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Review

Recent Updates on Diverse Nanoparticles and Nanostructures in Therapeutic and Diagnostic Applications with Special Focus on Smart Protein Nanoparticles: A Review

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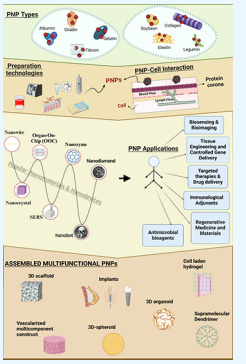
ABSTRACT: Nanomedicine enables advanced therapeutics, diagnostics, and predictive analysis, enhancing treatment outcomes and patient care. The choices and development of high-quality organic nanoparticles with relatively lower toxicity are important for achieving advanced medical goals. Among organic molecules, proteins have been prospected as smart candidates to revolutionize nanomedicine due to their inherent fascinating features. The advent of protein nanoarchitectures, which explore the biomolecular corona, offers new insights into their efficient tissue penetration and therapeutic potential. This review examines various animal- and plant-based protein nanoparticles, highlighting their source, activity, products, and unique biomedical applications in regenerative medicine, targeted therapies, gene and drug delivery, antimicrobial activity, bioimaging, immunological adjuvants, etc. It provides an extensive discussion on recent applications of protein nanoparticles across diverse biomedical fields as well as the evolving landscape of other nanoproducts and nanodevices for sensitive medical procedures. Furthermore, this review introduces different preparation technologies of protein nanoparticles, emphasizing how their design and construction significantly influence loading capacity, stability, and targeting effects. Additionally, we delve into the construction of different user-friendly multifunctional modular bioarchitectures by the assembly of protein nanoparticles (PNPs), marking a significant breakthrough in therapies. This review also considers the challenges of synthetic nanomaterials and the emergence of natural alternatives, which provides insights into protein nanoparticle research.



1. INTRODUCTION

Technology is still maturing by continuing research and inventions. Medical advancements are crucial from disease diagnosis to periodic post-treatment check-ups. The branch proteomics has identified the structure, functions, analysis, and regulation of various proteins, and it is now integrated with other technologies to enhance clinical implementation.[1] Nanotechnology is one such clinical implementation that is aimed at improving patient outcomes. The combination of proteomics with nanotechnology has paved the way for the emergence of effective and patient-specific medical tools. Such modifications will enhance the physicochemical properties of proteins like penetration ability, signal transduction, stability, specificity, enzyme catalysis, etc.[2] As proteins are macromolecules that impart various physiological regulations and biological functions to the body, they can be an active candidate in making potential biological materials or devices at the nanoscale. However, the limitation of the half-life of proteins due to their higher risk of denaturation makes them unstable to use in various biomedical applications. Like proteins, peptides have also been widely applied in nanobiopharmaceutical products. Both are made of amino acids of different sizes and have tremendous merit over other nanomaterials. The better specificity, lower toxicity, advanced hydrophilicity, targeted delivery, versatility, reduced complications, high drug binding efficiency, nonimmunogenicity, ease of availability, biocompatibility, biodegradability, improved pharmacokinetic profile, and potency of α-helix, β-sheet structures of proteins to form unique nanostructures offer more exposure to protein nanostructures.[3] The first FDA-approved nanomedicine called Doxil for the treatment of AIDS-associated Kaposi's sarcoma and the first FDA-approved RNAi therapy called patisiran laid two important milestones in clinical translation. By rectifying the limitations like inefficient permeation, rapid clearance, and accumulation by recombinant protein technologies, protein nanoparticles (PNPs) offer promising solutions for various nanomedicine applications.[4] This review focuses on the role of natural proteins in medical nanotechnology, exploring their interactions with the body and the methods employed to develop PNPs, highlighting their advantages, limitations, and examples of successful applications. We also offer a thorough discussion on nanotechnological applications of proteins in therapeutic and diagnostic uses, highlighting trends and identifying gaps in their respective fields.

