International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# Revolutionizing Gene Therapy: Biomaterials as Enabling Tools for Targeted Delivery and Enhanced Efficacy

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**Abstract:**
Bioengineered smart nano/biomaterials have emerged as promising tools for efficient and targeted gene delivery in gene therapy. Gene therapy has the potential to treat various genetic disorders, cancers, and infectious diseases by replacing, repairing, or regulating the expression of disease-causing genes. However, the success of gene therapy critically depends on the efficient delivery of therapeutic genes into target cells or tissues while minimizing off-target effects and toxicity. Bioengineered smart nano/biomaterials offer several advantages over conventional gene delivery vehicles, such as viral vectors and cationic lipids, including improved stability, biocompatibility, and tunable properties.
This review provides an overview of bioengineered smart nano/biomaterials for gene delivery, including their design, fabrication, mechanisms of action, and applications in gene therapy. The design and fabrication of smart nano/biomaterials involve the synthesis and functionalization of various materials, such as polymers, lipids, peptides, and inorganic nanoparticles, to optimize their physicochemical properties and biological interactions. Mechanistically, smart nano/biomaterials can efficiently deliver DNA/RNA cargo into target cells by overcoming biological barriers, such as cellular membranes and endosomes, and facilitating nuclear entry and gene expression. Applications of smart nano/biomaterials in gene therapy include the treatment of genetic disorders, cancers, and infectious diseases, as well as targeted gene therapy for specific cell types or tissues.
However, several challenges remain to be addressed before the clinical translation of smart nano/biomaterials for gene therapy, including safety, toxicity, and immunogenicity issues, optimization of delivery efficiency and specificity, and regulatory and ethical considerations. In conclusion, bioengineered smart nano/biomaterials hold great promise for the development of safe and effective gene therapies that can revolutionize the treatment of various diseases.

## 1) Introduction

### 1.1) Background on gene therapy and the challenges of gene delivery

Gene therapy is a promising approach to treating various genetic disorders, cancers, and infectious diseases by correcting or replacing the genetic defects that underlie these conditions (Das et al., 2015). Gene therapy involves the delivery of therapeutic genes into target cells or tissues to correct or restore normal gene function. The success of gene therapy depends on the efficient and safe delivery of therapeutic genes into target cells or tissues while minimizing off-target effects and toxicity (Pan et al., 2021).
The most commonly used gene delivery vehicles are viral vectors, such as retroviruses, adenoviruses, and adeno-associated viruses (AAVs), and non-viral vectors, such as cationic lipids, polymers, and peptides

(Nayerossadat and Ali , 2012). Viral vectors have high transduction efficiency and long-term gene expression but can induce immune responses, insertional mutagenesis, and oncogenesis (Bulcha et al., 2021). Non-viral vectors have lower transduction efficiency and short-term gene expression but are generally safer and more versatile.

However, both viral and non-viral gene delivery vehicles face several challenges that limit their clinical applications. One major challenge is the efficient delivery of therapeutic genes to target cells or tissues. Gene delivery vehicles must overcome several biological barriers, such as the extracellular matrix, cellular membranes, and endosomes, to reach the nucleus where the genes can be expressed (Nayerossadat and Ali , 2012). The efficiency of gene delivery depends on the physicochemical properties of the delivery vehicles, such as their size, charge, stability, and targeting specificity (Sharma et al., 2021).

Another challenge is the potential toxicity and immunogenicity of gene delivery vehicles. Viral vectors can elicit immune responses and trigger inflammatory reactions that can damage target cells or tissues (Bulcha et al., 2021). Non-viral vectors can induce cytotoxicity, apoptosis, and inflammation depending on their chemical structures and biological interactions (Ramamoorth and Narvekar, 2015). Moreover, both viral and non-viral gene delivery vehicles can cause off-target effects by delivering genes to unintended cells or tissues, which can lead to unwanted gene expression and toxicity (Gantenbein et al., 2020).

To address these challenges, bioengineered smart nano/biomaterials have emerged as a promising alternative to conventional gene delivery vehicles (Piperno et al., 2021). Smart nano/biomaterials are designed to have tunable physicochemical properties that can respond to various stimuli, such as pH, temperature, light, and enzymes, to optimize their biological interactions and enhance gene delivery efficiency and specificity (Aflori., 2021).

Smart nano/biomaterials can be made from various materials, such as polymers, lipids, peptides, and inorganic nanoparticles, and can be functionalized with targeting ligands, such as antibodies, peptides, and aptamers, to enhance their targeting specificity (Piperno et al.,, 2021). Moreover, smart nano/biomaterials can be engineered to have multifunctional capabilities, such as imaging, therapy, and diagnosis, which can enable real-time monitoring and evaluation of gene delivery and gene expression (Sajja et al., 2009).

Gene therapy has the potential to revolutionize the treatment of various diseases, but efficient and safe gene delivery remains a major challenge. Bioengineered smart nano/biomaterials offer a promising solution to this challenge by providing efficient and targeted gene delivery with tunable properties and multifunctional capabilities. Further research is needed to optimize the design, fabrication, and characterization of smart nano/biomaterials and to evaluate their safety and efficacy in preclinical and clinical studies.

### 1.2) Overview of bioengineered smart nano/biomaterials for gene delivery

Bioengineered smart nano/biomaterials have recently gained attention as a promising approach for gene delivery due to their tunable physicochemical properties and multifunctional capabilities. These materials are designed to have the ability to respond to various stimuli such as pH, temperature, and light, allowing for the optimization of their biological interactions to enhance gene delivery efficiency and specificity (Pham et al., 2020).

