Nanotechnology Application in tissue regeneration and gene therapy

Ayano Shanko MD[1], Devinder Kumar[2], Dr Deepak Prashar[3], Nikita rawat[4], Pooja Negi[5], Susheel Sharma[6]

[1]Assistant Professor, Internal Medicine, College of Medicine and Health Sciences, Nigist Eleni Mohammed Memorial Comprehensive Specialized Hospital, Wachemo University, Hosanna, Ethiopia. East Africa

[2] Assistant Professor Department of Pharmacology, Amar Shaheed Baba ajit singh jujhar singh memorial college of pharmacy, Bela, Punjab, India

[3]PhD, Green Hills Pharmacy College, Solan HP India.

[4] Department of Pharmacy, Kukreja institute of pharmaceutical sciences, dehradun, Uttarakhand, India.

[5]Assistant Professor (PhD Scholar), Amrapali Institute of Pharmacy and Sciences, Shiksha Nagar, Lamachaur, Haldwani, Nainital, Uttarakhand, India.

[6]Department of Pharmacy, Kukreja institute of pharmaceutical sciences, dehradun, Uttarakhand, India.

Corresponding Author

Devinder Kumar

Abstract

There've been a number of studies done on the materials and how they can be used in modern medicine. Medical marijuana has a wide range of uses. The biocompatibility of nanotechnology has piqued the curiosity of scientists. Soft or hard, depending on the use, these materials may exhibit the so-called "quantum effect." As a result of this quantum event, precise control over protein adsorption and subsequent cellular interactions can be achieved. Nanoparticles are a hot topic in science right now because of their multiple applications. Semiconductor, metallic, and insulator-like nanoparticles are all possible. MONPs exhibit exceptional chemical and physical properties because of their small size and high density. Materials chemistry, agriculture, medicine, and catalysis are just a few of the domains in which metal oxide nanoparticles have been studied by researchers.

Keywords: Nano-Particle, Microbes, mammalian, DNA, Gene-therapy

Introduction

Nano-biomaterials are becoming a vital medicinal tool due to their compatibility and innovative effects. The use of biomaterials has enabled new applications in a wide range of sectors. Modern medicine requires the ability to deal with difficult and complex situations. Nanotechnology has aided in the development of novel viruses and tissue engineering. Important and potentially impactful issues were picked for this overview. Nanotechnology is a new field of study that focuses on manipulating materials at the smallest scale imaginable. Materials were nanomanipulated using biotechnology, genetic engineering, and other fields. Nanoscale biomaterials have proven to be a powerful tool for obtaining precise and smart features like medication delivery, a size-dependent effect, or a light-triggered feature.

This study will identify the most promising treatments and vaccines for COVID-19. The first step is to thoroughly explain nanostructures to narrow the range of probable nanomaterials. Then, administrative procedures are outlined to better comprehend materials and architecture. Two novel pharmacological targets are indicated as the key application difficulties. Tissue engineering has several applications in regenerative medicine, including the current COVID 19 pandemic. Material, medicine, geometry, and other features are included into the nano biomaterial cues to accomplish desired functionality.

One of the most cutting-edge fields in human health research is stem cell research. However, the lack of reliable means for monitoring differentiation and long-term survival of engrafted cells or tissues has limited their application. By combining nanotechnology and SC research, we intend to learn more about how unique SCs control themselves, leading to new therapy possibilities for human illness prevention and treatment. To help differentiate and regenerate stem cells, scientists have developed nanotechnology-based methods using polycaprolactone, Tri-CaPSO4, carbon nanofiber, graphene oxide nanoparticles, and auto-assembled peptides. Fe3O4 NPs could be employed to label grafted cells for MRI analysis (MRI). Differentiation, tracking, and monitoring of stem cell populations, notably human mesenchymal stem cells, have considerably benefited from polymer-based scaffolds (hMSCs). Nanopatterning is a newer stem cell nanotechnology approach. These nanopatterned coverings or forms may help direct pluripotent stem cell adhesion, spreading, self-renewal, and differentiation without specialist media or chemical checks.

Brief Background of Nanoparticles

In one dimension, nanoparticles can range in size from 1 to 100 nm in any shape. Because of their unusual size, these particles have features that can be attributed to bulk materials as well as molecular architectures. Thus, nanoparticles might be considered the "bridge" between the macro and micro scales. Because of their small size, they have a high surface-to-volume ratio, which is one of their most appealing inherent qualities. When nanoparticles are unbound, they move rapidly, resulting in a very slow sedimentation rate (Fig 1). The nature of these materials can range from soft to hard, depending on the application, and they may display the so-called "quantum effect." The surface energy of these particles may be completely manipulated via this quantum phenomenon, allowing for precise control of protein adsorption and subsequent cellular interactions.