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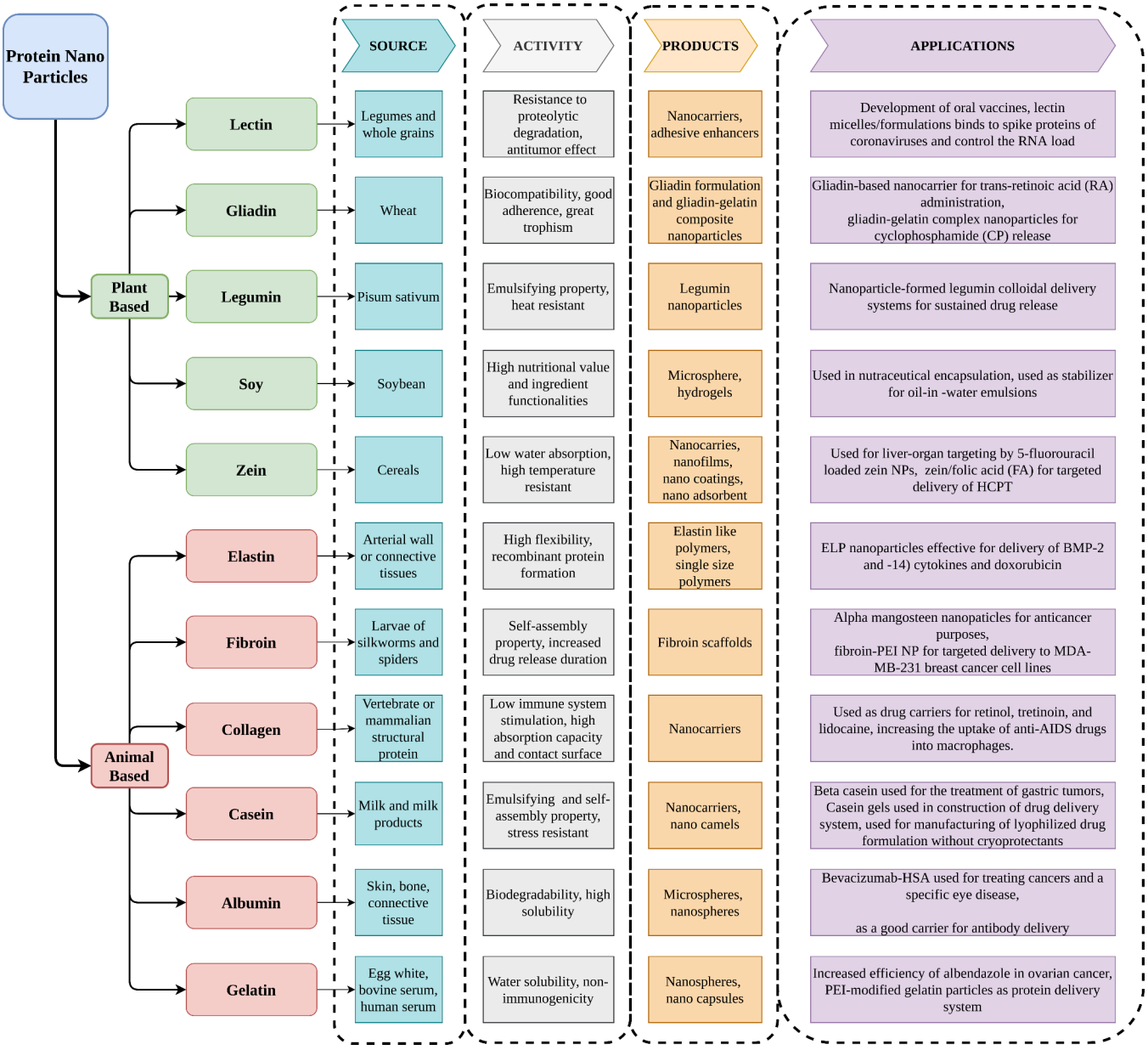

Figure 1. Different activities and applications of each protein according to its source of origin and nature of nanomaterial[6−10] (created with draw.io).

We emphasize diverse nanomaterials or devices to present an overview of the advanced technologies in nanomedicine and examine the era of multifunctional protein assemblies in the evolving field of nanomedicine. We summarize the challenges of synthetic nanoparticles and highlight the emergence of natural protein nanoparticles as a safer and more effective alternative in nanomedicine.