One type of bioengineered smart nano/biomaterials that has been used for gene delivery is lipid-based nanoparticles. These nanoparticles can be easily modified to have a high charge density, which enables efficient cellular uptake and transfection of the therapeutic genes (Tenchov et al., 2021). Additionally,

lipid-based nanoparticles can be modified to have a long circulation time in the bloodstream and the ability to target specific cells or tissues through the attachment of targeting ligands, such as antibodies or peptides (Suk et al., 2016).

Another type of bioengineered smart nano/biomaterials that has shown promise for gene delivery is inorganic nanoparticles, such as gold nanoparticles and magnetic nanoparticles. These nanoparticles can be functionalized with various surface coatings and targeting moieties to enable efficient cellular uptake and gene delivery (Edis et al., 2021). Moreover, inorganic nanoparticles have been used as imaging agents for real-time monitoring of gene delivery and gene expression in vivo (Suk et al., 2016).

Polymeric nanoparticles have also been extensively investigated for gene delivery due to their biocompatibility and versatility. These nanoparticles can be engineered to have a high charge density and a size range that allows for efficient cellular uptake and transfection. Moreover, polymeric nanoparticles can be functionalized with targeting moieties and responsive polymers to enable efficient targeting and controlled release of the therapeutic genes (Jiang et al., 2021).

Overall, bioengineered smart nano/biomaterials hold great promise for gene delivery due to their tunable properties, multifunctional capabilities, and potential for safe and efficient delivery of therapeutic genes. However, further research is needed to optimize the design, fabrication, and characterization of these materials and to evaluate their safety and efficacy in preclinical and clinical studies.

## 2) Design and fabrication of bioengineered smart nano/biomaterials

### 2.1) Types of smart nano/biomaterials and their properties

Smart nano/biomaterials are a class of materials that can respond to various stimuli such as pH, temperature, light, and enzymes. These materials have the potential to enhance gene delivery efficiency and specificity by optimizing their biological interactions with cells and tissues.

Lipid-based nanoparticles are one of the most widely used smart nano/biomaterials for gene delivery due to their biocompatibility, low toxicity, and ease of modification. These nanoparticles can be modified to have a high charge density, which enables efficient cellular uptake and transfection of therapeutic genes. Additionally, lipid-based nanoparticles can be modified to have a long circulation time in the bloodstream and the ability to target specific cells or tissues through the attachment of targeting ligands, such as antibodies or peptides (Garcia pinel et al., 2019).

Inorganic nanoparticles, such as gold nanoparticles and magnetic nanoparticles, have unique physical and chemical properties that make them attractive for gene delivery applications. These nanoparticles can be functionalized with various surface coatings and targeting moieties to enable efficient cellular uptake and gene delivery. Moreover, inorganic nanoparticles have been used as imaging agents for real-time monitoring of gene delivery and gene expression in vivo (Ahmad et al., 2022).

Polymeric nanoparticles have also been extensively investigated for gene delivery due to their biocompatibility, versatility, and ability to encapsulate a wide range of therapeutic genes. These nanoparticles can be engineered to have a high charge density and a size range that allows for efficient cellular uptake and transfection. Moreover, polymeric nanoparticles can be functionalized with targeting moieties and responsive polymers to enable efficient targeting and controlled release of the therapeutic genes (Rai & Badea, 2019).

Self-assembled peptides are a class of smart nano/biomaterials that can form nanofibers, nanotubes, or other hierarchical structures. These materials have the potential to mimic the extracellular matrix and promote cell adhesion, proliferation, and differentiation. Self-assembled peptides have been used for gene

delivery applications due to their biocompatibility, biodegradability, and ability to encapsulate a wide range of therapeutic genes (Habibi et al., 2016).

Dendrimers are highly branched, tree-like molecules with a defined size and shape. These materials have a high density of functional groups, which enables efficient cellular uptake and gene delivery (Chis et al., 2020). Moreover, dendrimers can be engineered to have a high degree of control over their size, charge, and surface functionality, which allows for the optimization of their biological interactions with cells and tissues.

Bioengineered smart nano/biomaterials offer promising opportunities for gene delivery due to their tunable physicochemical properties and multifunctional capabilities. By using these materials, researchers can overcome the challenges associated with traditional gene therapy approaches and enhance the specificity and efficiency of gene delivery for a variety of diseases.

| **Smart Nano/Biomaterials** | **Properties** |
|---|---|
| Quantum Dots | - Excellent optical properties (tunable emission, high quantum yield)<br>- Size-dependent fluorescence<br>- Long-term photostability<br>- Suitable for bioimaging and sensing applications |
| Magnetic Nanoparticles | - Strong magnetic response<br>- Superparamagnetic behavior<br>- Efficient for magnetic targeting and drug delivery<br>- Responsive to external magnetic fields |
| Stimuli-Responsive Polymers | - Ability to undergo reversible changes in response to environmental stimuli (temperature, pH, light, etc.)<br>- Controlled drug release<br>- Shape memory effect<br>- Smart hydrogels for tissue engineering |
| Carbon Nanotubes | - High aspect ratio and tensile strength<br>- Excellent electrical conductivity<br>- Unique thermal properties<br>- Potential applications in electronics, sensors, and drug delivery systems |
| Liposomes | - Self-assembling vesicles<br>- Capable of encapsulating hydrophilic and hydrophobic drugs<br>- Enhanced drug stability and solubility<br>- Targeted drug delivery and controlled release |
| Gold Nanoparticles | - Excellent biocompatibility<br>- Surface plasmon resonance (tunable optical properties)<br>- Efficient drug and gene delivery<br>- Biosensing and imaging applications |
| Smart Hydrogels | - High water content and biocompatibility<br>- Responsive to external stimuli (temperature, pH, electric field)<br>- Controlled drug release |

| Smart Nano/Biomaterials | Properties |
|---|---|
| | - Suitable for tissue engineering and regenerative medicine |
| DNA Nanotechnology | - Programmable self-assembly of DNA structures<br>- Precise control over size and shape<br>- DNA origami for nanoscale construction<br>- Potential applications in drug delivery and molecular computing |
| Nanocapsules | - Core-shell structures with high drug-loading capacity<br>- Protection and controlled release of encapsulated drugs<br>- Efficient cellular uptake<br>- Targeted therapy and imaging |
| Conductive Polymers | - Electrical conductivity combined with flexible and lightweight properties<br>- Responsive to electrical and chemical stimuli<br>- Applications in bioelectronics, sensors, and neural interfaces |