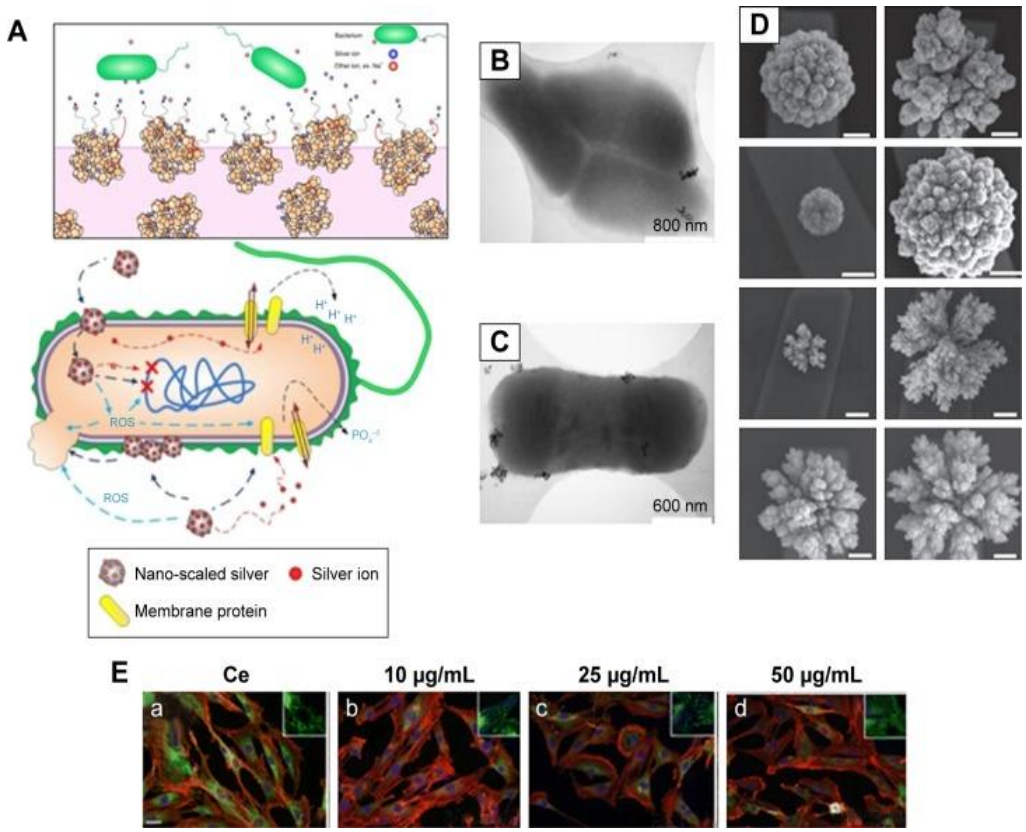


Fig 1: Microbes and mammalian cells interact with nanoparticles.

Notes : (A)Nanoparticles have been shown to affect bacterial cells in schematic form. E. coli interacts with cerium oxide nanoparticle samples at various magnifications, as shown in (B and C) representative electron microscopy photographs. (D) The production of programmable nanomaterials with GNPs under various deposition settings. Effects of CeO2 nanoparticles on the appearance and development of cardiac progenitor cells. A comparison of cells that have been exposed to 10 g/mL of CeO2, 25 g/mL, and 50 g/mL of CeO2 (d). Engineered cerium oxide nanoparticles affect bacterial growth and viability, American Society for Microbiology (A–C). Reprinted with permission. (D) Nanostructuring biosensors to alter their detection limitations. (E). Cardiac progenitor cells are protected from oxidative stress by nanoparticles of cerium oxide.

Depending on their shape, nanoparticles can be 0D, 1D, 2D, or 3D structured. These nanoparticles can be classified into groups based on their source or type of material. Nanoparticles can be made in both bottom-up and top-down ways. Pulsed laser, thermolysis, and solution combustion are chemical and biological syntheses, respectively. Other approaches include chemical, physical, and sol–gel. Depending on the substance, many methods can be used to characterise nanoparticles. The nanoparticles' morphology was studied using SEM, TEM, AFM, XPS, IR, XRD, Raman spectroscopy, Brunauer–Emmett–Teller (BET), and the Zeta size analyzer. Biological, electrical, optical, and other applications of nanoparticles are possible due to their unique properties. The use of nanoparticles in TE is still in its infancy, and is hampered by severe difficulties. Our purpose in this paper is to examine the usage of nanoparticles in TE and the associated issues, then discuss how these challenges might be overcome by using nanoparticles in more applications.

Some research shows therapeutic cells can anticipate transmitters during active medicine administration. SC therapies can benefit from drug-NP coatings on therapeutic cells to boost clinical efficacy. Any SC therapy should be able to study mobe (cell) transit and distribution to their biological targets. Using NPs with specific surface coatings can increase stem cell uptake and long-term monitoring. The impact of nanotechnology on TERM has resulted in more sophisticated and efficient systems. Other nanoscale technology items, such as nanofibers and nanopatterned surfaces, have been utilised to influence cell function in TERM. They can distribute numerous bioactive compounds, such as growth factors, to control stem cell destiny and morphogenesis, modify the mechanical strength of scaffolds for hard tissue applications, and reduce toxicity while boosting biocompatibility (Fig 2).