2. DIFFERENT TYPES OF PROTEIN NANOPARTICLES IN DRUG DELIVERY

The treatment of any disease is associated with diagnosis, proper prescription of drugs by clinicians, and stable association of drugs at the correct target sites. With drug delivery being an important step in treatment, there is a high demand for efficient delivery mechanisms. The sort of nanoparticles in this field can bring about smart methods of drug delivery. Synthesis of these particles in the nanoscale using nucleic acids, polysaccharides, lipids, proteins, etc., in drug delivery is very common nowadays because of their enhanced solubility, stability, theranostic ability, prolonged release, and efficient targeted site delivery. Among these, PNPs became a more popular and stronger candidate in drug delivery systems. There are natural protein nanoparticles of sizes ranging from 1 to 100 nm from both animal and plant sources. Some examples of protein nanoparticles derived from animal origins are gelatin, albumin, casein, collagen, fibroin, and elastin. Plant-derived protein nanoparticles include zein, soybean, legumin, gliadin, and lectin[5] (Figure 1). Different materials produced from these proteins have made a revolution in the clinical field.

3. UNDERSTANDING OF BIOMOLECULAR/PROTEIN CORONA ASSOCIATED WITH NANOTECHNOLOGY

After entering the biological system, nanoparticles interact with the biomolecules in the body and will give rise to a layer called the "corona". This happens within seconds of nanoparticle exposure by a process called the Vroman effect. This layer

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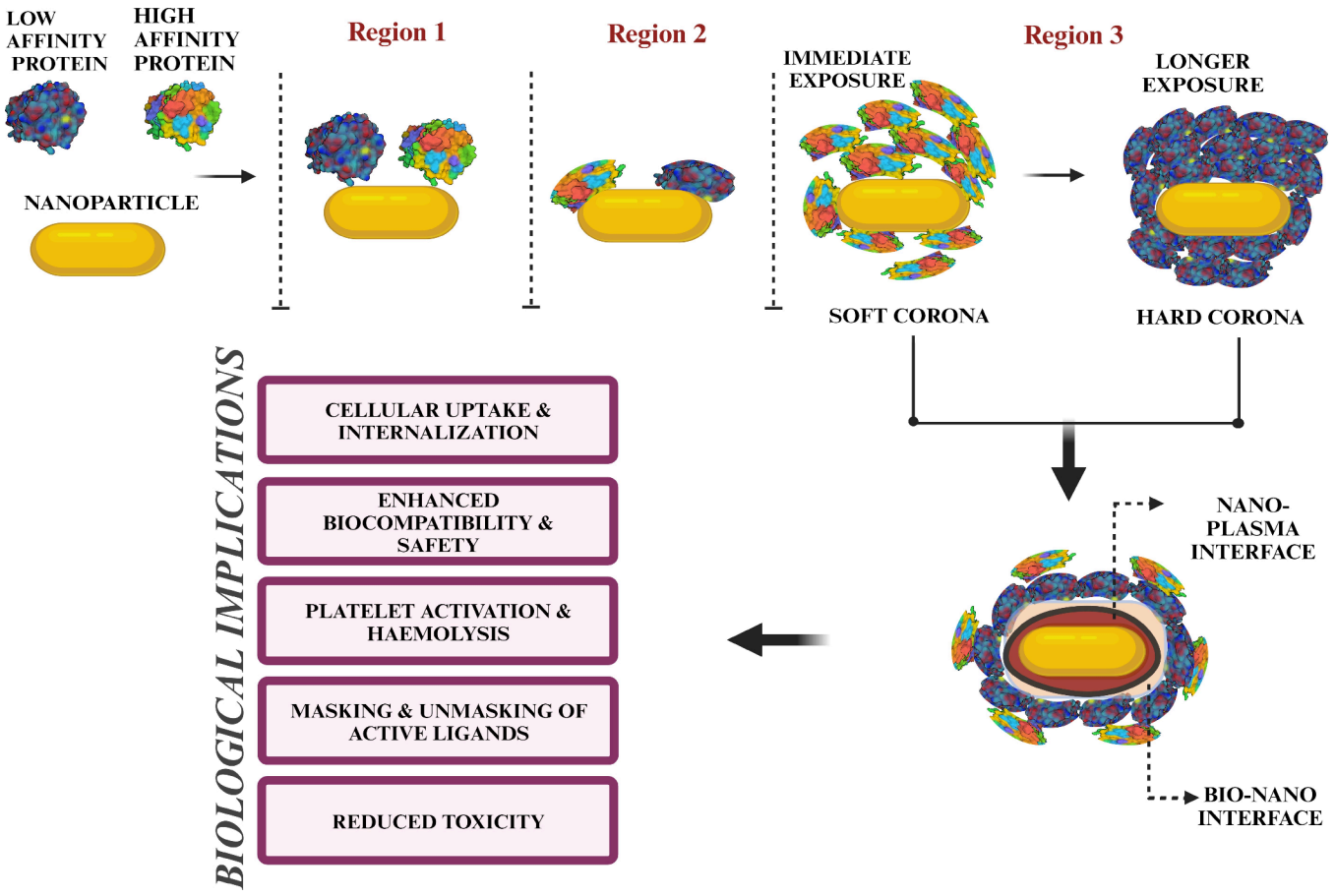