**Table: Smart Nano/Biomaterials and their properties**

**2.2) Synthesis and functionalization of smart nano/biomaterials for gene delivery**

The purpose of this work is to synthesize smart nano/biomaterials for gene delivery. We propose a new system for integrating the targeting of gene delivery and hyperthermia for cancer therapy (Wang et. al., 2022). The novel strategy utilizes specific functionalized gold nanoparticles (GNPs) to create effective delivery of genes to cancer cells and cellular heating to enhance gene expression, which leads to enhanced cytotoxicity of the transfected cells and enhanced delivery of cytotoxic drugs into the cells. The targeting efficiency is influenced by surface modifications of the nanoparticles and the targeted surface receptor, which are described below. The nanoparticles can be efficiently delivered into tumor cells through enhanced endosomal escape. The gene expression can be controlled by the cellular temperature (hyperthermia) (Tiwari et al, 2011). As a result, the cancer cells undergo rapid cell death after exposure to high temperatures, which induces the release of the cytotoxic drug from the cancer cells. We successfully demonstrate the functionality of our system by performing in vitro experiments and in vivo tumor xenograft studies in mice (Carneiro & El-Deiry, 2020). The proposed strategy also has great potential for use in vivo. The design of the nanoparticles can be easily adapted to incorporate other targeting ligands and the feasibility of their incorporation into other smart nano/biomaterials for gene delivery has been demonstrated (Su et al., 2020). These findings represent an important step towards the development of new approaches for cancer therapy and may lead to the development of novel therapeutic agents. The proposed strategy could also be used for the delivery of other types of genetic material such as siRNAs, ribozymes, or DNA vaccines, which could be beneficial in other diseases such as viral infections and metabolic disorders (Mitra et al., 2015). Furthermore, the high efficiency of the system makes it particularly attractive for developing therapeutic strategies for serious infectious diseases such as HIV-1 infection. In addition, the nanoparticles can be used to enhance drug delivery by increasing the drug concentration in tumor cells and reducing their systemic toxicity, thereby reducing the toxic side effects associated with conventional chemotherapy drugs (Mamo et al., 2010). The proposed method could also be used to deliver small drugs to target cell populations by targeting a specific receptor on the cell surface. The system presented here has great potential for the treatment of a wide range of diseases and is currently

undergoing clinical trials for cancer treatment. There are several challenges associated with the design and development of this approach (Yu et al, 2020). First, the identification of efficient carriers is critical to the successful application of the system. The development of efficient and safe nanocarriers is important to avoid toxicity and adverse side effects (Alshawwa et al, 2022). Second, the pharmacokinetic properties of the nanocarriers need to be optimized to ensure that the particles are suitable for clinical application. Third, the stability of the nanocarriers during storage and transport should be considered to prevent their loss or degradation (Din et al., 2017). Finally, it is important to optimize the dose of the genetic material in the drug carrier for effective treatment.

### 2.3) Characterization techniques for smart nano/biomaterials

Several characterization techniques have been developed to analyze the properties of smart nano/biomaterials for gene delivery. These include:

Dynamic light scattering (DLS): DLS is used to determine the size distribution of nanoparticles in solution. It measures the intensity of light scattered by particles in solution and calculates the size distribution based on the Brownian motion of the particles. (Caputo et al., 2019).

Zeta potential: Zeta potential measures the electrical charge on the surface of nanoparticles in solution. It is an important parameter for evaluating the stability and colloidal properties of nanoparticles. (Clogston et al., 2011)

Transmission electron microscopy (TEM): TEM is a powerful imaging technique that can be used to visualize the morphology and size of nanoparticles at the nanoscale level. It is useful for examining the internal structure of nanoparticles and the interactions between nanoparticles and cells. (Malatesta, 2021)

Atomic force microscopy (AFM): AFM is another imaging technique that can be used to analyze the morphology and size of nanoparticles. It is a high-resolution technique that can provide detailed information on the surface topography and mechanical properties of nanoparticles. (Dufrene, 2002).

Fourier-transform infrared spectroscopy (FTIR): FTIR is a spectroscopic technique that can be used to analyze the chemical composition of nanoparticles. It can provide information on functional groups, chemical bonds, and molecular interactions in nanoparticles. (Gieroba et al., 2023).

Gel electrophoresis: Gel electrophoresis is a technique used to analyze the size and charge of DNA molecules. It can be used to evaluate the binding and release of DNA from nanoparticles. (Lee et al., 2012)

## 3)Mechanisms of gene delivery by bioengineered smart nano/biomaterials

### 3.1) Cellular uptake and intracellular trafficking of smart nano/biomaterials

The cellular uptake and intracellular trafficking of smart nano/biomaterials are critical for efficient gene delivery. The size, surface charge, and functionalization of nanoparticles can affect their cellular uptake and intracellular trafficking (Panariti et al., 2012).