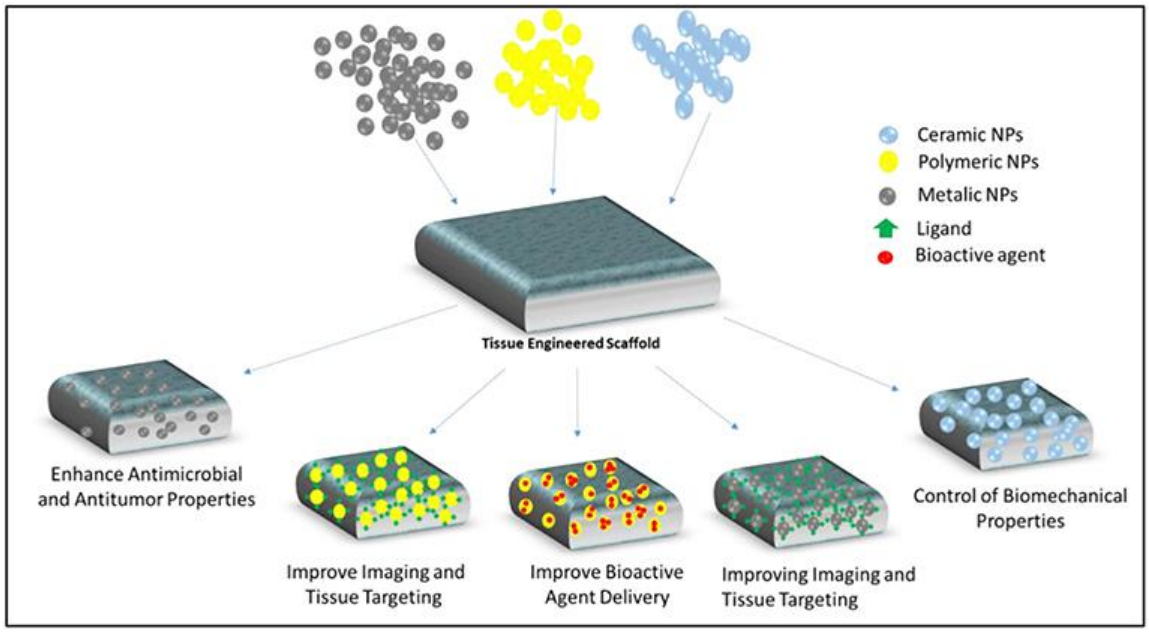


Fig:2 Different nanoparticles (ceramic, polymeric, metallic) can be used in TERM for a variety of purposes, including as tissue targeting and imaging, bioactive chemical administration, altering mechanical characteristics of scaffolds, giving antibacterial and anticancer capabilities.

Metal Nanoparticles

These nanoparticles have been studied extensively in medicine. No one was surprised when Faraday (1857) discovered metal nanoparticles in solution. Later, Kumar et al. (2018) investigated metallic nanoparticle colour and form. Currently, chemical groups that aid in antibody binding can be changed to improve nanoparticles. Noble metal nanoparticles (Ag, Au, Pt) have several biological applications, including anticancer and radiotherapy enhancement, antibacterial and antifungal diagnostics, and gene transport. The unique properties of noble metal nanoparticles add value. Metal nanoparticles can carry peptides, antibodies, RNA, and DNA, as well as biocompatible polymers like polyethylene glycol (Fan et al., 2018). Here are a few major nanoparticles and their biological uses. The ability to transport gold nanoparticles into target cells' nuclei aids in cancer detection. In this scenario, nanoparticles play a crucial role in biomedical applications.

Antimicrobial resistance in pathogenic microorganisms is a growing issue in medicine. Rai et al. (2009) investigated how antibacterial activity interacted with Ag nanoparticles. The antibacterial activity of amoxicillin, penicillin G, clindamycin, vancomycin, and erythromycin was increased by Ag nanoparticles. Keat et al. (2015) describe the use of Ag nanoparticles in biomedicine for cell imaging, cancer therapy, gene transfer, pharmaceutical delivery, and disease diagnostics. Ag nanoparticles have had a direct and indirect impact on the medical profession and health care system. Fellahi et al discovered antibacterial capabilities in silicon nano substrates with Ag or Cu nanoparticles (2013). They discovered improved antibacterial activity of produced nanoparticles against E. coli and want to examine this further. Cancer epithelial cells from the human lung were found to be biocompatible with Ag-coated silicon wires, but cytotoxic to Cu-coated Si nanowires. The researchers tried to enhance the quantity of Pd nanoparticles used in medical applications. Pd nanoparticles are anticancer and stabilising agents in medicines.

Nanoparticle research is currently a prominent issue in science due to its numerous applications. Nanoparticles can be semiconductor, metallic, or insulator-like. Due to their small size and high density, MONPs have remarkable chemical and physical properties. Scientists have been studying metal oxide nanoparticles in many fields, including materials chemistry and agriculture, as well as medicine and catalysis. CuO, ZnO, SnO_2, Al_2O_3, MgO, ZrO_2, AgO, TiO_2, CeO_2, etc. nanoparticles have been discovered to alter cell properties via structural modifications. A decrease in the number of surface and contact atoms causes strain or stress and structural changes. Size affects magnetic, conductive, chemical, and electrical properties of nanoparticles. The controllability of the size and shape of magnetic metal oxide nanoparticles has caught the curiosity of researchers. Smaller nanoparticle materials have better magnetic, electrical, and chemical characteristics. Materials scientists are researching iron oxide uses such as magnetic storage devices and MRI contrast agents. - At 55 nm, Fe_2O_3 nanoparticles are ferromagnetic, but 12 nm nanoparticles are superparamagnetic with no hysteresis. As particle size decreases, anisotropy reduces and superparamagneticity increases. To manufacture nanoparticles with the desired magnetic, electrical, and chemical properties, innovative and simple processes are required. The electrical properties (conductance) of oxides like SnO_2, WO_3, and In_2O_3 are highly reliant on particle size. The modulable electrical/ionic conductivity of TiO_2 materials is useful in optical, optoelectronic, and photovoltaic applications. TiO_2 decreases rapidly at high temperatures, affecting conductivity. 1-D ZnO with wurtzite-type structure offers a lot of potential in gas sensors and varistors. Due to their tiny size, nanostructured oxides have no long-range Madelung field influences.

