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Polymeric Nanohybrids as a New Class of Therapeutic Biotransporters

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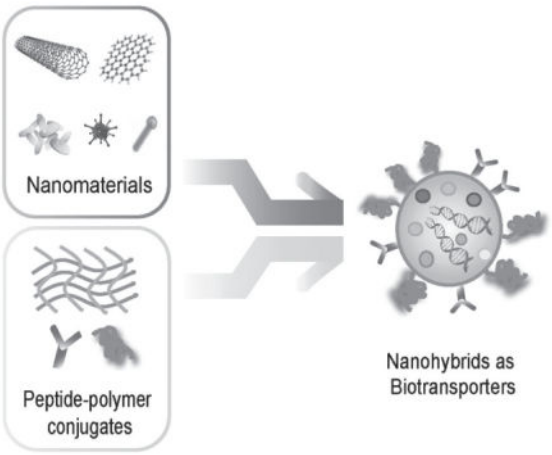
Abstract

A possible solution to enhance existing drug and gene therapies is to develop hybrid nanocarriers capable of delivering therapeutic agents in a controlled and targeted manner. This goal can be achieved by designing nanohybrid systems, which combine organic or inorganic nanomaterials with biomacromolecules into a single composite. The unique combination of properties along with their facile fabrication enables the design of smart carriers for both drug and gene delivery. These hybrids can be further modified with cell targeting motifs to enhance their biological interactivity. In this Talents and Trends article, an overview of emerging nanohybrid-based technologies will be provided to highlight their potential use as innovative platforms for improved cancer therapies and new strategies in regenerative medicine. The clinical relevance of these systems will be reviewed to define the current challenges which still need to be addressed to allow these therapies to move from bench to bedside.

Graphical Abstract



Keywords

biomaterials; cardiovascular therapy; medical devices; nanomedicine; regenerative medicine

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1. Introduction

The therapeutic effects of a drug or gene are dependent upon its rate of administration as well as its ability to target a specific tissue or organ. This concept particularly holds true for the eradication of tumors, since targeted delivery of chemotherapy drugs can localize the drug's toxicity to the hypoxic tumor tissue rather than surrounding tissues.[1] Moreover, the pharmacological activity that defines the overall success of a therapy is directly influenced not only by control over the release rate, but also by the dose or quantity of cargo delivered to specific tissues. Nanocarriers can be designed to both increase the bioavailability of drugs that are poorly water-soluble and to promote stability of their cargo as in the case of genetic materials that are generally susceptible to biodegradation.[2] In recent decades, these exciting properties have spurred a rapidly growing field of research focused on engineering smart nanomaterials that improve upon the delivery and targeting mechanisms of existing drug and gene therapies.[3–5]

To design this type of carrier, the selection of the appropriate combination of nanomaterials is fundamental in introducing unique and favorable properties that are not typically found in single components. For this reason, nanohybrids, a combination of different classes of biomaterials at the nanoscale level, are presented as a possible solution to address multiple bottlenecks for successful therapies, such as controlling the rate of cargo diffusion, increasing drug stability, and selectively targeted delivery.[6] This emerging class of nanocomposite materials combines synthetic or natural polymers including polysaccharides, proteins and nucleic acids together with inorganic or organic compounds in a 3D architecture.[7] This new type of carrier offers a versatile platform that can be easily tuned and modified by changing the type of nanomaterial or polymer. Among the wide variety of nanoscale compounds available to construct nanohybrids, both inorganic materials such as clay minerals and organic materials including carbon nanotubes (CNTs), graphene oxide (GO), and nanodiamonds (NDs) offer a valid alternative. In fact, each one of them has unique nanoscale properties that are favorable for the design of new and improved therapeutic carrier systems.[9–12] Nanohybrids composed of these materials have been applied over the past decades as smart carriers for the delivery of drugs and genes, especially for targeted cancer treatment. A successful design of this type of bionanohybrid material requires an understanding of the superficial properties of the nanoscale component, such as surface area, charge density and distribution of reactive functional groups. Furthermore, tissue or cell selectivity can be introduced by incorporating ligand-binding molecules with nanohybrids. Another important property to consider in the development of these nanohybrids is the affinity of the biopolymer and nanoparticle (NP) to self-assemble, as this step is fundamental in defining the final stability of the biocomposite and its loading efficiency. In fact, the corresponding 3D arrangement of the nanohybrid substrate can also influence the loading mechanisms and release behavior of its cargo.

This review focuses on the recent strategies available to engineer smart nanohybrids in order to achieve a better control over drug delivery as well as gene therapy (Figure 1). The first part of the review will focus on smart drug delivery approaches for the treatment of cancer, followed by a discussion including innovative regenerative medicine strategies that utilize biological gene delivery vectors. Finally, an overview over the possible clinical translation of

these nanohybrid materials is also proposed to delineate their future in regards to drug and gene delivery.

2. Nanohybrids for Drug Delivery

Nanohybrids can be categorized according to the types of the biomaterials employed. The selection of material dictates the types of interaction between the material and the drug, influencing the corresponding loading efficiency. For example, nanostructures carrying positive or negative charges can adsorb ionic drugs on their surface by ion exchange. At the same time the presence of planar nanostructure sheets composed of sp^2 carbon can load therapeutic agents with steroidal or aromatic structures by π–π stacking. Alternatively, nanoparticles carrying nucleophilic groups can be exploited to form either hydrogen or covalent bonds with the loaded cargo, modulating the kinetic release profile. The following sections focus on the developments of carbon-based nanohybrids for cancer therapy. Moreover, a discussion on hybrid nanoclays and other types of innovative nanohybrids will be provided to highlight the future trends of these promising carriers.

2.1. Carbon-Based Nanohybrids for Drug Delivery

CNTs represent one of the possible materials to engineer nanohybrids into drug delivery carriers. CNTs are composed of single or multiple layers of graphene sheets rolled into cylindrical tubes of sp^2 carbon, which are capped at both ends with networks known as fullerenes. These fullerenes can serve as drug delivery platforms since they can be easily modified to improve their water solubility and partially avoid the formation of aggregates.[11]

CNTs are categorized by structure as either single walled carbon nanotubes (SWNTs) or multi-walled carbon nanotubes (MWNTs). Their potential in this field is in part accredited to their affinity towards internalization by cells due to their unique nanostructure properties. CNTs are able to penetrate cells using several endocytosis pathways or simply by diffusion through the lipid bilayer. The route of cellular uptake is attributed to the tube length or the presence of polymeric coatings on their surface.[13] Once internalized, they generally localize in cell endosomes and lysosomes[14] or in other subcellular compartments including mitochondria[15] and the nucleus.[16]

Due to their poor thermodynamic stability in water, CNTs have a strong tendency to stabilize into aggregates. For this reason, side wall functionalization of CNTs is commonly performed to decrease the extent of bundle formation among tubes and improve their biocompatibility. Since the long term cytotoxicity of CNTs is a widespread concern for researchers and scientists, CNTs are most commonly hybridized with biodegradable polymers to increase their biocompatibility and decrease their ability to form reactive oxygen species inside cells.[17]