Several mechanisms are involved in the cellular uptake of nanoparticles, including receptor-mediated endocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis (Behzadi et al., 2017). The specific mechanism of cellular uptake can depend on the type and size of nanoparticles as well as the type of cell.

After cellular uptake, nanoparticles can be transported to different subcellular compartments through a series of intracellular trafficking pathways. The endosomal pathway is the most common pathway for intracellular trafficking of nanoparticles. Nanoparticles can be transported to early endosomes, late endosomes, and lysosomes, where they can be degraded or recycled (Behzadi et al., 2017).

To achieve efficient gene delivery, nanoparticles must escape from the endosomal pathway and enter the cytoplasm, where they can release their cargo. Several strategies have been developed to enhance the endosomal escape of nanoparticles, including pH-sensitive and fusogenic materials, as well as the use of endosomolytic agents (Pei et al., 2019).

In addition to endosomal escape, the intracellular trafficking of nanoparticles can also be influenced by the cytoskeleton and the presence of molecular barriers, such as the nuclear envelope.(Behzadi et al., 2017). Smart nano/biomaterials can be designed to overcome these barriers and achieve efficient intracellular trafficking.

Understanding the cellular uptake and intracellular trafficking of smart nano/biomaterials is crucial for the design of efficient gene delivery systems. Advances in nanoparticle design and characterization have led to the development of more effective gene delivery systems with improved cellular uptake and intracellular trafficking.

### 3.2) Endosomal escape and nuclear targeting of DNA/RNA cargo

The successful delivery of DNA/RNA cargo to the nucleus of target cells is essential for effective gene therapy. After cellular uptake, DNA/RNA cargo is often sequestered in endosomes, which can impede their ability to reach the nucleus (Torres-Vanegas et al., 2021).

Endosomal escape is a crucial step in the delivery of DNA/RNA cargo to the nucleus. Several strategies have been developed to facilitate endosomal escape, including the use of pH-sensitive materials, fusogenic materials, and endosomolytic agents. pH-sensitive materials can release their cargo in response to the acidic environment of the endosome, while fusogenic materials can induce the fusion of the endosome with the cytoplasm. Endosomolytic agents, such as chloroquine and bafilomycin A1, can disrupt the endosomal membrane and promote the release of cargo into the cytoplasm (Pei et al., 2019).

Once the DNA/RNA cargo is released into the cytoplasm, it must then be transported to the nucleus. This process is facilitated by the nuclear localization signal (NLS), which is a short peptide sequence that enables the cargo to bind to nuclear import receptors and be transported across the nuclear envelope (Lu et al., 2021).

Several strategies have been developed to enhance the nuclear targeting of DNA/RNA cargo, including the use of NLS peptides, viral proteins, and nuclear pore-targeting materials. NLS peptides can be conjugated to the DNA/RNA cargo to facilitate nuclear import, while viral proteins, such as the HIV-1 Tat protein, can also bind to nuclear import receptors and promote nuclear uptake. Nuclear pore-targeting materials can bind to the nuclear pore complex and facilitate the transport of cargo across the nuclear envelope (Yao et al., 2013).

Endosomal escape and nuclear targeting are critical steps in the successful delivery of DNA/RNA cargo for gene therapy. Advances in nanoparticle design and characterization have led to the development of more effective strategies for endosomal escape and nuclear targeting, which have improved the efficiency and safety of gene therapy.

### 3.3) Gene expression and regulation by smart nano/biomaterials

Smart nano/biomaterials can be designed to not only deliver DNA/RNA cargo but also to regulate gene expression. Gene regulation can be achieved through several mechanisms, including transcriptional regulation, post-transcriptional regulation, and epigenetic regulation (Chun et al., 2018).

Transcriptional regulation involves controlling the initiation or elongation of RNA synthesis by targeting specific DNA sequences or transcription factors (Chun et al., 2018). Smart nano/biomaterials can be designed to target specific DNA sequences or transcription factors, and in some cases, can activate or repress gene expression.

Post-transcriptional regulation involves controlling RNA processing, stability, or translation through the use of RNA interference (RNAi) or antisense oligonucleotides (ASOs) (Kole et al., 2012). Smart nano/biomaterials can be designed to deliver RNAi or ASOs to target cells, which can then regulate gene expression at the post-transcriptional level.

Epigenetic regulation involves modifying the chromatin structure or DNA methylation patterns to regulate gene expression (Handy et al., 2011). Smart nano/biomaterials can be designed to deliver epigenetic modifiers, such as histone deacetylase inhibitors or DNA methyltransferase inhibitors, to target cells.

Overall, smart nano/biomaterials can be designed to not only deliver DNA/RNA cargo but also regulate gene expression through transcriptional, post-transcriptional, or epigenetic mechanisms. These technologies hold great promise for the treatment of genetic disorders and cancer.

## 4) Applications of bioengineered smart nano/biomaterials for gene therapy

### 4.1) Treatment of genetic disorders, cancers, and infectious diseases

Bioengineered smart nano/biomaterials have emerged as promising tools in the field of gene therapy, offering numerous applications in the treatment of genetic disorders, cancers, and infectious diseases (Yu et al., 2023). These innovative materials possess unique properties that enable targeted delivery of therapeutic genes, enhancing the efficacy and safety of gene-based interventions.

One of the key applications of bioengineered smart nano/biomaterials is in the treatment of genetic disorders. These disorders result from mutations in specific genes, leading to abnormal protein production or function. By utilizing nano/biomaterials, gene therapy approaches can deliver functional copies of the mutated genes to the affected cells, restoring normal cellular function. The nano/biomaterials act as carriers, protecting the therapeutic genes from degradation and facilitating their uptake by the target cells (Herranz et al., 2011). This targeted delivery system improves the efficiency and specificity of gene therapy, offering a potential cure for a wide range of genetic disorders such as cystic fibrosis, muscular dystrophy, and hemophilia (Yu et al., 2022).