For example, germanium (GeO_2) nanoparticles could enhance the performance of optical fibres and other optoelectronic devices. For new electrical or optical applications, these nanoparticles can be used in surface-catalyzed systems. Ga_2O_3 is now being researched for its potential in optoelectronic applications due to its huge band gap. Smaller nanoparticles have more surface area per unit mass, promoting chemical reactivity. CuO nanoparticles are used as redox and oxidation catalysts, photoconductive and photothermal applications, and microwave irradiation processes. As a scrubber material for gaseous pollutants (CO_2, CO, NOx, SOx) and a catalyst in organic synthesis in many chemical industries, MgO nanoparticles are widely employed. Because of its unusual structure, Al_2O_3 is used as a

support for active phases covered with other materials. These nanoparticles act as an electrolyte, catalyst, and gas detecting material. CeO2 nanoparticles are used in catalysis, gas sensing, electrochemistry, biomedical, and material chemistry. Photocatalysis for pollution removal, solar cells, and many more applications in materials and engineering use TiO2 nanoparticles. ZnO is a broad bandgap semiconductor material that has been extensively researched for its intrinsic properties, including UV-blocking in sunscreens and lotions, integrated varistors and solar cells, optoelectronics and gas sensors.

The structure, size, and shape of nanostructures influence MONP characteristics and applications. Many factors influence the structural and functional features of functional devices, affecting their performance. As an example, combining two different nano-oxides (p–n junctions or dopants) can result in poor optical or electrical properties. When creating MONPs with configurable structural parameters and properties, it is critical to optimise all process parameters that may impact the final product. The construction and performance of functional devices are affected by so many variables that it is impossible to reproduce the results.

Nanotechnology in gene therapy for musculo-skeletal regeneration

Musculoskeletal problems are the second greatest cause of disability globally, according to the WHO. Pathogenic and age-related disorders such osteoporosis, osteosarcoma, sarcopenia, ischemia, tendinitis, and trauma can cause such conditions, which are more common among athletes.

In the moderate and severe stages of MSDs, there are few good therapeutic outcomes with local or systemic medicine or surgery. Advanced therapeutic (AT) procedures like stem cell and gene therapy can improve clinical outcomes. Rather than depending on recombinant proteins, gene therapy could replace or correct faulty genes. Gene therapy can promote tissue regeneration and functional repair by infusing therapeutic genetic material into host cells. Gene therapy tries to change the expression of the disease-linked gene. Despite the fact that gene therapy is mainly used to treat inherited diseases like cancer, MSDs benefit from it (fig 3).

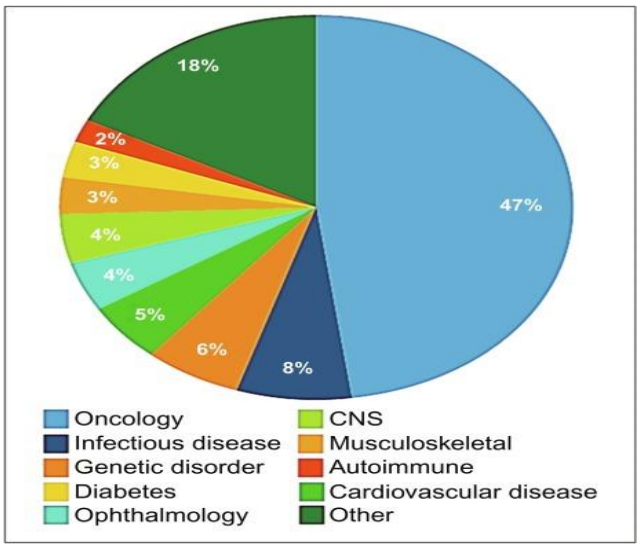


Fig: 3 Targeting therapeutic areas of advanced therapies (ATs) such as cell and gene therapy

In order to deliver therapeutic genes, such as deoxyribonucleic acid (DNA), messenger RNA (mRNA), small interfering RNA (siRNA), microRNA (miRNA), and antisense oligonucleotides (AONs), to the target cells and tissues, various ways must be used after the genetic target has been identified.. The genetic material must reach the intended cells, internalise the cell efficiently, and then integrate into the specified intracellular compartment regardless of the genetic material's kind or structure (in cytoplasm or nucleus). Ex vivo and in vivo delivery of genetic cargo to the target tissue or cell are both options (fig 4). To begin, the patient's own cells are taken from the patient's body, transfected in vitro, and then transplanted back into the patient. As a result, it requires a lot of time and resources (such as a GMP cell facility). To the contrary, in an in vivo technique, genes are directly delivered to the target tissue or cell by systemic or local injection. As a result of the possible extracellular impediments, such as enzymatic breakdown and immunological responses, unwanted protein interactions, and precipitation, it is usually less efficient to reach the intended location.

