e. SAMs with methyl terminal functional groups). In addition to transfection, cellular viability, shape, cytoskeletal features, and focal adhesions were analyzed as a function of surface chemistry and then correlated to transfection success. Successful transfection performed with LF2000 complexes was significantly correlated to the viability of cells induced by surface chemistry, but no other morphological factors. Conversely, successful transfection performed with bPEI complexes was highly correlated with substrate-induced cellular behaviors including cell density, spreading, cytoskeletal organization, and focal adhesions. The authors proposed that cytoskeletal reorganization was strongly affected by focal adhesions in response to the substrate environment; therefore, successful transfection may have been facilitated by cytoskeletal elements that attach to focal adhesions that in turn contribute to endocytosis and intracellular trafficking (i.e. stress fibers). Together, these studies on coatings and functional group modifications suggest that such features may help to overcome intracellular barriers of transfection via bolus delivery by activating endocytic pathways (that internalize DNA complexes) through integrin binding and upregulation of focal adhesions that influence the organization of cytoskeletal features, which may further enhance intra-cytosolic trafficking. The cell-material interface can also be used to overcome the extracellular barrier of mass transport by allowing primed cells to directly interact with complexes through substrate-mediated gene delivery, which will be further discussed in the next section.

Natural material coatings and chemical side groups to prime substrate-mediated gene delivery

Substrate-mediated gene delivery (SMD), also known as "reverse transfection" or "solid phase delivery," is a method of immobilizing naked or complexed nucleic acids to substrates for delivery to cells adhered to the substrate.[71] SMD can be accomplished by immobilizing DNA complexes through electrostatic interactions or covalent bonding[72–74] to facilitate local delivery to cells cultured onto the substrate. Often SMD substrates are coated with natural or synthetic materials to enhance the immobilization of nucleic acids and modulate the cellular response to the genetic material. For example, Bengali *et al.*[75] studied the effect of coating TCPS substrates with different ECM and serum proteins (fetal bovine serum, fibronectin, collagen I, laminin, and bovine serum albumin (BSA)) to prime cells for enhanced SMD transfection efficiency using complexes formed with bPEI and a DNA plasmid encoding for EGFP and LUC. First, the authors quantified DNA complex immobilization to substrates coated with fetal bovine serum, fibronectin, collagen I, laminin, and BSA, and showed that the amount of complexes immobilized was similar for most protein-coated substrates (except laminin, which had significantly less complexes immobilized compared to the other protein coatings), suggesting that, in general, the protein coating did not affect loading of DNA complexes onto the substrate. However, when NIH/3T3 fibroblasts were seeded on the aforementioned protein-coated substrates with immobilized DNA complexes, there were significantly more DNA complexes taken up by cells cultured on fibronectin and collagen I compared to the other protein coatings. When SMD transfection was analyzed on these surfaces, cells cultured on fibronectin had the highest level of reporter gene expression compared to cells cultured on the other proteins or control surfaces. Given these observations, the authors hypothesized that the presence of fibronectin on the surface may promote integrin binding, which may lead to the assembly of focal adhesions and cytoskeletal rearrangement, which can in turn affect endocytosis and downstream intracellular trafficking of the complexes. To test part of this hypothesis, the authors investigated endocytic pathways in cells cultured on the protein-coated substrates with immobilized DNA complexes by inhibiting caveolae- and clathrin-mediated endocytosis with genistein and chlorpromazine, respectively. For all protein coatings investigated, internalization of DNA complexes and the resulting transfection were both decreased significantly when cells were cultured with genistein (compared to cells cultured with chlorpromazine), suggesting that culturing cells on protein coatings may upregulate caveolae-mediated endocytosis. Thus, in this study, similar to those described earlier in this review in the context of bolus delivery, protein coatings, and in particular fibronectin, may have primed the cells

(presumably through integrin binding, focal adhesion formation, and cytoskeletal rearrangement) that resulted in biasing toward the potentially more efficient caveolae-mediated endocytosis mechanism[22] to enhance transfection.