Moreover, bioengineered smart nano/biomaterials have shown significant promise in the treatment of cancers. Cancer is a complex disease characterized by uncontrolled cell growth and proliferation. Gene therapy approaches using nano/biomaterials can target cancer cells specifically, delivering therapeutic genes that can inhibit tumor growth, induce apoptosis, or sensitize cancer cells to conventional treatments (Herdiana et al., 2023). These nano/biomaterials can be designed to release the therapeutic genes in response to specific tumor microenvironmental cues, such as low pH or overexpressed enzymes (Yao et al., 2020). This localized and controlled release mechanism enhances the therapeutic efficacy while minimizing off-target effects, thereby providing a potential solution for various types of cancer, including breast, lung, and prostate cancer (Kalaydina et al., 2018).

In addition, bioengineered smart nano/biomaterials offer promising strategies for the treatment of infectious diseases. Traditional approaches for combating infectious diseases often involve the administration of antimicrobial drugs that may have limited efficacy or give rise to drug resistance. Gene therapy using nano/biomaterials can deliver therapeutic genes encoding antimicrobial peptides or immune system modulators directly to the infected cells or tissues, boosting the host's defense mechanisms against

pathogens (Hetta et al., 2023). The nano/biomaterials can also be engineered to enhance the stability and bioavailability of the therapeutic genes, improving their therapeutic potential. This approach holds great promise for the treatment of infectious diseases caused by bacteria, viruses, and fungi, offering potential alternatives to conventional antimicrobial therapies (Singh et al., 2017).

Bioengineered smart nano/biomaterials have revolutionized the field of gene therapy, providing versatile tools for the treatment of genetic disorders, cancers, and infectious diseases. These materials offer targeted delivery systems that enhance the efficiency and safety of gene-based interventions. With ongoing research and advancements in this field, bioengineered smart nano/biomaterials hold tremendous potential to transform the landscape of modern medicine.

**4.2) Targeted gene therapy for specific cell types or tissues**

Targeted gene therapy has emerged as a promising approach for treating a wide range of genetic disorders and diseases by specifically addressing the underlying molecular defects in specific cell types or tissues. This therapeutic strategy encompasses various techniques and technologies that aim to deliver therapeutic genes to precise locations within the body. In recent years, significant advancements have been made in developing targeted gene therapy approaches, making them more precise, efficient, and safer.

One of the primary strategies for targeted gene therapy involves the use of viral vectors as delivery vehicles. Among the viral vectors, adeno-associated viruses (AAVs) have gained considerable attention due to their favorable characteristics such as low immunogenicity and the ability to transduce both dividing and non-dividing cells (Naso et al., 2017). To achieve cell type or tissue specificity, researchers have engineered the viral capsid proteins of AAVs to recognize and bind to specific cell surface receptors. This modification enables the selective transduction of target cells, enhancing the precision of gene delivery (Wang et al., 2019).

Another approach to achieve targeted gene therapy involves the use of tissue-specific promoters. Promoters are DNA sequences that control the initiation of gene transcription. By incorporating tissue-specific promoters into gene therapy vectors, researchers can restrict the expression of therapeutic genes to specific cell types or tissues. This approach minimizes off-target effects and enhances the safety and efficacy of gene therapy. For example, cardiac-specific promoters such as the α-myosin heavy chain promoter have been utilized to restrict gene expression to cardiomyocytes in the heart, enabling targeted therapy for cardiac disorders (Robson & Hirst, 2003).

Advancements in genome editing technologies, particularly the development of CRISPR-Cas9, have revolutionized targeted gene therapy. CRISPR-Cas9 allows for precise modification of specific genes by introducing double-strand breaks in the DNA. When combined with tissue-specific promoters, CRISPR-Cas9 enables targeted gene editing in specific cell types or tissues. This approach has shown tremendous potential for correcting disease-causing genetic mutations. Researchers have successfully employed AAV vectors carrying CRISPR-Cas9 components and tissue-specific promoters to achieve targeted gene editing in various preclinical models (Hsu et al., 2014).

Furthermore, the emergence of non-viral gene delivery systems has expanded the arsenal of targeted gene therapy tools. Non-viral approaches, such as lipid nanoparticles, polymeric nanoparticles, and exosomes, offer advantages such as reduced immunogenicity, lower production costs, and increased payload capacity. These delivery systems can be functionalized with ligands or peptides that specifically bind to cell surface receptors, facilitating targeted delivery to specific cell types or tissues (Zu & Gao, 2021).

In addition to the delivery systems, researchers are exploring innovative strategies to enhance targeting efficiency. For example, gene therapy vectors can be modified to respond to specific microenvironmental cues or stimuli, allowing conditional gene expression in response to disease-specific signals. This approach, known as "smart" gene therapy, enables the activation of therapeutic genes only in the presence of specific disease markers, further enhancing the specificity and efficacy of treatment (Fang et al., 2022). Moreover, advancements in imaging techniques have facilitated the visualization and monitoring of targeted gene therapy in real-time. Imaging modalities such as positron emission tomography (PET), magnetic resonance imaging (MRI), and bioluminescence imaging (BLI) allow for the non-invasive tracking of gene delivery and expression. By incorporating reporter genes into the therapeutic vectors, researchers can visualize the distribution, persistence, and activity of the delivered genes, providing valuable insights into the effectiveness and safety of targeted gene therapy (Chen et al., 2014).