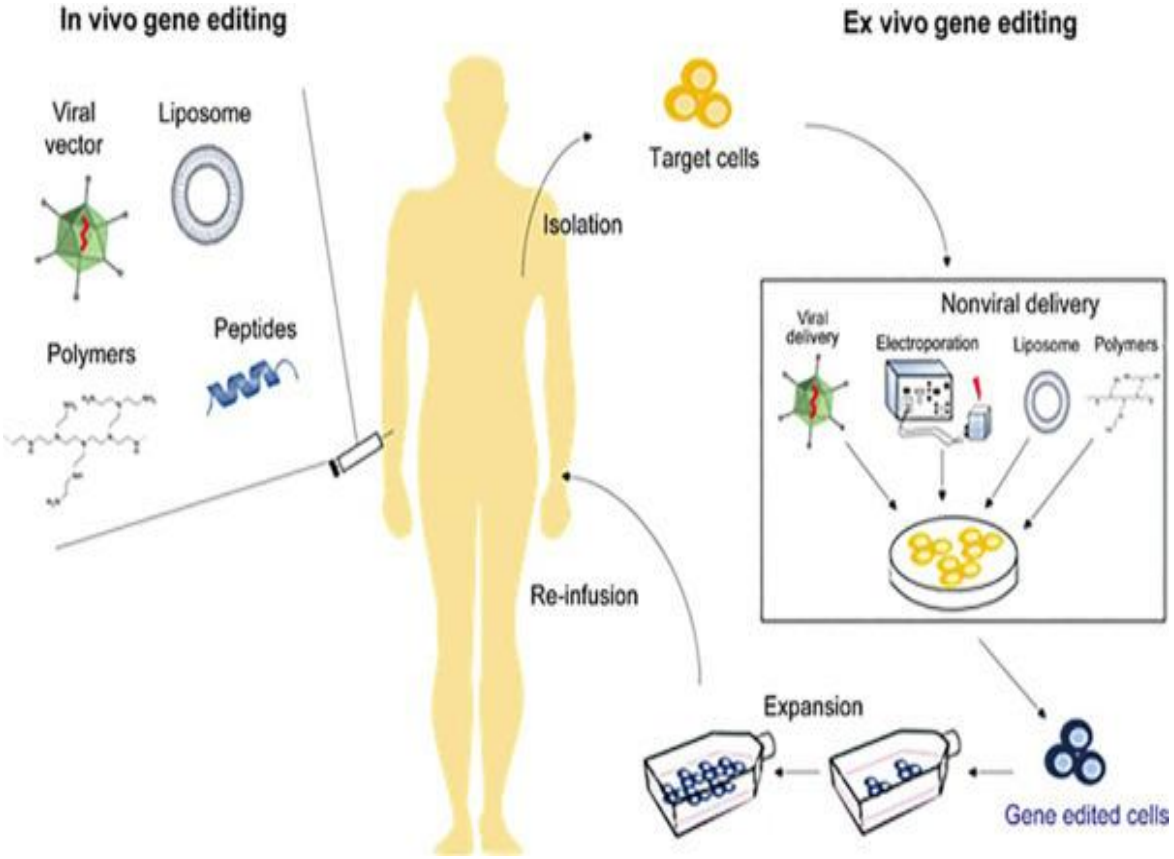


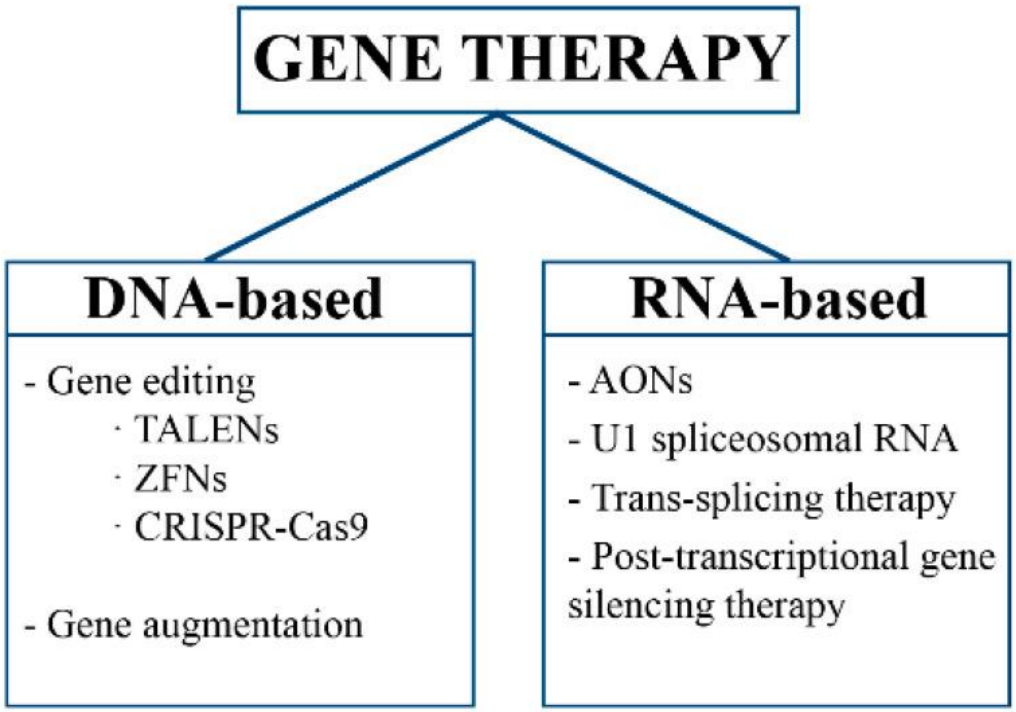
Fig:4 In vivo and ex vivo approaches to gene editing are depicted in this diagram. A viral or nonviral carrier could be used to deliver the therapeutic gene.