In addition to using natural coatings to both promote complex immobilization for SMD, as well as to enhance cellular responsiveness to DNA transfer, chemical surface modifications like SAMs can also be used to enhance SMD.[73,76] In a recent study, SAMs were used by Sun *et al.*[77] with different functional groups on the substrate, further functionalized with DNA-doped nanocomposites. Terminal functional groups on the SAMs included $-CH_3$ and $-COO^-$ groups, as well as blends of the carboxyl and methyl groups, in addition to –OH, and $-SO_3Na$. DNA doping was accomplished by submerging SAMs into a mineralizing solution (comparable to the extracellular environment around bone[78]) containing DNA. After 4 h in the solution, all SAM-modified surfaces were shown to have the same amount of DNA plasmid deposited, with similar surface morphology (investigated via scanning electron microscopy (SEM)). HEK293 (human embryonic kidney) cells were cultured on the modified surfaces to determine the effect of SMD on transfection success. Cells cultured on DNA-loaded SAMs functionalized with –OH groups produced the highest gene transfer efficiency (~7000 ng β-gal/mg protein) and the highest level of intracellular DNA plasmid compared to cells cultured on the other modified SAMs; both measurements were ~5-fold higher relative to surfaces that supported the lowest transfection and internalized DNA plasmid (i.e. SAMs functionalized with CH_3:COOH at a 1:1 ratio). The authors cited increased transfection success as a result of weak interactions between the DNA-doped nanocomposites and surface functional groups; in addition, gene transfer efficiency increased nearly linearly with the increase in intracellular DNA levels, suggesting that internalization may be primed by cell interactions with the hydrophilic SAMs, as previously suggested in this review.

Along with reducing the barrier to transfection of mass transport and modulating the cellular response through cell-substrate interactions, SMD can also increase the amount of DNA available for transfection within the local microenvironment as shown in many previous investigations.[72–76,79] SMD may also enhance transfection by releasing DNA or DNA complexes encapsulated within a hydrogel,[80–85] or within polymer films[86–93] (e.g. co-block polymers or polyelectrolytes formed layer-by-layer). In an investigation by Zhang *et al.*,[94] murine MSCs were cultured on Ti substrates with immobilized chitosan-siRNA (CS/siR) complexes. The Ti substrates were treated with thermal alkali (TA) and imaging with SEM showed that Ti-TA had a porous, fibrous network. Furthermore, cells cultured on Ti-TA had enhanced proliferation compared to cells cultured on polished Ti, possibly due to the porosity, which has been shown on other surfaces to improve proliferation.[95] The authors showed that TA-treated substrates increased the loading of CS/SiR complexes compared to polished titanium, thereby increasing the amount of complexes available to cells. Indeed, cells cultured on Ti-TA surfaces with immobilized CS/siR coating showed a significant decrease (40%) in intracellular mRNA compared to the controls, which suggests a successful silencing effect mediated by delivery of the siRNA. The enhancement of siRNA delivery on Ti-TA surfaces was attributed to increased loading of complexes onto the requisite substrate, yet given that the porosity of the surface induced by the TA was shown to improve proliferation, these surfaces may also serve to prime the cellular response to improve transfection.[29]

In summary, modification of surface chemistry can be accomplished by incorporating materials that are components of the ECM (e.g. collagen, fibronectin) or mimic the components of the ECM (e.g. chitosan or functional groups) (Table 1). The surface chemistry may prime cells to overcome intracellular barriers to nonviral gene delivery, potentially mediated by integrin binding, which facilitates focal adhesion formation and cytoskeletal arrangement that subsequently can affect endocytosis and intracellular trafficking. SMD may be able to exert a similar influence on the cellular response to transfection, yet also provide direct contact of the DNA with the cell to help overcome extracellular barriers to mass transport. In the next section, various studies will be discussed that highlight the physical substrate modifications that similarly modulate the cellular response to nonviral gene delivery.