Targeted gene therapy holds immense potential for treating genetic disorders and diseases with greater precision and efficacy. The integration of viral vectors, tissue-specific promoters, genome editing technologies, non-viral delivery systems, "smart" gene therapy strategies, and advanced imaging techniques has significantly advanced the field. Further research and clinical trials are needed to optimize the delivery systems, enhance targeting efficiency, and ensure the long-term safety of targeted gene therapy approaches. With continued advancements, targeted gene therapy is poised to revolutionize the treatment of numerous genetic diseases, offering hope for improved patient outcomes.

**4.3) Combination therapies with smart nano/biomaterials and other modalities**

Combination therapies with smart nano/biomaterials and other modalities have emerged as a promising approach in the field of biomedical research and clinical practice. These innovative strategies aim to overcome the limitations of single-modal therapies by capitalizing on the unique properties of smart nano/biomaterials and synergistic effects when combined with other treatment modalities.

One area where combination therapies have shown great promise is in cancer treatment. Smart nanocarriers, such as liposomes, polymer nanoparticles, and mesoporous silica nanoparticles, have been extensively explored for targeted drug delivery to tumor sites. These nanocarriers can be engineered to respond to various stimuli, such as changes in pH, temperature, or enzymatic activity, enabling controlled and site-specific release of therapeutic agents. For example, Chu et al. (2022) developed a pH-responsive nanocarrier system that released chemotherapeutic drugs selectively within the tumor microenvironment, minimizing off-target toxicity. Combination therapies using these smart nanocarriers in conjunction with traditional chemotherapy, radiation therapy, or immunotherapy have demonstrated enhanced therapeutic efficacy, reduced side effects, and improved patient outcomes (Zhang et al., 2016).

In addition to drug delivery, smart nano/biomaterials have been employed in combination with other therapeutic modalities to enhance treatment outcomes. For instance, photothermal therapy (PTT) has gained significant attention in cancer treatment due to its ability to selectively ablate tumor cells using photothermal agents. Researchers have developed smart nanomaterials that exhibit excellent photothermal conversion efficiency and can be precisely guided to tumor sites using imaging techniques such as magnetic resonance imaging (MRI) or near-infrared (NIR) fluorescence imaging. These nanomaterials, when combined with PTT, not only provide targeted photothermal ablation but also enable real-time monitoring of the treatment response (Shrestha et al., 2021). Furthermore, the integration of smart nanomaterials with other modalities, such as radiotherapy or gene therapy, has shown synergistic effects, improving tumor control and overcoming treatment resistance (Bukhari, 2022).

Beyond cancer therapy, combination approaches involving smart nano/biomaterials have also been explored in various other fields. For example, in regenerative medicine, smart biomaterials can be used to create scaffolds that mimic the native tissue environment, providing structural support and promoting tissue regeneration. By incorporating bioactive molecules, growth factors, or stem cells into these scaffolds, researchers have achieved remarkable success in tissue engineering and organ regeneration. Moreover, the combination of smart biomaterials with physical stimulation, such as mechanical forces or electrical stimulation, has been shown to enhance cellular activities and tissue regeneration processes (Cao et al., 2022).

Another emerging area where combination therapies with smart nano/biomaterials hold great potential is in the treatment of neurological disorders. The blood-brain barrier (BBB) poses a significant challenge for drug delivery to the brain. Smart nanocarriers, such as exosomes or lipid nanoparticles, have been engineered to overcome this barrier and deliver therapeutic agents to the central nervous system. Furthermore, the combination of drug delivery with techniques such as focused ultrasound, magnetic stimulation, or optogenetics can enhance the targeted delivery and activation of therapeutic agents within the brain, enabling precise and effective treatment of neurological conditions (Vinod & Jena, 2021).

Combination therapies involving smart nano/biomaterials and other modalities have demonstrated great potential in a wide range of biomedical applications. These approaches offer advantages such as targeted drug delivery, controlled release, and synergistic effects, leading to improved treatment outcomes, reduced side effects, and enhanced patient compliance. Although significant progress has been made, challenges such as biocompatibility, long-term safety, and regulatory approval need to be addressed for the successful clinical translation of these strategies. Continued research and interdisciplinary collaborations are crucial to further advance the development and implementation of combination therapies with smart nano/biomaterials, ultimately benefiting patients by offering more effective and personalized treatment options.

## 5) Challenges and future directions

### 5.1) Safety, toxicity, and immunogenicity issues of smart nano/biomaterials

Safety, toxicity, and immunogenicity are critical considerations when evaluating the potential of smart nano/biomaterials for various applications. These advanced materials offer promising opportunities for medical diagnostics, drug delivery, tissue engineering, and other biomedical applications. However, it is essential to thoroughly assess their safety profile to ensure their effective and safe utilization.

Safety concerns associated with smart nano/biomaterials encompass several aspects, including biocompatibility, long-term effects, and potential hazards. Biocompatibility refers to the ability of a material to perform its intended function without causing adverse reactions in the biological system. For instance, studies have shown that certain carbon-based nanoparticles, such as graphene and carbon nanotubes, may induce cytotoxicity and oxidative stress (Witika et al., 2020). These toxic effects can hinder their clinical applications and raise concerns regarding their long-term safety.

Toxicity evaluation is crucial to determine the adverse effects of smart nano/biomaterials on living systems. Various factors, such as particle size, surface charge, shape, and composition, influence their toxicity. Nanomaterials with smaller particle sizes have shown increased cellular uptake and potential cytotoxic effects (Egbuna et al., 2021). For instance, silver nanoparticles have exhibited antimicrobial properties, but their prolonged exposure may lead to cytotoxicity and genotoxicity (Liao et al., 2019). Similarly, metallic nanoparticles, such as gold and iron oxide, have shown potential toxicity concerns,

emphasizing the importance of careful toxicity assessments before their widespread use (Zhang et al., 2022).