Nanotechnology in gene therapy

An experimental procedure known as gene therapy is used to treat or prevent disease by altering genes or gene expression. Cells can be given new abilities or their function can be restored in cases of monogenic diseases. Instead of relying on pharmaceuticals, gene therapy achieves its goals through editing, replacing, or modifying the expression of specific genes (Fig 5). In order to reach their target, gene therapeutics must overcome a number of

biological hurdles. As a first step, they must avoid degradation as they travel through the body. Because of the existence of endo- and exonucleases, oligonucleotides in physiological medium have a relatively limited half-life.

Fig. 5: Gene therapy nucleic acids.



Biological barriers must be crossed based on the organ or tissue targeted and the delivery method. Intravenous therapy is widespread (IV). To work, gene treatments must pass the endothelium. Organ and tissue characteristics differ. Blood flow to the spleen, liver, and tumours is fenestrated.

Neurological gene therapy is undergoing numerous studies. Brain gene therapy necessitates blood–brain crossover (BBB). The brain has an endothelial barrier. The tight junctions between endothelial cells keep poisons and germs out.

Now, 67.3 percent are for cancer. Cancer cells in solid tumours require a large extracellular collagen matrix to reach. High interstitial fluid pressures (IFPs) prevent drug uptake, especially high molecular weight pharmaceuticals like gene therapies. Gene therapies must penetrate through extremely complicated cellular membranes. Nucleic acids are very heavy. Their phosphate backbone makes them highly water soluble. As a result, they cannot pass biological membranes. Endenosis is the main intracellular pathway. Endosomes are intracellular vesicles that contain nucleic acid digestion enzymes. En route from early to late endosomes, nucleic acids' pH lowers. So gene therapies should be able to avoid low pH and catalytic enzymes as soon as possible. A silencing RNA works in the cytoplasm of cells. In contrast, DNA-based gene therapy needs penetration of the double nuclear membrane. Gene therapies urgently require a delivery strategy that can bypass endosome degradation and bridge membranes. To ensure gene therapy product safety and efficacy (Figure 5). Gene therapy is helpful for many illnesses, but lack of delivery vehicles keeps it out of clinic.

.