Physical modifications that influence cellular responsiveness to gene delivery

Similar to chemical modifications, physical modifications of the substrate impact cell-material interactions by altering the interface, yet maintain bulk material properties such as biocompatibility and hardness. Physical characteristics of the substrate have been shown to affect cellular adhesion, spreading, migration, proliferation, and morphology, presumably by spatially confining adsorbed ECM proteins and cells[40,96–99] or by mediating cytoskeletal tension.[100–103] Physical modifications are typically accomplished through the addition of topographical features and tuning the stiffness of the substrate.

Modifying substrate topography to prime cells for nonviral gene delivery

Physical modifications often are performed through the addition of topographical features,[104,105] which are designed to mimic the physical cues of the ECM[37,106] to influence cell-material interactions. Micro- and nanotopographical features have been shown to be innately patterned on native ECM and basement membrane by proteins forming complex hierarchically structured microscale and nanoscale pores, grooves, ridges, and fibers.[37] Cell-material interactions with similar topographical features on biomaterials have been shown to affect cellular behaviors, including motility,[107,108] focal adhesions formation,[109,110] and actin fiber alignment,[111] which may affect transfection success as discussed throughout this review. Physical surface modifications can be accomplished by etching,[112] lithography or imprinting,[113] or depositing[114] topographical features in a variety of architectures, such

Table 1. Summary of chemical substrate modifications highlighted in this review and their effect on the cellular response.

Substrate modification	Ref.	Delivery (del.) method of nucleic acid	Response on transfection	Biological mechanism proposed for cell priming
Natural materials				
Chitosan on glass	[59]	Bolus del. of naked DNA plasmid	Increase in transfection of ADAS by 3-fold in comparison to those cultured on TCPS	Enhancing proliferation, focal adhesion formation, RhoA activation (influencing intracellular trafficking), clathrin-mediated endocytosis bias
Chitosan-HA on glass	[59]	Bolus del. of naked DNA plasmid	Increase in transfection of ADAS by 7-fold in comparison to those cultured on TCPS	Enhancing proliferation, focal adhesion formation, RhoA activation (influencing intracellular trafficking), caveolae-mediated endocytosis bias
Chitosan on Ti-TA	[94]	SMD of chitosan-siRNA complexes	Knockdown of intracellular mRNA of mMSCs by 40% in comparison to those cultured on control groups	Enhancing proliferation
Fibronectin on TCPS	[45,63,64]	Bolus del. of linear PEI-plasmid DNA complexes	Increase in transfection of mMSCs by up to 10-fold in comparison to those cultured on uncoating TCPS	Enhancing proliferation, integrin binding, focal adhesion formation, RhoGTPase activation, stress fiber formation, cellular contractility, clathrin-mediated endocytosis bias
Fibronectin on TCPS	[75]	SMD of bPEI-plasmid DNA complexes	Increase in transfection of NIH/3T3 fibroblasts by 4 orders of magnitude to those cultured on uncoating TCPS	Enhancing internalization, caveolae-mediated endocytosis bias
Collagen I on TCPS	[75]	SMD of bPEI-plasmid DNA complexes	Increase in transfection of NIH/3T3 fibroblasts by 3 orders of magnitude to those cultured on uncoating TCPS	Enhancing internalization, caveolae-mediated endocytosis bias
RGD motif islands on alginate hydrogel	[67]	Bolus del. of linear PEI-plasmid DNA complexes	Increase in transfection of MC3T3-E1 preosteoblasts on dense RGD island by 5-fold in comparison to those cultured on spread RGD islands	Enhancing proliferation, stress fiber formation, enhancing internalization
Synthetic materials				
SAMs (hydrophilic) on gold	[70]	Bolus del. of bPEI (or LF2000)-plasmid DNA complexes	Increase in transfection of NIH/3T3 fibroblasts by ~2-fold in comparison to those cultured on hydrophobic SAMs	Cell density and spreading, focal adhesion formation, cytoskeletal organization
SAMs (hydrophilic) on gold	[77]	SMD of naked DNA plasmid	Increase in transfection of HEK293 cells by ~5-fold in comparison to those cultured on the lowest transfecting surface	Enhancing proliferation, enhancing internalization