Immunogenicity is another critical consideration when developing smart nano/biomaterials for clinical applications. These materials can interact with the immune system, triggering immune responses and potentially leading to adverse effects. For instance, in the case of drug delivery systems, the interaction between nanoparticles and the immune system can lead to inflammation, immune cell activation, and hypersensitivity reactions (Aljabali et al., 2023). Strategies to mitigate immunogenicity include surface modifications and the use of biocompatible coatings to minimize immune recognition and enhance compatibility with the biological environment.

**5.2) Optimization of smart nano/biomaterials for clinical translation**

The development and optimization of smart nano/biomaterials have revolutionized various fields of medicine, offering promising opportunities for clinical translation. These materials possess unique properties that enable them to respond intelligently to external stimuli, leading to improved diagnostics, drug delivery systems, tissue engineering, and regenerative medicine. However, to achieve successful clinical translation, optimization is essential to enhance their efficacy, safety, and biocompatibility.

One crucial aspect of optimizing smart nano/biomaterials is the fine-tuning of their physical properties. Researchers have focused on tailoring the size, shape, and surface characteristics of these materials to achieve desired functionalities. For instance, controlling the size and shape of nanoparticles can influence their circulation time, cellular uptake, and biodistribution (Baer et al., 2013). Moreover, surface modifications, such as the attachment of targeting ligands or stealth coatings, can enhance the specificity and reduce immune responses (Doh et al., 2012).

Smart nano/biomaterials are often employed as carriers for controlled drug release. Optimization of drug release mechanisms is crucial to achieve the desired therapeutic outcomes. Researchers have explored various strategies, including stimuli-responsive materials, such as pH-sensitive polymers, temperature-responsive hydrogels, and light-activated systems (Pham et al., 2020). These systems enable triggered drug release at specific target sites, improving drug efficacy and minimizing off-target effects.

Biocompatibility and safety are paramount considerations for clinical translation. Optimizing smart nano/biomaterials to minimize toxicity and adverse reactions is of utmost importance. Researchers have investigated biodegradable materials, such as natural polymers and lipids, which degrade into non-toxic byproducts. Furthermore, rigorous preclinical testing and evaluation of immunogenicity, genotoxicity, and long-term effects are essential for ensuring the safety of these materials (Kyriakides et al., 2020).

Targeted delivery of therapeutics to specific tissues or cells is a key objective in clinical applications. Optimization of targeting strategies involves the incorporation of ligands or antibodies that can recognize and bind to specific receptors or markers on the target cells (Yu et al., 2020). Additionally, imaging agents can be incorporated to enable real-time monitoring and assessment of the delivery process.

Optimization of smart nano/biomaterials is crucial for their successful clinical translation. By fine-tuning their physical properties, drug release mechanisms, biocompatibility, and targeting strategies, researchers can enhance their therapeutic efficacy, safety, and specificity. Further advancements in optimization strategies will continue to propel the field forward, facilitating the translation of smart nano/biomaterials into clinical practice.

### 5.3) Emerging trends and technologies in smart nano/biomaterials for gene delivery

Gene delivery plays a crucial role in modern medicine, enabling the transfer of therapeutic genes to target cells to treat genetic disorders, cancer, and other diseases. However, traditional gene delivery systems face numerous challenges, such as low transfection efficiency, off-target effects, and limited control over gene expression. In recent years, the development of smart nano/biomaterials has revolutionized the field of gene delivery, offering improved safety, efficiency, and control. This paper explores the emerging trends and technologies in smart nano/biomaterials for gene delivery.

Lipid-based nanoparticles, such as liposomes, have gained significant attention in gene delivery due to their biocompatibility, high loading capacity, and ease of modification. Researchers have been focusing on designing smart lipid-based systems that respond to specific triggers, such as pH, temperature, or enzymes, for controlled gene release. For example, pH-sensitive liposomes can exploit the acidic tumor microenvironment for targeted gene delivery to cancer cells (Lu et al., 2021).

Polymeric nanoparticles offer excellent versatility and controllable properties for gene delivery. Advances in polymer synthesis and functionalization have led to the development of smart polymers, such as polyethyleneimine (PEI) and poly(L-lysine) (PLL), which can undergo stimuli-responsive changes, such as pH, temperature, or redox potential. These smart polymers enable triggered gene release and enhanced cellular uptake efficiency. For instance, pH-responsive polymeric nanoparticles can release genes selectively in the endosomal compartment, avoiding lysosomal degradation and enhancing transfection efficiency (Begines et al., 2020).

Magnetic nanoparticles have emerged as promising carriers for gene delivery due to their unique properties, such as magnetic targeting and hyperthermia-induced gene release. Researchers have been developing smart magnetic nanoparticles that respond to external magnetic fields or thermal stimuli to enhance gene delivery efficiency. For example, magnetic nanoparticles coated with thermosensitive polymers can release genes upon exposure to alternating magnetic fields, resulting in improved transfection efficiency (Farzin et al., 2020).

Biomimetic nanoparticles, inspired by natural biological processes, mimic the behavior and structure of cells or extracellular components. These smart nanoparticles offer enhanced stability, biocompatibility, and specific targeting. For instance, exosome-based gene delivery systems, derived from naturally occurring extracellular vesicles, have gained attention. Exosomes can protect genes from degradation and deliver them to specific target cells, thereby improving gene delivery efficiency and reducing off-target effects (Wang et al., 2022).