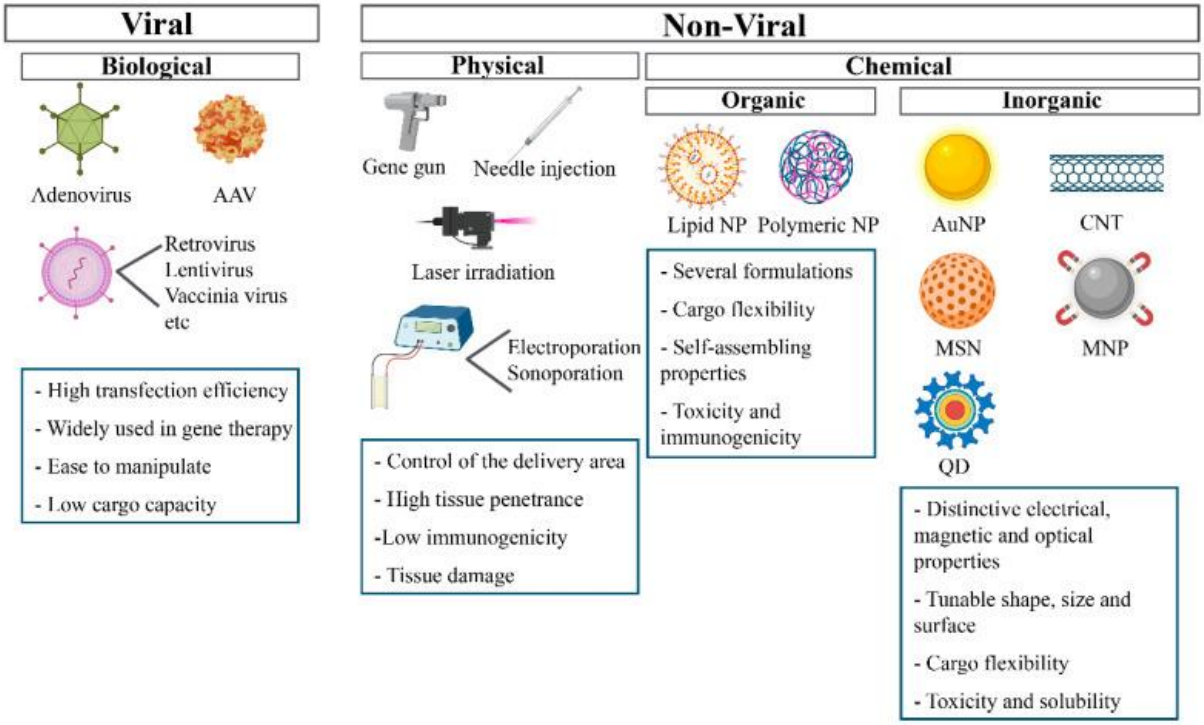


Fig: 6 This number includes viral and nonviral delivery methods. Adeno-associated virus (AAV). NP stands for nanoparticles. A CNT is a carbon nanotube. Magnetic nanoparticle (MNP) an MSN, a mesoporous silica nanoparticle Quantum dot (QD)

Conclusion:

We review recent advances in theranostics, an emerging discipline combining nanotechnology, gene therapy, and imaging techniques. Researchers in this discipline should remember three things. Preliminary physical-chemical properties of the nanobioconjugate. This characterization might facilitate probe design. Second, the new conjugation must be explained in detail. Once in tissue, nanobioconjugates should be continuously examined. New developments in nanoparticle manufacturing and functionalization are expected, allowing for greater material and synthesis diversity. New gene therapy delivery vectors have been developed. These include transgenic toxicity, immunological reactions, selective gene expression, and vector injection. New nanoparticle vectors and imaging tools will improve gene therapy. Finally, combining MRI and PET may improve diagnostic accuracy.

References

Henderson M.C., Azorsa D.O. High-throughput RNAi screening for the identification of novel targets. *Methods Mol. Biol.* 2013;986:89–95.

2. Venter J.C., Adams M.D., Myers E.W., Li P.W., Mural R.J., Sutton G.G., Smith H.O., Yandell M., Evans C.A., Holt R.A., et al. The sequence of the human genome. *Science.* 2001;291:1304–1351. doi: 10.1126/science.1058040.

3. Dang C.V., Premkumar Reddy E., Shokat K.M., Soucek L. Drugging the 'undruggable' cancer targets. *Nat. Rev. Cancer.* 2017;17:502–508. doi: 10.1038/nrc.2017.36.

4. Ginn S.L., Amaya A.K., Alexander I.E., Edelstein M., Abedi M.R. Gene therapy clinical trials worldwide to 2017: An update. *J. Gene Med.* 2018;20:e3015. doi: 10.1002/jgm.3015.

5. Vázquez-Domínguez I., Garanto A., Collin R.W.J. Molecular Therapies for Inherited Retinal Diseases-Current Standing, Opportunities and Challenges. *Genes.* 2019;10:654. doi: 10.3390/genes10090654.
6. LaFountaine J.S., Fathe K., Smyth H.D. Delivery and therapeutic applications of gene editing technologies ZFNs, TALENs, and CRISPR/Cas9. *Int. J. Pharm.* 2015;494:180–194. doi: 10.1016/j.ijpharm.2015.08.029.
7. Kim H., Kim J.-S. A guide to genome engineering with programmable nucleases. *Nat. Rev. Genet.* 2014;15:321–334. doi: 10.1038/nrg3686.
8. Naso M.F., Tomkowicz B., Perry W.L., Strohl W.R. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs Clin. Immunother. Biopharm. Gene Ther.* 2017;31:317–334. doi: 10.1007/s40259-017-0234-5.
9. Yin H., Kauffman K.J., Anderson D.G. Delivery technologies for genome editing. *Nat. Rev. Drug Discov.* 2017;16:387–399. doi: 10.1038/nrd.2016.280.
10. Yip B.H. Recent Advances in CRISPR/Cas9 Delivery Strategies. *Biomolecules.* 2020;10:839. doi: 10.3390/biom10060839.
11. Bai H., Lester G.M.S., Petishnok L.C., Dean D.A. Cytoplasmic transport and nuclear import of plasmid DNA. *Biosci. Rep.* 2017;37:BSR20160616. doi: 10.1042/BSR20160616.
12. Coutinho M.F., Matos L., Santos J.I., Alves S. RNA Therapeutics: How Far Have We Gone? *Adv. Exp. Med. Biol.* 2019;1157:133–177.
13. Garanto A. RNA-Based Therapeutic Strategies for Inherited Retinal Dystrophies. *Adv. Exp. Med. Biol.* 2019;1185:71–77.
14. Tanner G., Glaus E., Barthelmes D., Ader M., Fleischhauer J., Pagani F., Berger W., Neidhardt J. Therapeutic strategy to rescue mutation-induced exon skipping in rhodopsin by adaptation of U1 snRNA. *Hum. Mutat.* 2009;30:255–263. doi: 10.1002/humu.20861.
15. Berger A., Lorain S., Joséphine C., Desrosiers M., Peccate C., Voit T., Garcia L., Sahel J.A., Bemelmans A.P. Repair of rhodopsin mRNA by spliceosome-mediated RNA trans-splicing: A new approach for autosomal dominant retinitis pigmentosa. *Mol. Ther.* 2015;23:918–930. doi: 10.1038/mt.2015.11.
16. O'Reilly M., Palfi A., Chadderton N., Millington-Ward S., Ader M., Cronin T., Tuohy T., Auricchio A., Hildinger M., Tivnan A., et al. RNA interference-mediated suppression and replacement of human rhodopsin in vivo. *Am. J. Hum. Genet.* 2007;81:127–135. doi: 10.1086/519025.
17. Naessens S., Ruysschaert L., Lefever S., Coppieters F., De Baere E. Antisense Oligonucleotide-Based Downregulation of the G56R Pathogenic Variant Causing NR2E3-Associated Autosomal Dominant Retinitis Pigmentosa. *Genes.* 2019;10:363. doi: 10.3390/genes10050363.
18. Blanco E., Shen H., Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* 2015;33:941–951. doi: 10.1038/nbt.3330.
19. Tsui N.B., Ng E.K., Lo Y.M. Stability of endogenous and added RNA in blood specimens, serum, and plasma. *Clin. Chem.* 2002;48:1647–1653. doi: 10.1093/clinchem/48.10.1647.
20. Chiou H.C., Tangco M.V., Levine S.M., Robertson D., Kormis K., Wu C.H., Wu G.Y. Enhanced resistance to nuclease degradation of nucleic acids complexed to