Figure 2. Diagrammatic representation showing the adsorption mechanism of protein to nanoparticles (created with BioRender.com).

consists of many biomolecules with increased adsorption and enhanced properties compared to those in the normal biological environment. Thus, this has a high impact on the ultimate performance of these nanoparticles. The liquid profile and protein composition of this corona layer have been extensively studied with respect to various works. According to the adsorption rate of proteins to their surface, the corona can be classified into two main categories: a hard corona and a soft corona. High affinity proteins tightly and directly associated with nanoparticles will form a hard corona, and low affinity proteins loosely and indirectly associated with nanoparticles will form a soft corona. While taking this into consideration, a hard corona is more convenient in terms of nanotechnology. The structure and composition of the corona layer can be studied by various techniques, such as DLS, SEC, DCS, TEM, PAGE, LC-MS/MS, DC, SPR, ITC, etc. Parameters like the number of bonds, thermodynamic feasibility of interaction, rearrangements of molecules, temperature, conformational changes, solvation, influence of other proteins, etc., determine the nanomaterial–protein adsorption effect.[11] Generally, there are 3 steps in the protein adsorption to nanoparticles, especially for gold nanoparticles (AuNPs) (Figure:2):

- Region 1: characterized by protein–NP surface interaction only with original protein conformation.
- Region 2: characterized by protein–protein interactions and protein–NP interactions with changes in its structural conformations.
- Region 3: characterized by protein–protein interactions only, along with structural alterations and protein multilayer formation.

The composition of the corona is formed by different types of plasma proteins, viz., immunoglobulins, tissue leakage proteins, complement factors, coagulants, lipoproteins, and other serum components. They are collectively called "adsorbomes" after the adsorption mechanism. They deal with specific functions such as antigen recognition, transport of biomolecules and ions, complement activation, and agglutination. A few examples of such adsorbomes are keratin, plasminogen, albumin, kininogen, fibrinogen, haptoglobin, vitronectin, and complement factors like C3, C4, etc.[11,12] The adsorption will lead to the formation of two interfaces called the nanoplasma interface and the bionano interface. The nanoplasma interface will help in the size change, permeability, condensation, and interactions of NPs. The bionano interface mainly includes dynamic physiochemical interactions and interchanges in organelles, membranes, endocytic vesicles, etc. Corona formation directly and indirectly helps in different biological activities like cellular uptake, hemolysis, reduced toxic exposure, and activation of platelets.[13]

4. PREPARATION METHODS OF PROTEIN NANOPARTICLES

The design and construction of nanoparticles highly influence loading capacity, stability, and targeting effect. PNPs are made of naturally occurring or engineered proteins and have to induce phase separation from the solution. Various physicochemical methods that initiate this separation include self-assemblage,