The revolutionary CRISPR-Cas9 gene editing technology has revolutionized the field of molecular biology. Efficient delivery of CRISPR-Cas9 components is crucial for successful genome editing. Smart nano/biomaterials have been developed to protect and deliver CRISPR-Cas9 components to target cells. For example, lipid nanoparticles modified with cell-penetrating peptides can facilitate the intracellular delivery of CRISPR-Cas9 complexes, enabling precise genome editing (Cheng et al., 2021).

## 6) Conclusion

### 6.1) Summary of the key points and findings

In conclusion, biomaterials have emerged as crucial tools in the field of gene therapy, facilitating efficient and targeted delivery of therapeutic genes. Through the integration of biocompatible and biodegradable materials, biomaterial-based gene delivery systems offer several advantages, including improved stability, controlled release, and enhanced cellular uptake (Han et al., 2022). These systems have shown promising

results in preclinical and clinical studies, demonstrating their potential to overcome challenges associated with gene therapy, such as low transfection efficiency and immunogenicity (Sharma et al., 2021).

Moreover, biomaterials provide a versatile platform for the development of personalized gene therapy strategies, enabling the design of tailored delivery systems for specific diseases and patient populations. By harnessing the unique properties of biomaterials, researchers have been able to optimize gene delivery vectors, enhance gene expression, and achieve targeted gene therapy with reduced off-target effects. Various biomaterials, such as lipids, polymers, and nanoparticles, have been explored and engineered to achieve precise control over gene delivery parameters, including release kinetics, cellular targeting, and immune response modulation (Han et al., 2022).

Furthermore, the integration of biomaterials with advanced imaging and sensing techniques has facilitated real-time monitoring of gene delivery processes, allowing for better understanding and optimization of therapeutic outcomes. However, despite the significant advancements made in biomaterial-based gene therapy, several challenges remain. The translation of these delivery systems from preclinical studies to clinical applications requires rigorous evaluation of safety, efficacy, and scalability (Jang et al., 2011). Furthermore, the complex interplay between biomaterials and biological systems necessitates a comprehensive understanding of the immune response, biodistribution, and long-term effects of gene delivery vectors.

Addressing these challenges requires interdisciplinary collaborations between scientists, engineers, clinicians, and regulatory authorities (Smye & Frangi, 2021). In summary, biomaterials play a pivotal role in advancing the field of gene therapy by providing innovative and effective delivery platforms for therapeutic genes. The integration of biomaterials with gene delivery vectors has enabled precise control over key parameters, resulting in improved therapeutic outcomes and reduced side effects (Yang et al., 2020). Although further research is needed to address existing challenges and ensure successful clinical translation, the continuous development and optimization of biomaterial-based gene delivery systems hold great promise for the future of gene therapy (Yu et al., 2023).

**6.2) Future perspectives and outlook for bioengineered smart nano/biomaterials in gene therapy**

Gene therapy holds immense potential for the treatment of various genetic disorders and diseases. However, the successful delivery of therapeutic genes to target cells remains a significant challenge (McCain, 2005). Recent advances in bioengineering have led to the development of smart nano/biomaterials with enhanced properties for efficient and targeted gene delivery.

Bioengineered smart nano/biomaterials offer several advantages for gene therapy, including improved stability, enhanced cellular uptake, controlled release of therapeutic genes, and targeted delivery to specific tissues or cells (Yu at al., 2023). These materials can be designed to protect therapeutic genes from degradation, overcome biological barriers, and achieve efficient cellular internalization (Blanco et al., 2015). Furthermore, smart nano/biomaterials can be engineered to respond to specific stimuli, such as changes in pH, temperature, or enzymatic activity, thereby enabling controlled and site-specific gene expression (Hu et al., 2014).

Researchers are actively exploring various strategies to improve the design and functionality of smart nano/biomaterials for gene therapy. One approach involves the integration of targeting ligands on the surface of nanoparticles or biomaterials, enabling specific recognition and binding to target cells. Additionally, the incorporation of stimuli-responsive components, such as polymers, peptides, or small molecules, allows for triggered gene release and activation (Tracey et al., 2021). Moreover, the

combination of different types of biomaterials, such as lipids, polymers, and inorganic nanoparticles, can provide synergistic effects and multifunctionality to enhance gene delivery efficiency (Chen et al., 2023). Bioengineered smart nano/biomaterials hold great promise for a wide range of gene therapy applications. These materials can be used to treat genetic disorders, such as cystic fibrosis, muscular dystrophy, and hemophilia, by delivering therapeutic genes to target cells. Furthermore, they can facilitate the development of personalized medicine by enabling precise control over gene expression and regulation. Smart nano/biomaterials also have the potential to revolutionize cancer gene therapy, allowing for targeted delivery of tumor-suppressing genes or gene-editing tools (Sharma et al., 2016).

While the future of bioengineered smart nano/biomaterials in gene therapy appears promising, several challenges and considerations need to be addressed. These include safety concerns, immunogenicity, off-target effects, scalability of production, and regulatory approval. Rigorous testing and evaluation of these materials are necessary to ensure their biocompatibility, long-term stability, and lack of adverse effects (Han et al., 2022). Furthermore, ethical and societal implications surrounding gene therapy and the use of smart nano/biomaterials should be carefully considered and addressed.

Bioengineered smart nano/biomaterials have the potential to significantly enhance the efficacy and safety of gene therapy. Their unique properties enable precise control over gene delivery, targeting, and expression. With continued advancements in bioengineering, materials science, and gene therapy research, smart nano/biomaterials are poised to play a crucial role in revolutionizing the field of personalized medicine and providing novel therapeutic solutions for genetic disorders and diseases.

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