TCPS: tissue culture polystyrene; SMD: substrate-mediated gene delivery; SAMs: self-assembled monolayers; RGD: arginyl-glycyl-aspartic acid.

as columns, grooves, islands, pits, pores, wires, and more, fabricated through wide variety of techniques to produce disordered (e.g. acid-etching[112]) and ordered structures (e.g. lithography[115] and glancing angle deposition[116]).

The interactions of cells with nanotopography on biomaterials have previously been shown to be a promising technique to improve nonviral gene delivery, as described in the review by Adler and Leong,[117] but many novel investigations have been performed since their publication describing the effect of topography (i.e. micro- and nanoscale) on enhancing transfection through priming of cell responsiveness. For example, in a study by Teo *et al.*,[118] topographical features on the micro- and nanoscale were investigated for their effect on the cellular response to nonviral gene delivery. For their investigations, poly(methyl methacrylate) (PMMA) was spin coated onto silicon substrates and imprinted to produce 2 μm and 200 nm pillars, and 250 nm grating. Bone marrow-derived hMSCs, breast cancer (MCF7) cells, and COS7 simian fibroblasts were seeded onto the substrates, and morphological analysis revealed that all types of cells exhibited more spreading on pillars compared to cells cultured on substrates with grating. Cells on both types of pillars were more spread, but only cells on the 2 μm pillars exhibited intracellular actin regions localized around the tops of the pillars, while the cells cultured on the 250 nm grating showed stress fiber alignment along the nanofeatures, which suggested that cytoskeletal organization and focal adhesion formation were affected by the substrate topography. After analyzing cell morphology, transfection studies were first performed with fluorescein isothiocyanate (FITC)-labeled dextran, a large molecule frequently used to monitor macropinocytosis, which showed COS7 cells and hMSCs cultured on 2 μm pillars had significantly more internalized dextran (~70 and ~14% of cells, respectively) compared to cells on flat PMMA controls (~50 and ~4% of cells, respectively), suggesting that macropinocytosis was stimulated in fibroblasts and hMSCs, a finding further supported by the observation of actin localization around the tops of the 2 μm pillars that suggested membrane ruffling may be occurring. Next, transfection studies with DNA complexes formed with GFP-encoding DNA plasmid and LF2000 vector were performed in cells cultured on 2 μm and 200 nm pillars, and 250 nm grating, and these studies showed a significant increase in transfection efficiency (5% of cells expressing the transgene) in hMSCs cultured on 200 nm pillars compared to cells cultured on flat PMMA (2% of cells expressing the transgene),

which the authors suggested was potentially due to stimulation of clathrin-mediated pathway by 200 nm pillars, citing conclusions from previous studies on the typical path of lipid-based particles,[18] although this claim was not investigated. Undoubtably, the size of the particle contributed to the endocytic pathway utilized by the cell (i.e. dextran is large and must be taken up through a pathway like macropinocytosis, whereas smaller complexes like DNA-LF2000 typically follow receptor-mediated endocytic pathways[18]), yet given the cytoskeletal-induced changes in the cells on these surface, it is reasonable to postulate that the cell response to topographical features may have contributed to the bias of the internalization pathway used to improve gene delivery as discussed throughout this review.