Drugs can bind with CNTs through different mechanisms such as physical absorption or covalent bonding with the functional groups on the walls of the CNTs.[18,19] Moreover, the introduction of a polymeric coating can also provide additional drug binding sites by the formation of ester or amide bonds, which are generally cleaved by hydrolysis in acidic

environments.[20,21] Since the microenvironments of solid cancerous tumors in the human body have a slightly acidic pH, a polymeric nanohybrid carrying therapeutic cargo would only release the drug in the hypoxic regions localized to tumor environments. In this sense, Liu et al. have proposed a system consisting of branched polyethylene glycol (PEG) chains on SWNTs to deliver paclitaxel (PTX) in vivo in mice. PTX was conjugated with PEG using a cleavable ester bond to form a water-soluble SWNT–PTX conjugate, and as a result, the nanohybrid showed higher efficacy in suppressing tumor growth in a breast cancer model with respect to the control treatment with Taxol, a chemotherapeutic agent used clinically.[21]

CNTs can also be surface-modified to introduce specific macromolecules, including growth factors, to improve the selectivity of action during cancer treatment. In a study by Bhirde et al., cisplatin, a common anticancer agent, was bound with epidermal growth factor (EGF) on SWNTs to target squamous cancer cells. In comparison to unmodified cisplatin, the hybridized drug demonstrated a signficantly higher efficacy in targeting and killing tumorous cells in vivo.[22]

Aside from covalent bonding, drugs and bioactive molecules can also be loaded onto the surface of the CNTs by π–π stacking. In another study by Huang et al., doxorubicin (DOX) was loaded onto the surface of SWNTs by π–π stacking interactions followed by inclusion of chitosan conjugated with folic acid (FA). Due to the higher expression of folate receptors on cancer cells, folic acid was proposed as a targeting mechanism. An increase in the release of DOX was achieved at a pH of 5.3 as a result of the reduced chemical interactions between doxorubicin and the surface of the CNTs in the acidic environment. Most importantly, the encapsulation of SWNTs with chitosanfolic acid provided a nanohybrid with better control over the release of DOX. The main factors behind this improvement are the additional diffusion through the chitosan shell and the possible hydrogen bonding between folic acid and DOX, which can hinder the diffusion of the drug from the nanohybrid.[23]

Among our research, an alternative solution has been proposed to improve the efficiency of drug loading onto CNTs using a lipid–drug approach.[24] Specifically, PTX was conjugated with docosanol and adsorbed onto the surface of SWNTs. Folic acid was also conjugated using the same strategy (Figure 2A). Our novel nanohybrid improved the effectiveness of PTX in vivo in a human breast cancer xenograft mouse model. Analogously, in a more recent study, we have proposed the conjugation of PTX with human serum albumin (HSA) nanoparticles which were further linked on the surface of SWNTs modified with a bifunctional PEG spacer.[25] The PTX delivered with the nanohybrid composed of albumin and SWNTs demonstrated a greater reduction in the activity of breast cancer cells compared to the PTX delivered by HSA nanoparticles.

In addition to CNTs, GO is another unique nanomaterial composed of sp^2 carbon sheet with specific physical and chemical properties that have been exploited for enhanced drug delivery, especially in cancer therapy.[26,27] The large superficial area combined with the π-conjugated structure allows higher loading efficiency of aromatic compounds through π–π interactions. At the same time, the surface can be modified with ligands to introduce selective targeting. Furthermore, GO in the reduced form also presents high optical

absorption in the near infrared spectrum, and this property has been explored for photothermal cancer treatments.[28,29]

However, GO presents a series of drawbacks including poor colloidal stability due to its tendency to aggregate in physiological conditions and its natural affinity for proteins.[30] To overcome these limitations, GO can be modified with water-soluble molecules to improve biocompatibility and colloidal stability in the presence of salt and serum. Erqun et al. have recently proposed a novel DOX delivery platform composed of GO coated with hyaluronic acid (HA) as a carrier of DOX.[31] The anticancer drug was loaded through π–π interactions onto the surface of GO followed by chemical conjugation with adipic acid hydrazide-modified HA. The complex showed higher stability, drug loading efficiency, biocompatibility and also pH sensitivity with a sustained release of DOX.

Among other natural polymers, dextran has also been widely used as agent to improve the efficacy of GO as a drug carrier. Jin et al. have proposed an innovative nanohybrid of GO and hematin-modified dextran. The hematin–dextran conjugate self-assembled with GO through π–π interactions and the dextran alone improved the overall stability. The group demonstrated that the nanohybrid exhibited improved water solubility as well as better cytocompatibility with respect to GO alone. When conjugated with DOX, the nanohybrid showed a greater ability to treat drug-resistant cancer cells (Figure 2B).[32]

GO can also be functionalized with synthetic polymers that contain both hydrophobic moieties capable of interacting with the carbon sp^2 sheets and hydrophilic blocks to increase their water solubility. In this sense, Hu et al. have proposed a nanohybrid with reduced GO and the amphiphilic pluronic F127 capable of loading DOX with high efficiency and pH sensitivity.[33]

Another example of carbon-based nanomaterials is NDs, which possess unique physical and chemical properties that render them ideal for use in nanocomposites. NDs have a truncated octahedral morphology and highly tunable surface properties that can be oxidized or reduced to modulate the presence of reactive functional groups. These functional groups, such as hydroxyl groups (-OH) or carboxylic groups (-COOH), can be utilized to establish hydrogen or covalent bonds with drugs and polymers. Moreover, the natural fluorescence of NDs can be used to monitor their location within cells, which is particularly useful when considering cell therapy with hybridized anticancer drugs. In a study by Huynh et al., different strategies have been proposed to load cisplatin on the surface of ND in the presence or absence of polymer coatings (Figure 2C). The nanohybrid systems outperformed the non-coated ND in terms of cytotoxicity against the ovarian cancer cell line A2780 because of the higher cellular uptake enabled by the polymer coating.[34] Xiao et al. also reported that the combination of a synthetic polymer coating can enhance the therapeutic effect of NDs loaded with DOX (ND–DOX). The synthetic polymer used in this study improved the dispersibility of the ND–DOX complex, allowing a higher loading efficiency and localized delivery of DOX to the nuclei of cancer cells.[35] In another interesting approach, Moore et al. designed a ND–lipid hybrid by rehydration of lipid thin films containing cholesterol and biotinylated lipid using ND solutions loaded with epirubicin. The new formulation was then targeted using biotinylated antibodies (anti-EGFR) to target and successfully treat triple

negative breast cancer. This platform could also be applied to the treatment of many other types of cancer simply by changing the type of antibody exposed on the surface of the ND–lipid nanohybrid.[36]

These few examples demonstrate the versatile and tunable properties of carbon-based nanohybrids that allow them to serve as smart and environmentally responsive delivery agents, especially for cancer therapies. Aside from carbon-based nanohybrids, other types of nanohybrids composed of inorganic compounds such as clay minerals are also very promising candidates and their potential in drug delivery will be described briefly in the following section.