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Table 1. Preparation Technologies of Protein Nanoparticles[10,14−24]

method	PNP involved	advantages	disadvantages	achievements
self-assemblage	zein, albumin	in vivo gene editing, tailored smart delivery systems, cancer therapy, high molecular precision	ineffective for weak electrostatic interactions	AstraZeneca COVID vaccine, zein-PEG micelles, albumin-octaldehyde core−shell nanomicelle
denaturation	bovine serum albumin (BSA)	easy encapsulation of hydrophobic drugs, good stability, and long-term storage	uncontrollable particle size, loss of bioactivity	minor toxicity and sustained release of 2,6-divanillylidene cyclohexanone (DVH)-BSA nanoparticles
cross-linkage	albumin HRP/GOX	increased cellular uptake and hydrophilicity, better chemical flexibility	possibility of denaturation due to surfactants used and resulting nonfunctionality	BSA nanocapsules
hydrophobic assemblage	BSA	increased drug uptake, used for intracellular delivery of drugs	risk of protein denaturation due to the lack of common solvent, polymers can cause nondegradability and cellular toxicity	polymethyl methacrylate (PMMA)-BSA nanoparticles
coacervation	zein, casein	enhancement in oral bioavailability, simple to implement, high loading capacity, low processing costs, recovery of native proteins after particle disassembly	difficult to control morphology, cross-linker toxicity, causes aggregation, centrifugation is required for separation, needs surface charge for stability attainment	gitoxin, quercetin
desolvation	β-lg, *N*-acetyl cysteine modified bovine serum albumin, legumin	good mucus penetration, cellular uptake and bioavailability, superior mucoadhesion	large amounts of organic solvents are required, toxicity by harmful cross-linkers	anthocyanins, curcumin
emulsification	dextran, zein, lactoferrin	stable in simulated gastric fluid and improved antitumor activity, high dispersibility	uncontrollable particle size, need for large protein quantities	insulin, gambogic acid
nanospray drying	zein	superior pharmacodynamics, fast processing time, smoother surface, and uniform size distribution	risk of unnecessary protein degradation by shear forces, particle collision during the drying phase	praziquantel with HSA
nanoparticle albumin-bound (NAB) technology	HSA, lactoferrin	minimize protein denaturation, less toxicity, long-term storage, no external cross-linking required	needs suitable stabilizing agents with natural sulfhydryl and/or disulfide cross-linking groups	Abraxane (nab-paclitaxel) in anticancer treatment
electrospraying	zein	high encapsulation and loading efficacy, does not require a tedious separation process	requires high voltage for spraying, dependent on structure and interactions associated with proteins	curcumin, tamoxifen, β-carotene, resveratrol-loaded zein-chitosan nanocarriers
electrohydrodynamic (EHD) co-jetting	human serum albumin (HSA), human serum transferrin (hTf)	preservation of protein biological activity and structure, fabrication of multicompartmental protein particles, enhanced tunability	limited throughput, complex setup	preparation of synthetic protein nanoparticles (SPNPs) for delivery of RNAi-based therapeutics
gelation	soy, α-lactalbumin	enhanced intestinal transport, no organic solvent-associated toxicity	not suitable for heat-sensitive drugs	vitamin B12
supercritical antisolvent process	zein	allows greater control over particle size, reduced solvent presence in the yield	specific and expensive setting	riboflavin, δ-tocopherol, and β-carotenein zein vitamin complex microcapsules
flash nanoprecipitation	BSA, gelatin	enhanced tumor regression, less toxicity	ineffective for hydrophilic drugs	sorafenib, chitosan-sodium caseinate-polyelectrolyte nanocomplex for nutraceutical encapsulation
antisolvent salt precipitation	zein, gliadin	improved bioaccessibility and stability, high encapsulation efficiency	a large amount of organic solvent is used for preparation, optimized fabrication conditions needed	resveratrol
built-in ultrasonic dialysis process (BUDP)	zein	smaller particle size and narrower size distribution, no need for additional organic solvent separation	physical instability upon storage, agglomeration	polydopamine-coated zein-indomethacin particles for colon-targeted drug delivery
microfluidic technology	HSA	consistent flow rate and high mixing efficiency, good repeatability	chance of clogging, expensive manufacturing	cabazitaxel-HSA NPs

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denaturation, cross-linkage, hydrophobic assemblage, coacervation, dissolution, emulsification, nanospray drying, nanoparticle albumin-bound (NAB) technology, electrospraying, electrohydrodynamic (EHD) co-jetting, gelation, supercritical antisolvent process, flash nanoprecipitation, antisolvent salt precipitation, built-in ultrasonic dialysis process (BUDP), and microfluidic technology (Table 1) and have led to significant advancements in the structure of PNPs.