In addition to its ability to potentially influence endocytosis to overcome intracellular barriers to transfection, substrate topography can also be used to load DNA on the substrate for SMD, or topography can be used to "inject" DNA into the cell.[119–122] For example, an investigation by Elnathan *et al.*[123] studied transfection in cells cultured on substrates with different formations of silicon nanowire arrays (SiNW), using four different cell lines including HEK293, HeLa (cervical cancer cells), human dental pulp stem cells (hDPSCs), and human foreskin fibroblasts (HFF). The arrays were fabricated over a Si substrate and wet etched to produce a variety of arrays ranging from 330 to 600 nm for columnar diameter, 400 nm–6.3 μm for columnar height, and 0.6 to 4.0 SiNW per μm^2. Transfection was performed by coating SiNWs with DNA plasmids encoding for EGFP and the transfection efficiency for all cell types (hDPSCs, HEK293, HFF, and HeLa cell lines) was highest in cells cultured on arrays with 3.5 μm columnar height (up to 80% higher, with optimal columnar diameter at 400 nm and optimal density at 1 SiNW per μm^2) compared to cells on any other array configuration. Cell viability and proliferation were measured, and both were highest in cells cultured on the SiNW array with 3.5 μm columns compared to cells on all other heights of SiNW arrays. Given that increasing proliferation and viability are both known to improve transfection and that cells on 3.5 μm columns had the highest transfection efficiency, cells may have been primed for transfection when cultured on the 3.5 μm columns. In order to further investigate the cell-material response to transfection when cells are cultured on substrates with 3.5 μm columns, the authors performed SEM investigations of cellular morphologies on these features, which showed highly spread cells with many long filamentous filopodia, both of which may influence transfection either by the assembly and disassembly of focal adhesions[27] (and thus endocytosis and intracellular trafficking) or by "carrying" complexes into the intracellular environment of the cell body.[46] Finally, some cells were penetrated with the 3.5 μm tall nanowires without compromising the integrity of the cellular membrane, thereby physically injecting DNA into the cell, which could have further led to high transfection efficiency, but priming of the cellular response (i.e. proliferation, viability, spreading and filopodia production) presumably also contributed to transfection success.

Given the wide range of topographical parameters possible for investigation, it may be crucial to study platforms with many topographical features to identify key determinants to improve nonviral gene delivery transfection success. In a paper by Adler *et al.*,[124] a library of 160 different microtopographies were analyzed for their abilities to influence the cellular response of normal human dermal fibroblasts (NHDFs) to transfection with complexes formed with a GFP-encoding plasmid and LF2000. The topographies were formed with polydimethylsiloxane (PDMS) arrays (coated with fibronectin) with a uniform pit depth of 2.4 μm and 10 distinct pit morphologies (with patterned circles or squares) that varied in both pit size (width) and edge-to-edge spacing (1, 2, 4, or 6 μm). Through their investigations, cells cultured on 4 μm wide pits with small spacing (1 μm) showed increased transfection efficiency (25% improvement) compared to cells cultured on the smooth areas of the surface, although the transfection efficiency was not affected by topographical pattern (i.e. transfection efficiency was similar for cells on all topography patterns). Topographical features were also shown to influence cell morphology, wherein NHDFs cultured on 4 μm wide pits with small spacing (1 μm) exhibited spreading as well as alignment to the topography. While not studied, these results suggest that the pits may promote cellular adhesion and cytoskeletal reorganization, possibly influenced by integrin binding to the fibronectin coating, as well as the assembly and disassembly of focal adhesions related to cellular spreading,[27] which together can promote endocytosis and intracellular trafficking of complexes to the nucleus and thus prime cells for improved transfection.