2.2. Clay Nanohybrids for Drug Delivery

Clay minerals are silicates of aluminum or magnesium that are organized in layered or microfibrous tetrahedral and octahedral structures. Layered clays are classified as either natural smectites, such as montmorillonite and hectorite, or synthetic smectites including laponite.[6] To realize the importance of nanoclays in drug delivery and as building blocks for nanohybrid systems, an understanding of their chemical structure is imperative. These smectite clays are organized in two tetrahedral silica sheets, with the internal sheet composed of Al^{3+} or Mg^{2+} arranged in an octahedral structure.[37] Due to their composition, smectite clays are a hydrophilic material with an internal layer that is freely accessible to water molecules, allowing surface conjugation or intercalation with hydrophilic polymers. Among the smectite family, laponite is the clay most commonly investigated in combination with a variety of natural and synthetic polymers due to its higher surface area and ability to establish strong interactions with guest compounds. The presence of laponite can serve as a crosslinker and as a thickening agent in a polymeric network, which can then be used for the fabrication of injectable or prefabricated scaffolds for drug delivery (Figure 3).[38–43] Moreover, the charges on the laponite surface are negative while the edges of the nanoparticles are positively charged and pH dependent which can be useful for the design of pH-sensitive nanohybrid systems.[44] In a study by Gonçalves et al. a pH-responsive laponite-alginate nanohybrid formulation was investigated for the delivery of DOX. DOX was first loaded onto laponite nanodiscs through electrostatic interactions and then coated with alginate. The system showed pH sensitivity and a sustained in vitro release.[45] Using a different approach, Wang et al. proposed the design of a nanocomposite formulation based on laponite hybridized with a polyethylene glycol and polylactic acid copolymer (PEG–PLA) as pH-sensitive carriers of DOX.[46] In this case, a self-assembling process of the amphiphilic PEG–PLA copolymer on the surface of the laponite was achieved. PEG served as a protective shell to enhance the stability of the nanohybrid system and the hydrophobic region of the copolymer functioned as an anchor on the surface of the loaded nanodiscs. The study concluded a high loading efficiency of DOX combined with a pH-sensitive release profile.

Apart from the smectite group, there are other clays of interest that display different morphologies such as sepiolite and halloysite clays.[47] Sepiolite is a fibrous clay composed of an octahedral sheet of magnesium oxide/hydroxide placed between two tetrahedral silica layers. The periodic inversion of the SiO_4 tetrahedron creates a regular discontinuity of the

silica sheets along the axial extension of fibers, forming a structural tunnel which can be used to allocate drugs. Sepiolite presents a high surface density of silanol (Si-OH) groups on its external fibers that interact with polymers through hydrogen bonds to form nanohybrids as carrier of drugs.[48] On the other hand, an alternative morphology is displayed by halloysite clays which are alumosilicate sheets rolled in the form of tubes. With respect to smectite clays, they do not require exfoliation as they can be readily dispersed into polymeric solutions.[49] Their diameter is much larger than that of CNTs, which gives halloysite clays a high loading capacity for polymers and globular proteins. Moreover, the different chemistry in the external and internal regions of the tubes provides versatility in terms of chemical modifications. Drugs can be loaded using several strategies including the following: intercalation, adsorption onto the external and internal wall of the tubes, or internal loading followed by crystallization/condensation.[50] Nanohybrids composed of these clay nanotubes represent a very promising drug delivery platform for a vast array of drugs including antibiotics[51] and chemotherapeutic drugs.[52]

Finally, layered double hydroxides (LDHs) are another emerging class of clays that differ from the types previously mentioned, as they possess a higher charge density and anion exchange ability.[53] They can be functionalized with negatively charged polymers, and the layered structures within the resulting nanohybrid can be loaded with anionic drugs and compounds through ion exchange. By these very same mechanisms, LDHs can also be used to deliver genes.[54] Drugs and biomolecules can be bonded to these clays following several different approaches including exfoliation-restacking of the layers, intercalation, and pillaring reactions.[55] Among other studies, Kim et al. demonstrated that LDHs can be utilized as effective carriers of otherwise insoluble drugs, such as the anti-cancer drugs methotrexate (MTX) and 5-fluorouracil (5-FU). The in vitro studies between the drug carrier and cervical adenoma cancer cells verified that the LDH-mediated delivery of the drugs caused an immense reduction in tumor cell viability compared to the delivery of the drugs alone. These results are attributed to the enhanced cell internalization of the drugs facilitated by the LDH carriers.[56] In addition, cell or sub-cellular targeting can be introduced by the linkage of specific biomolecules such as folic acid. In a recent study by Yan et al., LDH nanoparticles were prepared by co-precipitation and covalent conjugation with folic acid. The modified LDH nanohybrids loaded with MTX showed an increased capacity to penetrate cell nuclei, resulting in the improved efficacy of MTX.[57] A more extensive description of other possible strategies in drug delivery using LDH nanohybrids can be found in other excellent reviews.[58–60]

2.3. Lipid-Polymer-Based Nanohybrids for Drug Delivery

Another important class of emerging nanohybrids is that of polymers and lipids, which are generally organized in a multilayered core–shell structure.[61] These nanocarriers combine both properties of liposomes and polymeric nanoparticles, to exhibit a higher drug loading efficiency and physical stability once administered in vivo.[62] The enhanced properties can be attributed to their unique composition, which generally consists of a polymeric drug loaded core enclosed in a lipid shell and surrounded by an additional layer of PEG. The PEG coating enables a prolonged in vivo circulation and increased steric stabilization. The polymer core can be composed of natural or synthetic polymers with different degrees of

crosslinking, allowing a precise control over the release profile of the loaded cargo. In a recent work by Petralito et al., the polymeric core was designed using a photo-crosslinked hydrogel composed of polyethylene glycol–dimethacrylate (PEG–DMA) that improved the mechanical stability of the lipid bilayer and modified the release kinetics of the model cargo with respect to liposomes composed of hydrogenated soybean phosphatidylcholine.[63] Additional structural integrity can be provided by modifying the lipid chemical structure, rather than the polymer core, leading to the fabrication of hybrid vesicles known as cerosomes.[64] In this case, the hybrid inorganic–organic bilayer is synthesized by the self-assembly of organoalkoxysilanes which resemble the chemical structure of lipids.[65] These nanocarriers present higher stability towards surfactant-induced dissolution and can be used for the delivery of anticancer drugs with a better control over their release behavior in respect to conventional liposomes.[66] Apart from improved mechanical integrity, lipid-polymeric nanohybrids can be precisely oriented to offer targeted delivery to localized tissues or cells as in the case of cancer treatment. To achieve this important goal, the hybrid system can be loaded with magnetic nanoparticles which can be used as magnetic resonance imaging (MRI) probes or as targeting devices in the presence of applied magnetic fields. As reported by Yang et al., the anticancer drug DOX and the monodispersed magnetic nanocrystals (Fe_3O_4) were simultaneously encapsulated within an amphiphilic block copolymer to form multifunctional magneto-polymeric nanohybrids (MMPNs) for the treatment of breast cancer. The presence of the magnetic nanocrystals enabled MRI detection in in vitro and in vivo models.[67] In a more recent study, citrate-stabilized ferrite nanoparticles (CA–MFNPs) were linked to polyethyleneimine (PEI), which was crosslinked with Pluronic F127 copolymer using ethyldicarbodiime and *N*-hydroxysuccinimide (EDC/NHS) chemistry. Targeting of DOX to human cervix adenocarcinoma cells was achieved by linking FA to the hybrid system that was uptaken through FA receptors via endocytosis.[68] The presence of magneto-nanoparticles can be used as smart approach to control the amount of drug released simply by regulating the intensity of the external magnetic field. Specifically, on and off release can be achieved by inducing motions of the magnetic nanoparticles embedded in the nanohybrid lipid system, enabling an on-demand release of the loaded cargo.[69] While the major focus thus far has been on nanohybrids for cancer therapy, many researchers are applying similar polymeric nanohybrids towards treatments for autoimmune disorders. In one such study, Carambia et al. have assessed the in vivo efficacy of antibody-targeted, polymer-coated nanoparticle carriers to treat autoimmune encephalomyelitis (AE). In this study, superparamagnetic Fe_2O_3 nanoparticles were coated with an amphiphilic polymer and conjugated with autoantigen peptides prior to administration to an experimental AE mouse model. This research concluded that the peptide-conjugated nanohybrids selectively targeted and delivered the autoantigen peptide to the hepatic endothelial tissues affected by the autoimmune disease.[70] This selective targeting mechanism could also be employed for the treatment of a variety of other autoimmune diseases that are currently difficult to cure.