5. RECENT APPLICATIONS OF PROTEIN NANOPARTICLES IN THE BIOMEDICAL FIELD

The wide application of protein nanoparticles in the therapeutic field is mainly due to their large surface areas, high diffusion rate, rapid absorption, controlled release, controlled particle size, and surface characteristics. Not only protein nanoparticles but also other biopolymers like polysaccharides, lipids, polypeptides, nucleic acids, etc., are used in the biomedical field.[25]

5.1. Regenerative Medicine and Materials. Several degenerative disorders need proper attention and medication that aim to repair damaged and disorganized tissues. Thus, regenerative medicine has a high demand for curing such diseases. The better interaction of the material surface and body is to be considered more, and hence, the protein intervention of nanotechnology will contribute for better interaction than synthetic materials. Nanostructured surfaces, nanodelivery materials, nanocoatings, nanofiber scaffolds, etc., are various tissue engineering methods involving nanotechnology and regeneration.[26] Both in vivo and in vitro methods of living tissue regeneration had effective cures in the biomedical field. Many protein nanoengineered material surfaces are used in the manufacture of intervertebral discs, tendons, nerves, etc. The special features such as cell adhesion, proliferation, and differentiation of nanoparticles have aided cellular behavior. Particularly, in nerve tissue engineering, using 4D bioprinting technology, PU/gelatin-based self-healing hydrogels were developed. Also, in a novel branch called corneal tissue engineering, natural cornea was replaced by an *o*-nitrosobenzaldehyde group (NB)-modified gelatin (GelNB) layer.[27] Bioactivity, cell adhesion, calcium deposition, etc., of protein nanocoatings have wide functionalization in bone regeneration treatments. Delivery system nanomaterials, including microcapsules, micelles, dendrimers, etc., have facilitated the development of drug carrier systems. In addition to that, nanofiber scaffolds mimic damaged tissues and will trigger the colonization of cells.[28] Some of the notable PNP contributions include bioink-based 3D scaffold construction that gained great attention for the differentiation of neural stem cells (NSCs) by bioprinted NSCs in a PU/SPI hybrid hydrogel, angiogenesis by VEGF-loaded PCL NPs,[29] development of bone grafts by collagen-infilled 3D printed scaffolds, treatment of dermatological ailments by silk fibroin film, and used as bioinks for hydrogel 3D printing. The collagen-based scaffolds Integra, Collaplug, and Ultrafoam have wide skin and bone regeneration applications.[30]

5.1.1. Advantages. PNPs possess known cell-binding sites that help in the regeneration of desired tissues, thus facilitating cartilage formation[30] and having the possibility of high-resolution 3D printing without organic solvent.[31] Specifically, collagen proteins are reported to induce regeneration and bone remodeling by stromal cell differentiation and shall be utilized as osteoid in the mineralization process. Collagen became an important candidate in regenerative medicine because of its capacity to form fibrils with high tensile strength.[32]

5.1.2. Limitations. There exists difficulty in tailoring porosity and mechanical properties in 3D scaffolds, as well as limited supply, high cost, and the chance of pharmaceutical agent degradation during 3D printing.[31] Collagen particles still suffer from a high rate of degradability and low mechanical strength, but this gap is covered by the use of collagen with bioactive glass nanofibers, which forms a biomedical device that mimics bone composition.[32]

5.2. Antimicrobial Bioagents. Protein nanoparticles can be also incorporated into antiviral drugs. Several adjuvants, such as viral glycoproteins, toll-like receptors, or protein fractions, are used as carriers for vaccines like Inflexal V and Epaxal for hepatitis A and influenza treatment, respectively.[33] Also, monoclonal antibodies report the capability to target microbial phenotypes. Examples include 3E9-11, which targets the O25b O-antigen of the *Escherichia coli* ST131 O25b:H4. The emergence of caplacizumab, a bivalent nanobody with efficiency in the treatment of thrombotic thrombocytopenic purpura, proved its efficacy to target microbial strains.[34] Antimicrobial activity has been shown by LYZ−AuNCs (lysozyme−gold nanoclusters), a popular metal nanocluster, against antibiotic-resistant *Acinetobacter baumannii* and *Enterococcus faecalis* with better interaction. Another MNC bioagent, papain-protected CuNCs, has demonstrated significant results in treating wound infections. *Staphylococcus aureus* associated infection in the mice model has been reduced by albumin-based ciprofloxacin@R-BSA-S treatment because of its enhanced antibiofilm activity.[35,36] From a study, ketoconazole-loaded lecithin−zein NPs have been designed to treat skin fungal infections.[37]