In two papers published by Hsu's group in 2015, many distinct topographies and materials were tested for their influence on transfection success in hMSCs, including nanosheets fabricated from layered silica on silicon and etched using different chemical etching mixtures with a base of sodium borohydride (NS), as well as surfaces coated with chitosan modified with HA, polyurethane (PU) flat films, PU random and aligned electrospun fibers, and PU microgrooves. Transfection efficiency was measured for hMSCs cultured on the nanosheets[125] or the coated substrates[126] using a naked GFP-encoding plasmid or the GATA binding protein 4-encoding plasmid, respectively. Both studies showed that substrates that produced the highest transfection efficiency in hMSCs (nanosheets etched with NS alone[125] or PU microgrooves[126]) also stimulated focal adhesion formation, suggested by enhanced hMSC migration on nanosheets etched with NS (as cell migration requires focal adhesion formation[48]) and cellular alignment to microgrooves, which may indicate that cytoskeletal arrangement may also be a factor in transfection success. In both papers, the authors tested whether focal adhesion was influenced by the substrate topography by measuring the endogenous expression of integrins αv and $\beta 3$, and focal adhesion kinase (FAK). Cells cultured on substrates that resulted in the highest transfection (i.e. nanosheets etched with NS alone[125] and PU microgrooves[126]) had the highest expression of both integrin subunits compared to cells on substrates with lower transfection levels, and cells cultured on microgrooves were also shown to have high expression of FAK, a protein induced by the binding of integrins to the ECM.[127] Therefore, the results

of these studies suggest the interplay of integrin activation, focal adhesion formation, and cytoskeletal arrangement as critical determinants of cellular transfectability, due to the ability of cytoskeletal features to influence endocytosis and intracellular trafficking. Notably, both papers showed successful transfection efficiency with a naked plasmid, which is typically considered a poor transfecting method, highlighting that tuning the cell-material interface through the addition of topography may be a promising technique to improve nonviral gene delivery systems compared to traditional vector modification studies. In summary, modification of surface topography can be used to prime cells for transfection through focal adhesion formation and cytoskeletal organization, which may alter internalization and endocytic pathways, and thus transfection (Table 2).

Modifying substrate stiffness to prime cells for nonviral gene delivery

One of the critical biomechanical properties of a substrate environment is the stiffness of a material. Native ECM stiffness is known to modulate the cellular response including cellular migration through focal adhesion attachment and disassembly, and intracellular actin tension,[130] all of which may factor into transfection success, as has been suggested by the many studies reviewed in this manuscript. Biomaterials can be engineered to exhibit a range of stiffness from several pascals (soft hydrogels) to 150 GPa (titanium oxide),[131] and the material stiffness has been shown to regulate cell behaviors such as integrin binding,[132] migration,[133] and adhesion.[134] In general, biomaterials are chosen to mimic the innate tissue stiffness for their application (e.g. bone ($>10^9$ Pa), or muscle (10^3–10^4 Pa)[131]).

Increasing substrate stiffness has been consistently shown to increase transfection success. For example, in an article by Kong *et al.*,[29] alginate hydrogels were crosslinked to produce a range of stiffnesses (20–110 kPa) and functionalized with the cell adhesion peptide RGD using carbodiimide chemistry. Transfection investigations were performed using complexes formed with rhodamine-labeled PEI and FITC-labeled plasmid DNA encoding β-galactocidase and LUC, which showed transfection in murine MC3T3-E1 preosteoblasts increased as the stiffness of the alginate increased (4-fold difference in transfection between the stiffest and softest substrates). The cell response to alginate stiffness was quantified using

Table 2. Summary of physical substrate modifications highlighted in this review and their effect on the cellular response.