3. Nanohybrids for Gene Therapy

Supplementary to drug delivery, gene delivery is an alternative strategy for diagnosing and treating diseases and other clinical ailments. Specific genes can be delivered and expressed

within cells to utilize their native machinery to produce therapeutic proteins. These therapies have not had a significant clinical impact for the treatment of human diseases thus far because of suboptimal gene expression capabilities and biosafety concerns resulting from the selection and design of vectors.[71] Genetic material can be delivered to cells by physical, chemical, and viral methods. Physical and chemical methods are often referred to as nonviral gene delivery, as they do not utilize native biological vectors such as viruses, but instead rely upon mechanical or chemical procedures to enable the transfer of genetic material across cell membranes. Both nonviral and biological (viral) gene delivery technologies hold promise for future clinical treatments, such as in the repair of damaged cardiac tissue after myocardial infarction but further advances are necessary for their clinical translation. In addition to gene therapy by means of therapeutic protein expression, gene silencing by RNA interference is a recent discovery that also has a vast therapeutic potential for the treatment of cancer, autoimmune diseases, and neurodegenerative diseases such as Alzheimer's. Small interfering RNA (siRNA) are 20–25 bp double-stranded RNA that form RNA-induced silencing complexes (RISCs) upon entering the cytoplasm of a cell. Subsequently, these RISCs pair with and cleave the complementary mRNA. By this mechanism, the protein expression of a specific gene sequence can be hindered by effective siRNA delivery. Current gene silencing therapies are limited by the fact that siRNA are easily inactivated by serum complement and they do not readily diffuse across the cell membrane.[72] As a result, their therapeutic effects are diminishing as they cannot accumulate in target tissues. Nanoparticle polymer and lipid vectors have been used to overcome these factors due to the enhanced cell penetration and nucleic acid shielding effects they provide.[73] The following section will focus on the emerging trends developed to enhance the efficiency and therapeutic potential of chemical vectors and nonpathogenic viral vectors for gene delivery. Additionally, this section will review the current research strides in the use of chemical vectors for gene silencing therapies.

3.1. Carbon-Based Polymeric Nanohybrid DNA Vectors

Chemical vectors are nonviral vectors that are desirable for clinical applications given their minimal immunogenicity. However, nonviral platforms historically have very low transfection efficiencies compared to viral systems and as a result are often incapable of eliciting gene expression at therapeutic thresholds. Common chemical vectors use cationic lipids or polymers to deliver genes. The shortcomings of these systems primarily arise from their inability to diffuse across the cell membrane and the instability of the genetic cargo.[74]

Recently, biofunctionalized carbonbased nanohybrids have been proposed as vectors that overcome these principle issues.[75] The unique properties of carbon nanomaterials enable the delivery of genetic material across the cell membrane into the cytosol and therefore enhanced gene expression. The local retention time of nanovectors can be even further augmented by controlled delivery from hydrogels. Controlled gene delivery is vital to the success of tissue-specific therapies. Our studies have shown GO in conjunction with PEI is a viable delivery vehicle for plasmid DNA and therapeutic effects are prevalent when the GO–PEI–DNA nanohybrids are delivered by methacrylated gelatin hydrogels. When injected intramyocardially in a rat model of myocardial infarction, vascular endothelial growth factor (VEGF) plasmid expressed by the GO vector significantly restored cardiac function through

the activation of neoangiogenic pathways. Thus, the hydrogel facilitated in vivo localized gene expression in cardiomyocytes within periinfarct regions.

Another strategy to maximize the transfection efficiency of a vector is to modify the outer surface of highly functional carbon nanoparticles with biologically responsive molecules such as a peptides. Nanomaterials that are otherwise biologically inactive can be further hybridized into stimuli-responsive, biointeractive materials. Graphene oxide, for instance, can be functionalized with cell-adhesive RGD peptides to grant the nanoparticle an affinity for cell binding.[76] This is an especially attractive feature for nanohybrid vectors, as interfacing the vector with its biological environment plays a large role in optimizing transfection. Our investigations have highlighted the utility of functionalizing nanovectors with the cell-penetrating transactivating transcriptional activator (TAT) peptides. TAT is an endosomolytic peptide derived from the HIV-1 virus and promotes both cell membrane penetration and endosomal escape.[77] To demonstrate this concept, a carbon nanotube and polyacrylic acid (PAA) nanovector was noncovalently conjugated with TAT/DNA nanoparticles. The vector dually expressed VEGF and angiopoeitin-1 (Ang1) cDNA. To apply these components towards a therapeutic model, the CNT–TAT/DNA hybrids were embedded in fibrin hydrogels and incorporated into a vascular stent device using layer-by-layer gelation assembly.[78] The hydrogel localized the expression of the transgenes, and the TAT peptides further increased the bioactivity of the stent by augmenting transfection efficiency. When employed in vivo in a canine femoral artery, the nanohybrid stent outperformed bare metal stents in terms of arterial re-endothelialization (Figure 4).[78] In addition to delivery of double-stranded, plasmid DNA, nanohybrids such as CNTs functionalized with PEI can efficiently deliver siRNA given the high loading capacity and cell penetrative abilities of CNTs in conjunction with the endosomolytic attributes of PEI.[79] Other groups have validated the efficiencies of alternative carbon nanoparticles, such as nanodiamonds, for use as hybrid siRNA vectors.[80,81]

3.2. Clay-Based Nanohybrid Vectors

As discussed previously, nanoparticle clays possess unique surface chemistries, high loading capacities, and the ability to form self-assembling hybrids for environmentally responsive drug delivery systems. These same properties can be exploited to develop self-assembling gene delivery nanohybrids. Layered double hydroxides are a class of anionic clays that can be directly loaded with nucleic acids, DNA, and RNA by intercalation. By anion exchange mechanisms, linear DNA fragments as large as 8000 bp and plasmid DNA are reported to self-assemble with LDHs to form LDH–DNA nanohybrids.[82] Ladewig et al. studied the transfection efficiency of LDH–DNA nanohybrids across various cell lines and determined a high efficiency accompanied by minimal to no cytotoxicity, in comparison to standard lipid-based carriers.[83] In fact, LDH complexes are proposed as favorable vectors over other nanoparticle vectors because rather than accumulating in cells and tissues upon internalization as observed with carbon-based and polymeric nanoparticles, LDHs instead dissolute into noncytotoxic ions.[82,84]

Recently, LDHs have been extensively applied in vitro as siRNA vectors for gene silencing. LDH hybrids, for example amine-functionalized, silicon dioxide-coated LDH–siRNA

complexes, are often surface modified to improve nanoparticle dispersion and therefore increase transfection efficiency.[85] LDH siRNA vectors have also been coupled with hydrogel scaffolds that could be utilized for localized regeneration of cartilage and the treatment of osteoarthritis by serving not only as cell scaffolds, but also to strongly express siRNA and effectively silence the human GAPDH gene.[86] LDHs have furthermore shown the ability to simultaneously function as both drug carriers as well as siRNA or DNA vectors. Li et al. have shown the vast therapeutic potential of this platform by studying the co-delivery by LDH complexes of chemotherapeutic drug 5-fluorouracil and delivery of apoptotic siRNA, concluding great success in its preclinical stages.[87] A platform such as this one, capable of both gene silencing and drug delivery, can be used to simultaneously suppress a pro-tumorigenic gene and deliver an anti-cancer drug to treat drug resistant tumors.