5.2.1. Advantages. Nanobodies have robust structure, high stability, antigen-binding affinity, cognate target specificity, water solubility, and reversible refolding. Hydrophobic drugs like curcumin can be encapsulated through adsorption to hydrophobic regions of the proteins, resulting in increased drug bioavailability, less toxicity, and protection against high temperatures.[18] The glycoconjugated *Helicobacter pylori* ferritin has been reported as an efficient carrier protein for antimicrobial glycoconjugate vaccine development by offering peptidic T-cell epitopes to the immune system, by improvement of the protective response.[38] In another study, ZE-Ag was confirmed as an enhanced antibacterial nanocomposite against *E. coli* and *S. aureus*.[39] By the use of PNPs, the burden of antimicrobial resistance can be lightened.[40] Cu-BSA-NPs showed better antibacterial activity by blocking the synthesis of peptidoglycan.[41]

5.2.2. Limitations. There exists the possibility of microbial contamination in fibroin-based hydrogels. Silk fibroin hydrogels lack mechanical strength and reduce the ability of drug carrying due to pH-related limitations.[42] Another limitation observed is for albumin nanoparticles, which have versatile application in this field, but some problems like denaturation during the preparation process, reaction of albumin with other body proteins, structural stability issues, etc., still need to be addressed.[43]

5.3. Targeted Therapies. Drug delivery is a risky strategy in biomedicine, so improved targeted therapies are employed in the fields of chemotherapy, radiotherapy, photodynamic therapy, photothermal therapy, etc. The two targeting strategies of therapeutic nanoparticles in chemotherapy include active and passive targeting. These are correlated and used for effective drug delivery to enhance the immune system and suppress tumor growth factors.[33] VLPs (virus-like particles) and CPs (caged proteins) can be efficiently utilized against cancer cells as

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nanocarriers. The use of antibacterial, antifungal, and antiviral drugs in the treatment of infection has negative effects like resistant strains, toxicity, etc. These can be reduced by combining them with a nanodelivery system. Lipoquin, amphotericin B, Inflexal, and Epaxal are a few nanodrugs used for infectious microbial treatment. Amoxicillin-loaded gliadin NPs were utilized for the elimination of *Helicobacter pylori*.[32] An HIV vaccine made from VLPs is also used in the treatment of autoimmune diseases like AIDS[33] and rheumatoid arthritis, accelerating this field. The inflamed tissues are targeted, which creates strong long-lasting immunity with reduced undesired effects, of which the nanoformulation of CZP (certolizumab pegol) derived from a monoclonal antibody fragment is an example. PTX-loaded bovine serum albumin (BSA) nanoparticles were used for targeting human prostate cancer cell lines.[44] Abraxane is an albumin-based nanoparticle drug that helps to initiate paclitaxel delivery. Apart from that, the therapies that take advantage of nanomedicine are those treatments related to cardiovascular diseases, ocular diseases, pulmonary diseases, neurodegenerative diseases, tissue degenerative diseases, etc.[45] Protein replacement therapy for the treatment of Gaucher's disease by administration of enzyme β-glucocerebrosidase (GCase) into VLPs marked another promising result.[18]

5.3.1. Advantages. BSA-propolis NPs (propolis-loaded targeted albumin NPs) showed cell death in both MCF-7 and A549 cells and have been utilized in targeted therapies for breast and lung cancers by cellular mucoadhesion, penetration, and the inhibition of cyclin D1.[46] Luteolin-loaded zein nanoparticles were reported to improve the bioavailability, efficacy, and antioxidant and apoptotic activity against SW480 colon cancer cells.[47]