Substrate modification	Ref.	Delivery (del.) method of nucleic acid	Response on transfection	Biological mechanism proposed for cell priming
Topography				
Micropillars (made from PMMA) on silicon	[118]	Bolus del. of FITC-labeled dextran	Increase in dextran internalization of hMSC and COS7 by ~20 and ~10%, respectively, in comparison to a smooth control (1 mg/mL)	Cytoskeletal organization, macropinocytosis bias
Micropillars (made from PDMS)	[124]	Bolus del. of LF2000-plasmid DNA complexes	Increase in transfection of NHDFs by 25% in comparison to cells cultured on smooth substrates	Cell spreading
Nanopillars (made from PMMA) on silicon	[118]	Bolus del. of LF2000-plasmid DNA complexes	Increase in transfection of hMSCs by 2% in comparison to cells cultured on smooth substrates	Cytoskeletal organization
Nanowires (made from silicon)	[123]	SMD of naked DNA plasmid	Increase in transfection of hDPSCs, HEK293, HFF, and HeLa on 3.5 μm tall columns by up to 80% in comparison to all other heights	Cell viability, enhancing proliferation, filopodia production, cell spreading
Nanosheets (made from silica on silicon)	[125]	Concurrent bolus del. (added with cells) of naked DNA plasmid	Increase in transfection of hMSCs on substrates etched with NS by ~60% in comparison to planar substrates	Enhancing migration, integrin activation
Microgrooves (made from PU)	[126]	Concurrent bolus del. (added with cells) of naked DNA plasmid	Increase in transfection of hMSCs by ~50% in comparison to TCPS	Cytoskeletal organization, integrin activation
Stiffness				
Alginate hydrogels with RGD	[29]	Bolus del. of PEI-plasmid DNA complexes	Increase in transfection of MC3T3-E1 preosteoblasts cultured on stiff substrates by 4-fold in comparison to those on soft substrates	Enhancing proliferation
PEGDA hydrogels	[128]	Bolus del. of linear PEI-plasmid DNA complexes	Increase in transfection of NIH/3T3 fibroblasts, D1 bone marrow stromal (BMSCs) cells, and C2C12 myoblasts cultured on stiff substrates in comparison to those on soft substrates	Cytoskeletal organization, enhancing internalization
Anhydride-functionalized silicone hydrogels	[129]	Bolus del. of LF2000-plasmid DNA complexes	Increase in transfection of hASCs cultured on stiff substrates by 3-fold in comparison to those on soft substrates	Cell spreading, focal adhesion formation, caveolae-mediated endocytosis bias

FITC: fluorescein isothiocyanate; PEGDA: poly(ethylene glycol) diacrylate; RGD: arginyl-glycyl-aspartic acid; PMMA: poly(methyl methacrylate); PEI: polyethylenimine; PU: polyurethane.

proliferation (as a rate with respect to time), and it was shown that proliferation, like transfection, was increased in response to the stiffness of the alginate substrate. Furthermore, when transfection was compared to transfection in cells adhered to other cell adhesion substrates of varying stiffnesses (but that did not contain conjugated RGD), it was shown that successful transfection was also dependent on the stiffness of the substrate, suggesting that stiffness rather than RGD presentation on the alginate hydrogel is the guiding influencer of transfection. Thus, the authors concluded that increased stiffness of alginate may prime the cellular response for transfection by increasing proliferation, which was further confirmed by cell cycle inhibitor studies performed in the context of substrate stiffness.