3.3. Biodegradable Polymeric Nanohybrid Vectors

Despite the promising outlook of the aforementioned nanohybrid siRNA vectors, recent concerns regarding nanoparticle toxicity have encouraged researchers to develop biocompatible and biodegradable nanovectors for siRNA delivery. These biodegradable nanohybrid vectors have been formulated with low molecular weight polymers[88] and polysaccharides such as dextran[89] and chitosan.[90] Proteins endogenous to the human body can also be used to deliver genetic cargo. Our reports have revealed for the first time the potential of PEI-coated human serum albumin nanohybrids as siRNA vectors.[91] Albumin, a binding protein abundant in human plasma, is ideal for in vivo delivery applications since it has a high binding affinity yet it lacks immunogenicity and is readily metabolized in the liver.[92] Results indicate that the PEI–albumin nanohybrids can transfect breast cancer cells in vitro with high efficiency and minimal cytotoxicity.[91]

3.4. Viral Gene Therapy with Polymeric Nanohybrids

Biological vectors, such as retrovirus, adenovirus, lentivirus, and adenoassociated virus (AAV), are also commonly used vectors for gene therapy applications. Viruses are highly efficient vectors because their capsids are surrounded by viral envelopes that enable the transduction of viral DNA across cell membranes. The development of therapeutic gene delivery applications with these viruses is hindered by issues regarding biosafety, immunogenicity, and potential of insertional mutagenesis.[93,94] In contrast to mammalian viruses, insectoriginated baculoviruses (Bac) are nonpathogenic to humans since they are unable to replicate in mammalian cells. However, the baculovirus still possesses viral envelope glycoproteins that facilitate cell membrane penetration and can transfer genetic material within cells. These attributes present the baculovirus as an ideal viral vector. In our investigations, we have explored the efficacy of baculoviral nanohybrids for stem-cell–gene therapies, localized gene delivery, and therapeutic intervention within biomedical devices. Beyond the topics of this discussion, hybridized baculoviruses are also excellent vectors for the delivery of siRNA, which is thoroughly reviewed by Makkonen et al.[95]

We have found that the baculovirus can be used to enhance cell-based therapies. An emerging therapy for restoring damaged cardiac tissue after myocardial infarction is transplantation of multipotent stem cells into infarct regions. The restorative capacity of

many of these therapies is not sufficient to warrant the use of this type of treatment in a clinical setting.[94] Many groups have improved the success potential of this therapy by genetically modifying stem cells prior to transplantation, but low transfection efficiency with nonviral vectors and biosafety concerns with viral vectors are current downsides.[96] The baculovirus by itself has a low transduction efficiency in vivo since it is susceptible to serum inactivation.[97] To mitigate this effect, baculoviruses can be surface modified with polymers such as polyamidoamine (PAMAM) dendrimers or PEI. We have found that baculoviruses noncovalently hybridized with PAMAM display increased transduction efficiency due to the properties of the dendrimer. PAMAM–baculovirus nanohybrids carrying VEGF transgene were able to efficiently transduce human adipose derived stem cells (hASCs) resulting in overexpression of the pro-angiogenic gene. Following the injection of the transduced hASCs into infarct sites of a murine myocardial infarction model, the infarct regions displayed increased vascularization and overall improved cardiac function compared to the control therapy with unmodified hASCs. Furthermore, transient expression of VEGF was observable for up to two weeks upon implantation.[98] Other groups have also implemented similar baculovirus-enhanced cell therapies for the treatment of myocardial infarction. Yeh et al. have recently developed VEGF-expressing, ASC cell sheets, genetically enhanced by hybridized baculoviruses. The study concluded that the transduced cell sheets significantly reversed the damage caused by myocardial infarction.[99] Other groups have also used baculovirus nanohybrids to modify stem cells to overexpress osteogenic and angiogenic growth factors for in vivo bone regeneration.[100,101] As with the nonviral applications, hydrogels can also be utilized as controlled and sustained release platforms for viral nanohybrid vectors. To illustrate this concept in a potential cell-based therapy, PAA coated CNTs hybridized with baculoviruses were embedded in a denatured collagen gel. The CNTs were introduced to both extend the release of the recombinant baculoviruses and to enhance the hydrogel's mechanical properties. The in vitro interactions between this hydrogel scaffold and rat bone marrow stromal cells (rBMSCs) revealed a sustained release profile of baculovirus from the hydrogel and a high transduction efficiency over two weeks.[102]

Since hydrogels facilitate sustained and localized gene delivery, and due to their versatile mechanical and chemical properties, they are ideal platforms for introducing baculovirus nanohybrids to biomedical devices. Analogously to our previous studies on CNT nanohybrid stents, we applied baculovirus nano-hybrid hydrogels to vascular stents to demonstrate the clinical potential of this viral gene therapy. To address the challenge of serum inactivation and to prolong transgene delivery, PAMAM–baculovirus complexes were microencapsulated in poly (glycolic-co-lactic acid) (PLGA). The microcapsules were subsequently applied to the stent within layers of a fibrin hydrogel. This fibrin-coated stent was implanted in canine denuded femoral arteries, and the pro-angiogenic effects of the baculovirusmediated VEGF expression were observable with prominent endothelial regeneration in injury sites, four months post-implantation. The fibrin hydrogel successfully sustained release of the microencapsulated nanohybrids, resulting in localized and controlled transgene expression (Figure 5).[103]

3.5. Combined Gene Therapy Strategies with Nanohybrids

Integrating nonviral vectors with viral vectors into a multipurpose delivery system is an effective strategy that combines the features of both types of vectors to synergistically maximize the potential of a gene therapy. Chemical vectors are advantageous due to their ease of production and minimal immunogenicity, yet their therapeutic effects are not as pronounced as viral vectors. The nonpathogenic baculovirus can express high transduction efficiencies, but not to the same extent as mammalian viral vectors. We developed a hybrid recombinant baculovirus linked with nonviral TAT/DNA nanoparticles to combine the strengths of both gene delivery platforms. Aimed towards myocardial therapy, we investigated the potential of a baculovirus expressing transgene Ang1 noncovalently linked with Ang1-expressing TAT peptide nanoparticles. The resulting Bac–NP nanohybrid displayed higher transduction efficiency and Ang1 expression than each vector alone. The angiogenic potential of this heightened Ang1 expression by Bac–NP system was studied in vivo in rat myocardial infarction models. Two weeks following the intramyocardial injection of the nanohybrid to infarct sites, the Bac–NP vector demonstrated sustained and localized Ang1 expression, up to 1.75 higher than that of the recombinant baculovirus alone. Cardiac repair was noted along with a reduction in infarct size.[104]

We further investigated the potential of the Bac–NP nanohybrid in genetically enhancing stem cell therapies. Bac–NP constructs expressing Ang1 both virally and nonvirally were used to transduce hASCs, which were implanted intramyocardially in rat models of myocardial infarction. The nanohybrid vectors effectively induced Ang1 overexpression from the hASCs, and just as in the previous study, transgene expression was significantly higher than baculovirus or TAT/DNA vectors alone. The transduced hASCs, one month post-infarction, restored cardiac function, reduced infarct size, and promoted vascular density in the infarct regions. The success of this combined viral/nonviral gene delivery platform in genetically engineering stem cells confirms the clinical relevance of this unique platform in cell-based therapies (Figure 6).[105]