asialoglycoprotein-polylysine carriers. *Nucleic Acids Res.* 1994;22:5439–5446. doi: 10.1093/nar/22.24.5439.

21. Sudhakar V., Richardson R.M. Gene Therapy for Neurodegenerative Diseases. *Neurotherapeutics.* 2019;16:166–175. doi: 10.1007/s13311-018-00694-0.

22. Berndt P., Winkler L., Cording J., Breitkreuz-Korff O., Rex A., Dithmer S., Rausch V., Blasig R., Richter M., Sporbert A., et al. Tight junction proteins at the blood-brain barrier: Far more than claudin-5. *Cell. Mol. Life Sci.* 2019;76:1987–2002. doi: 10.1007/s00018-019-03030-7.

23. Edelstein M. Gene Therapy Clinical Trials Worldwide. *J. Gene Med.* 2021;20:e3015

24. Henke E., Nandigama R., Ergün S. Extracellular Matrix in the Tumor Microenvironment and Its Impact on Cancer Therapy. *Front. Mol. Biosci.* 2020;6:160. doi: 10.3389/fmolb.2019.00160.

25. Heldin C.H., Rubin K., Pietras K., Ostman A. High interstitial fluid pressure—An obstacle in cancer therapy. *Nat. Rev. Cancer.* 2004;4:806–813. doi: 10.1038/nrc1456.

26. Kumari S., Mg S., Mayor S. Endocytosis unplugged: Multiple ways to enter the cell. *Cell Res.* 2010;20:256–275. doi: 10.1038/cr.2010.19.

27. Redpath G.M.I., Betzler V.M., Rossatti P., Rossy J. Membrane Heterogeneity Controls Cellular Endocytic Trafficking. *Front. Cell Dev. Biol.* 2020;8:757. doi: 10.3389/fcell.2020.00757

28. Dinis T.B.V., Sousa F., Freire M.G. Insights on the DNA Stability in Aqueous Solutions of Ionic Liquids. *Front. Bioeng. Biotechnol.* 2020;8:1207. doi: 10.3389/fbioe.2020.547857.

29. Bulcha J.T., Wang Y., Ma H., Tai P.W.L., Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduct. Target. Ther.* 2021;6:53. doi: 10.1038/s41392-021-00487-6.

30. Raper S.E., Raper S.E., Chirmule N., Lee F.S., Wivel N.A., Bagg A., Gao G.P., Wilson J.M., Batshaw M.L. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol. Genet. Metab.* 2003;80:148–158. doi: 10.1016/j.ymgme.2003.08.016.

31. McCormack M.P., Rabbitts T.H. Activation of the T-cell oncogene LMO2 after gene therapy for X-linked severe combined immunodeficiency. *N. Engl. J. Med.* 2004;350:913–922. doi: 10.1056/NEJMra032207.

32. Hacein-Bey-Abina S., Garrigue A., Wang G.P., Soulier J., Lim A., Morillon E., Clappier E., Caccavelli L., Delabesse E., Beldjord K., et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Investig.* 2008;118:3132–3142. doi: 10.1172/JCI35700.

33. David R.M., Doherty A.T. Viral Vectors: The Road to Reducing Genotoxicity. *Toxicol. Sci.* 2016;155:315–325. doi: 10.1093/toxsci/kfw220.

34. Wang D., Tai P.W.L., Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat. Rev. Drug Discov.* 2019;18:358–378. doi: 10.1038/s41573-019-0012-9.

35. Eggenhofer E., Doenecke A., Renner P., Slowik P., Piso P., Geissler E.K., Schlitt H.J., Dahlke M.H., Popp F.C. High volume naked DNA tail-vein injection restores liver function in Fah-knock out mice. *J. Gastroenterol. Hepatol.* 2010;25:1002–1008. doi: 10.1111/j.1440-1746.2009.06156.x.