Along with proliferation, other studies have shown increasing the stiffness of the substrate can enhance transfection and internalization of DNA complexes in cultured cells. For example, in Chu and Kong,[128] poly(ethylene glycol) diacrylate (PEGDA) hydrogels substrates were prepared on glass or TCPS at increasing percentages of polymer concentration (5–20%), which resulted in gels with elastic moduli varying from 10 to 670 kPa for transfection studies. Transfection of NIH/3T3 fibroblasts was performed with linear PEI complexed with a plasmid encoding for BMP-2, which showed transgene expression was linearly related to the increase in stiffness, and a similar increase was shown in the internalization of fluorescently labeled DNA complexes. The authors then investigated the cellular response to the hydrogel through imaging of cell morphology, which showed that increasing the stiffness of the substrates also resulted in increased area of the NIH/3T3 fibroblasts cells, as well as a larger nuclear aspect ratio, suggesting that the stiffness of the hydrogel influences cell morphology, potentially through reorganization of the cytoskeleton, as microtubules and stress fibers have been shown to influence nuclear shape,[135] which may affect intracellular trafficking[20] and thus transfection. A similar effect of stiffness on DNA internalization and cell morphology was also shown by Modaresi *et al.*[129] using anhydride-functionalized silicone hydrogels at 0.5 and 32 kPa stiffnesses, with human adipose-derived stem cells (hASCs) cultured onto the hydrogels. Transfection was performed using an EGFP encoding DNA plasmid complexed with an LF2000 vector; reporter gene expression was increased by 3-fold and plasmid internalization was slightly increased in cells cultured on stiff substrates compared to softer substrates. Morphological investigations showed that hASCs grown on stiffer hydrogels compared to soft hydrogels had significantly increased spreading and focal adhesion areas, suggesting that integrin binding and cytoskeletal reorganization may have been influenced by the stiffness of the substrate. The authors also showed that hASCs grown on stiffer hydrogels (compared to soft hydrogels) had significantly more stress fibers per cell, which anchor to focal adhesions[27] and may provide a pathway for the DNA complexes to be intracellularly trafficked to the nucleus. Using a quantitative polymerase chain reaction (qPCR) time course investigation, the authors showed that cells cultured on stiffer gels expressed integrin and caveolae genes that followed the typical progression of caveolae-mediated endocytosis, suggesting that gene delivery in cells cultured on stiffer hydrogels was being accomplished through upregulated caveolae-mediated endocytosis, an advantageous pathway that may avoid lysosomal degradation, as previously discussed in this review. Combined, these studies suggest that substrate stiffness may prime cells for transfection by affecting cytoskeletal reorganization, which in turn may alter internalization and intracellular trafficking of the complexes (Table 2), thereby suggesting that tuning the stiffness of a substrate may improve nonviral gene delivery.

While beyond the scope of this review, recent investigations have also studied the influence of stiffness on transfection success in 3D, by encapsulating DNA complexes and cells within a hydrogel.[136,137] Similar to the work presented in this review, the 3D investigations demonstrated correlation between features of the hydrogel (e.g. RGD presentation, stiffness) and endocytic pathway bias, trafficking along cytoskeletal elements, activation of RhoGTPases and transfection profiles, yet the cellular response to 3D requires further elucidation. The investigations in this section show physical modification of topography may modulate the cellular response through the interplay of integrin activation, focal adhesion formation, and cytoskeletal arrangement, while stiffness affects downstream activities such as cytoskeletal remodeling, nuclear shape and availability, and intracellular trafficking. In summary, understanding the relationship between physical substrate properties and cellular response to gene delivery is essential to designing biomaterials that promote transfection through cell priming.

Conclusions and future directions

The cell-material interface may represent an underexploited target to improve the success of nonviral gene delivery in a variety of applications where a substrate exists (e.g. diagnostics, vascular stents, bone implants, tissue engineering scaffolds). Physical and chemical characteristics of the substrate can work separately or in tandem to mimic ECM cues and modulate cellular behaviors that influence transfection efficiency. In this review, some of the underlying mechanisms that may modulate cellular responsiveness to transfection through cell-material interactions have been suggested (e.g. integrin binding and focal adhesion formation, cytoskeletal remodeling, intracellular trafficking, endocytic mechanisms); however, more investigations are necessary to confirm how to use such cell-material interactions to produce different cell transfection phenotypes. Furthermore, exploration into the presentation of nucleic acids (i.e. substrate-mediated delivery vs. traditional bolus delivery) may be used to overcome extracellular barriers to nonviral gene delivery, while also priming cellular responsiveness. Finally, while this review has focused on nonviral gene delivery, there is evidence to suggest similar mechanisms and information learned from nonviral studies may also be applicable to viral delivery systems.[138–141]

Authors' contributions: The authors contributed equally to the writing and editing of this document.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The Nebraska Research Initiative, University of Nebraska-Lincoln Tobacco Settlement Funds, National Science Foundation (CBET1254415), Center for Nanohybrid Functional Materials (NSF EPS1004094), USDA CSREES-Nebraska (NEB-21–146), and National Institutes of Health (1 DP2 EB025760-01) [DP2] are acknowledged for funding this work.

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