4. Prospects and Challenges

In recent years, bioengineered nanohybrids have come forth as a promising new therapeutic strategy for both drug and gene delivery. However, nanohybrids still face several challenges which are hindering the translation of these treatment platforms from bench to bedside. The concerns of long term accumulation, distribution, and cytotoxicity of nanoparticles present a major hurdle for the use of nanohybrids in the human body. This particularly holds true for carbon-based nanohybrids, as in the case of graphene oxide, which can cause in vivo mutagenesis at high concentrations.[106] In addition, the majority of the studies regarding their potential toxicity have been carried out on rodent animals and these results cannot be easily translated to primates and humans.[107] The current studies on biodistribution and accumulation of nanoparticles are not sufficient to predict the long-term effects of nanohybrids on the human body.[108]

On the contrary, numerous polymeric nanohybrid DNA vectors are currently undergoing clinical trials, primarily for cancer therapies and vaccines. A vast array of siRNA nanovectors are also currently being tested in clinical trials, with lipid or polymer conjugates

delivering siRNA to silence genes responsible for diseases ranging from macular degeneration to advanced cancers. Yin et al. have provided an in depth analysis on these recent clinical developments.[109]

Both carbon and clay-based nanohybrid vectors have shown favorable effects in vivo, but the materials must be tailored to optimize desired therapeutic effects. A greater understanding of the manner by which a nanocomposite's biological interactions can impact the loading and release of genetic material is necessary to unlock their vast potential in tissue or disease-specific treatments. Nuclear uptake of genetic material, which is essential for successful transfection, is a rate limiting step in kinetic gene expression models, and the ease of nuclear uptake varies according to the cell type and cell–material interactions.[110]

Baculoviral nanohybrids, on the other hand, are not under clinical development for human gene therapy at the present moment. While over 50% of clinical trials involving gene therapy utilize viral vectors, none of them employ the baculovirus. However, many pre-clinical studies have recently shown their potential, and upon further study of the effects of this virus in the human body, clinical trials are imminent.[102] From our own studies, we have concluded that baculovirus nanohybrids can be tailored for use in a wide variety of applications, ranging from gene expression in biomedical devices such as stents, to injectable hydrogels capable of delivering angiogenic genes for treatment of myocardial infarction. We envision from our work and from other research group studies that the intelligent design of baculoviral nanohybrids can give rise to an extraordinary variety of applications within the field of regenerative medicine. It is important to note that there is no universal nanohybrid platform that is superior for all applications. Each nanohybrid must be carefully tailored to best serve its intended purpose in a new device or treatment.

Future considerations must be taken in the design of new nanohybrids targeted towards clinical use. Since nanoparticle toxicity is a major concern, researchers must continue to study the effects of nanoparticle accumulation in the human body, especially for the development of nanohybrids intended for in vivo use. In addition, researchers can shift special focus to developing nanohybrids of purely biodegradable materials, as discussed previously regarding layered double hydroxides and albumin-based carriers for drug and gene delivery. Beyond the concerns of cytotoxicity, studies have yet to be conducted on characterizing the pharmacokinetics of nanohybrid delivery in the human body. For instance, the nanodiamond–polymer nanohybrid developed by Moore et al.[36] effectively targets and treats tumors in a small rodent model, but the efficacy and reproducibility of such a treatment in humans is virtually unpredictable at the present moment. Additionally, the study of hybridizing alternative nonpathogenic, biologically derived vectors, such as bacteriophages and virus-like particles, holds great merit in creating innovative and advanced gene delivery strategies.[74] Genetically engineered bacteriophages, for example, can be used to express genes in animals and humans for applications ranging from cancer treatments[111] to promoting vasculogenesis within 3D bone regeneration scaffolds.[112]

5. Outlook

Nanohybrid transporters offer a promising alternative with respect to other technologies for the preparation of smart devices capable of selective targeting in drug deliver and gene therapy. As discussed in the previous sections, they represent a field of research that holds the potential to improve the outcome of existing therapies by reducing the side effects associated with established treatments as well as increasing the effectiveness of the therapeutic agent. However, as for any new technologies that seek to improve the field of nanomedicine, several critical issues are still present and a continued refinement of their properties is required for their clinical success in the near future. One of these issues is the safety profile of nanohybrids within the human body. For this reason, biodistribution, accumulation and cytotoxicity in different organs and tissues are important clinical problems that need to be considered to better clarify their potential clinical use. In addition, the interactions of nanohybrids with proteins and components of the immune system is another essential aspect that needs particular attention. It is thus imperative to consider all of these issues and potential risks in the development of new nanohybrids in order to not only improve their design and efficacy but at the same time ensure that they do not pose any cytotoxic effects in vivo.

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Biography

Arghya Paul is an assistant professor in Chemical and Petroleum Engineering and Bioengineering at the University of bioactive materials and biotherapeutic devices for clinical Kansas. His BioIntel Research Laboratory works on developing advanced applications. In particular, his team works in the interdisciplinary research areas of regenerative medicine, nanotherapeutics, and medical implants for cardiovascular and orthopedic applications. Prior to this, Arghya earned his PhD in Biomedical Engineering from McGill University, Canada, followed by postdoctoral research at the Harvard-MIT Division of Health Sciences and Technology and Harvard Medical School.

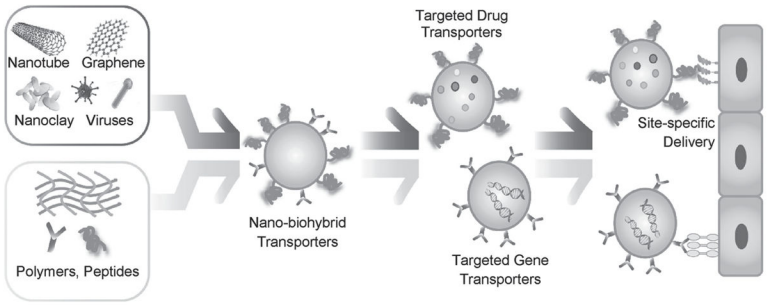

Figure 1.

Schematic representation of nanohybrid strategies to promote a targeted delivery of both drugs and genetic material. Nanohybrids combine both polymeric and other nanomaterials to enhance the therapeutic efficacy of existing therapies in both drug delivery and gene therapy.

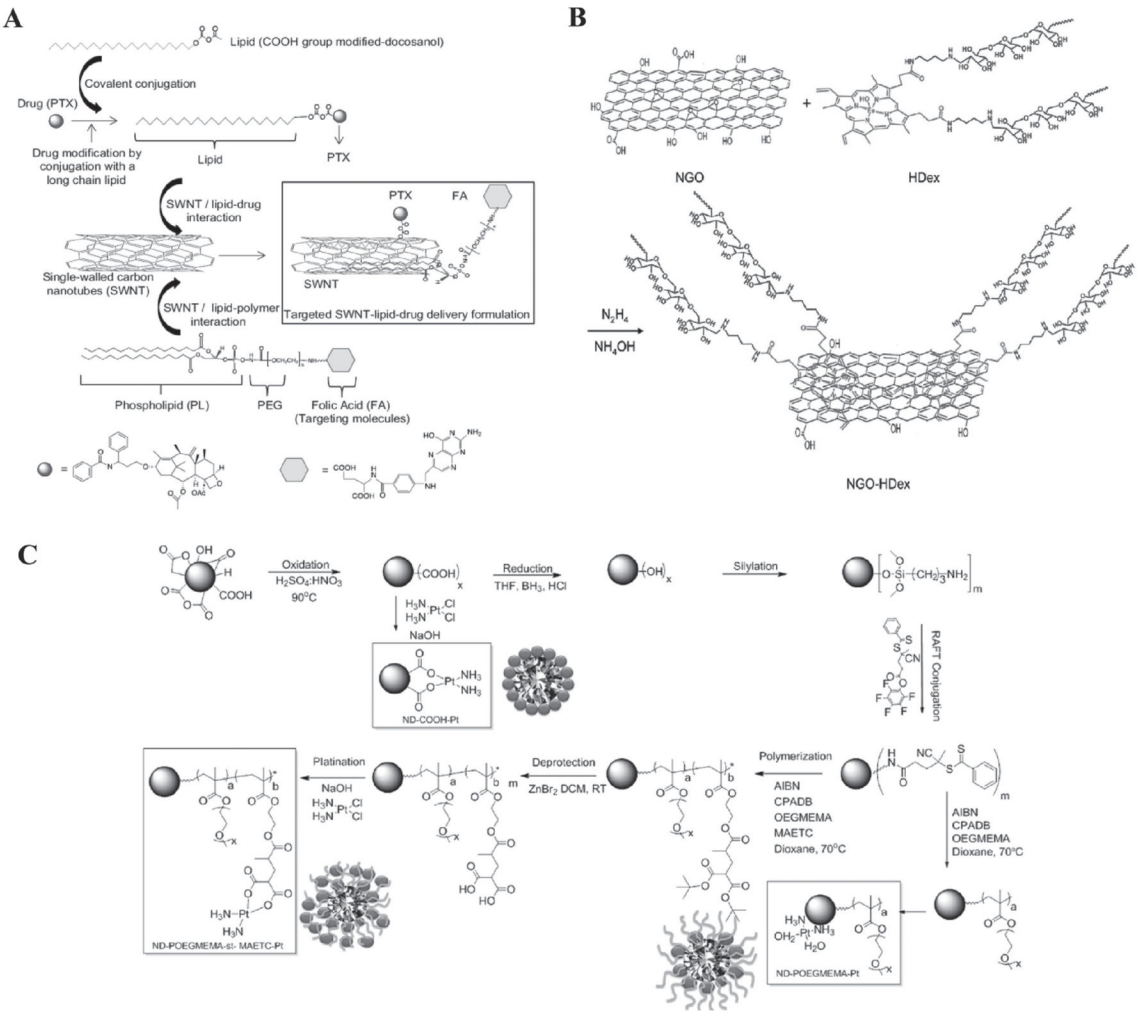

Figure 2.

Carbon-based nanohybrid surface modifications. A) Design strategy of a novel targeted SWNT–lipid–drug delivery system of PTX. The drug was chemically conjugated with a lipid tail through a reversible carbonate bond. The lipid tail is able to bind through hydrophobic interactions to the surface of the SWNT. Using a similar strategy, FA was linked with a phospholipidic tail Reproduced with permission.[24] 2013, Elsevier. B) Schematic of π–π interaction between graphene oxide and dextran modified with hematin (red). Reproduced with permission.[32] Copyright 2013 American Chemical Society. C) Possible chemical surface modifications of nanodiamonds to engineer polymeric nanohybrids as carriers for cisplatin. Reproduced with permission.[34] Copyright 2013, American Chemical Society.

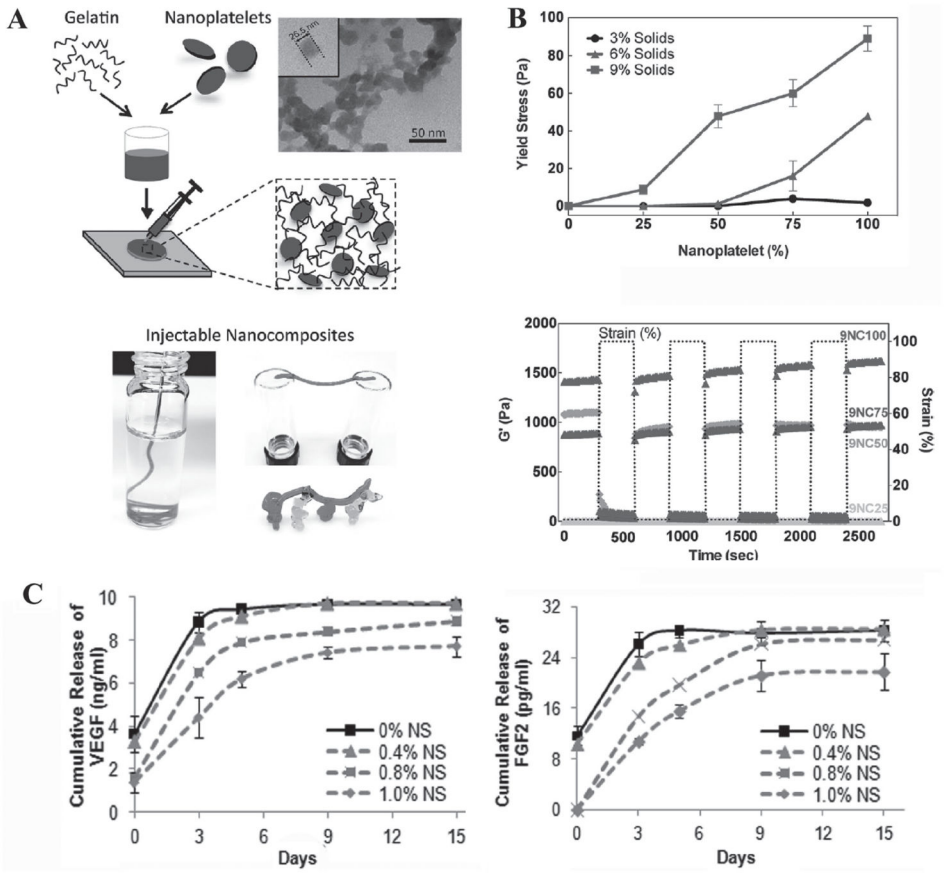

Figure 3.

Laponite interaction with gelatin polymer network to form injectable hydrogels. A) Schematic representation of injectable nanocomposite hydrogel made of gelatin and laponite along with transmission electron microscopy (TEM) images indicating the size of the nanoclay. Scale bar: 50 nm B) Yield stress of gels as function of the nanoclay concentration loaded in the hydrogels along with rheological characterization alternating low and high shear stress. For all of the nanocomposite hydrogels, more than 95% recovery was observed. C) Release profile of VEGF and fibroblast growth factor-2 (FGF_2) from gelatin methacrylate (GelMA) nanocomposite hydrogels containing different concentrations of laponite in the range of 0% up to 1.0% w/v. Adapted with permission.[42] Copyright 2016, The Royal Society of Chemistry.

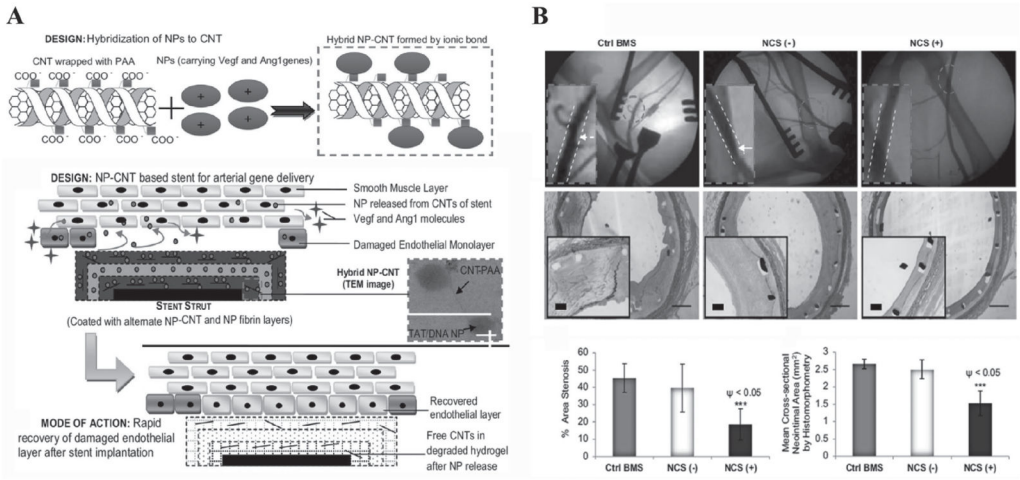

Figure 4.

Example of chemical vector for gene delivery used to promote re-endothelialization in vascular stents. A) Formation of an electrostatic complex between cationic nanoparticles loaded with VEGF and Ang1 genes and CNT wrapped with PAA. The hybrid NP–CNT system is coated over the stent surface by LbL fabrication using fibrin matrix to promote re-endothelialization. B) First row includes angiographic images of canine femoral arteries at 6 weeks post stent deployment of three different groups namely BMS (bare metal stent), NCS (−) (NP coated stent with no gene) and NCS (+) (NP coated stent with Ang1 gene). In the second row cross sectional images of elastic Van Gieson stained stented femoral arteries at 6 weeks post deployment. Scale bar: 0.5 and 100 mm (insert). Results on the bottom show significant reduction in the percentage of stenosis an neointimal area for the group containing genes NCS (+). The data represent the mean ± SD ($n = 8$); $^{***}p < 0.001$. p value on comparing NCS (+) and NCS (−) is denoted by ψ. Reproduced with permission.[78] Copyright 2012, Elsevier.

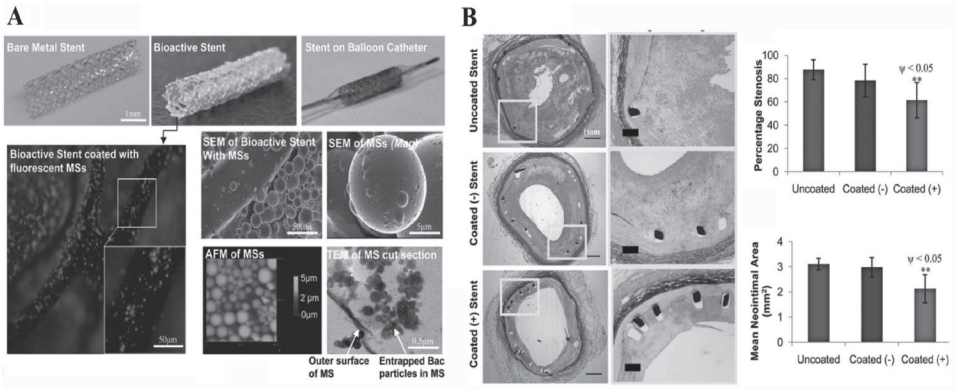

Figure 5.

Example of biological vector using baculovirus (Bac)-based stent therapy, as a strategy to promote vascular re-endothelialization. A) The first row includes images of the bare metal stent and the bioactive stent which contains Bac–PAMAM nanocomplexes before and after crimping of balloon catheter Scale bar: 1 mm. SEM, TEM, and fluorescent images to display the morphology of the microsphere (MS) of PLGA entrapping the Bac. Scale bar: 50 μm for fluorescent images. Scale bar: 50 μm (left) and 5 μm (right) for SEM pictures. Scale bar: 0.5 μm for TEM images. In addition the AFM image demonstrates the surface topography of MSs, encapsulating the nanohybrid baculovirus components. B) Representative cross-sectional images of elastic Van Gieson stained femoral arteries with uncoated bare metal stent and stents coated with BacNull–PAMAM and BacVegf–PAMAM at week 16 after stent deployment. Scale bar: 1 mm (left) and 100 μm (right). Results showed a decrease in the percentage of stenosis and neointimal area for the stents coated with BacVegf–PAMAM. The data represent the mean ± SD ($n = 8$). ANOVA: $^{**}p < 0.01$; p value on comparing COATED (+) and Coated (–) is denoted by Paul et al.[103] Reproduced with permission.[103] Copyright 2013, Nature Publishing Group.

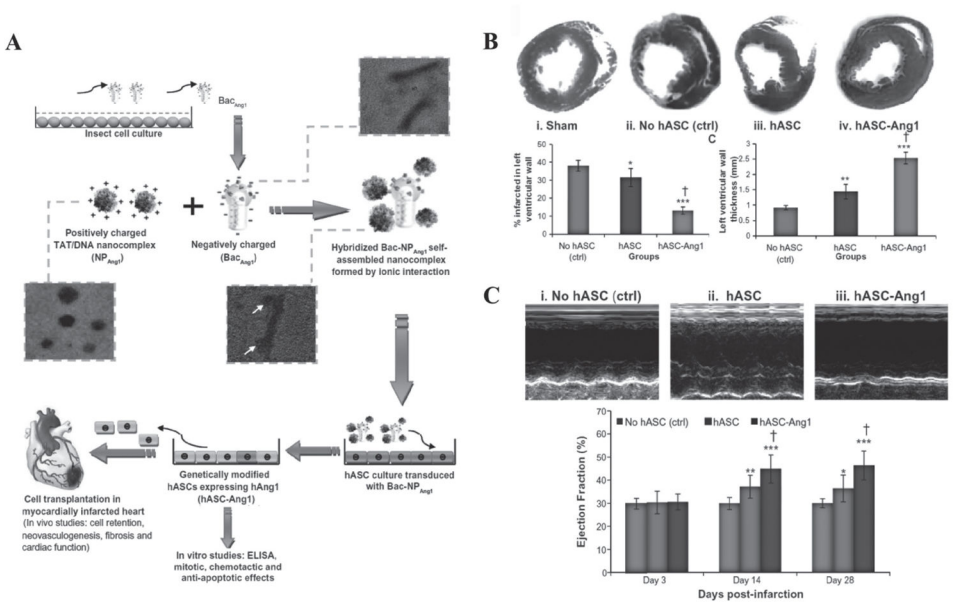

Figure 6.

Example of hybrid chemical/biological vector for gene delivery to enhance stem cell activity in myocardial therapy. A) Schematic representation of the steps necessary to generate the recombinant baculovirus (Bac-Ang1) and prepare the hybridized baculovirus with TAT/DNA nanoparticles necessary to transduce hASC for myocardial therapy. B) Representative images of the left ventricle myocardial section stained with Mason's trichrome showing a decrease in cardiac fibrosis after hASC and hASC–Ang1 transplantation. C) Echocardiographic assessment of cardiac function. Heart ejection fraction increased significantly after treatment with hASC and hASC–Ang1 groups after 28 d post-infarction. Data expressed as mean ± standard deviation. Statistically significant differences between groups compared to control no hASC are indicated as $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$. Significant difference between hASC and hASC–Ang1 is indicated by $^{\dagger}p < 0.001$. Reproduced with permission.[105] Copyright 2012, DOVE Medical Press.