5.3.2. Limitations. Using the surfactants and organic solvents can cause protein denaturation, and the need for a cross-linking step to stabilize the particles may reduce their degradability. This hinders the effectiveness of BSA in mixed BSA-antibody/ROS nanoparticles intended for tumor growth inhibition.[18] In the case of zein, the amount of payload is limited and may not been sufficient for tumor treatment.[48]

5.4. Tissue Engineering and Controlled Gene Delivery. PNPs are polymer-based colloidal protein nanocarriers for drug or gene delivery. For tissue engineering and delivery purposes, some features such as responsiveness and functionalization need to be taken care of. Protein nanoparticles with these features can be designed in such a way that they can be used for controlled assembly or delivery. Apart from these, they can be used for cell patterning, DNA transfection, and viral transduction. The proper selection of such NPs should be made by considering enhanced mechanical, biological, and electrical properties. This will further help in a high rate of cell proliferation, tissue regeneration, improved conductivity, etc.[49] Many protein categories like therapeutic genes, growth factors, noncoding RNAs, anti-inflammatory factors, etc., are used as delivery agents. A chitosan hydrogel-based system for osteoarthritis gene therapy was reported to be successful in repairing rabbit cartilage injuries.[50] PAMAM dendrimers are regarded as protein carriers of RNase A required for cervical cancer treatment.[51] HSA−protamine sulfate−DNA ternary particles (AlPrO) are successful nonviral gene delivery vehicles for oligonucleotide administration. Virus-based gene therapy, particularly for adenoviruses, is examined to be delivered by silk−elastin-like protein polymers (SELPs) for treating solid tumors.[52] Gene delivery is very important in gene therapy, mainly for diseases like AIDS and cancer. The defective genes of patients are corrected by the proper transfer of encapsulated genetic materials or gene elements to a particular site. Examples such as various patches, medical device coatings, time-release drugs, and wound dressings made of nitric oxide (NO) stand out in this context. Thus, the tissue engineering−gene delivery combination can be considered as a new paradigm for regeneration medicine.[25]

5.4.1. Advantages. PNPs are reported to have good ability for proliferation in comparison to other polymers and good biocompatibility for the growth.[53] Particularly, silk fibroin (SF)/TiO_2 and SF/TiO_2−F nanocomposite scaffolds with interconnected pores were found to be appropriate for bone tissue engineering because of the proliferation of osteoblast cells and the regeneration of the bone tissue.[54] In another work, the phosphatase and tensin homologue (PTEN) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) genes loaded to zein nanoparticles (ZNPs) were found to be a potential antitumor vector for gene therapy for hepatocellular carcinoma. Its nonviral nature, controlled release properties, self-assembling nature, and ability to protect and deliver genetic material into specific cells present a promising avenue for zein NPs.[55]

5.4.2. Limitations. Successful implementation of tissue scaffolds depends on the choice, thickness, and porosity, but the pore size and porosity of nanocomposite scaffolds depend on the content of nanoparticles.[54] Immunogenicity of zein NPs needs to be evaluated concerning the size and dosage, while their extraction and purification may influence characteristics and performance ability due to the composition and the presence of other substances.[56]

5.5. Biosensing. Biosensing is a diagnostic technique that incorporates both biological and sensory aspects that work as a combined system. This has important applications in the field of biomedicine. A biosensor carefully detects a biological compound (analyte) in a sample by converting it to different signals. Various parts of a biosensor include bioreceptors, transducers, amplifiers, electronic detectors, etc. According to the nature of analyte detection, there are different types of biosensors like immunosensors, DNA sensors, tissue-based sensors, magnetic biosensors, thermal sensors, piezoelectric sensors, optical biosensors, etc.[57] The influence of nanotechnology in biosensors will increase the sensitivity, specificity, selectivity, accuracy, and surface area of the system.[52] Different noble metal nanoparticles, nanomaterials, nanotubes, etc., are used for application. However, PNP modification and functionalization have caused wide applications in biocatalysis and biosensing. Qβ, HBV, Fd, MS2, and M13 are commonly used VLPs as biosensors. Nanocages like E2, lumazine synthase, reencapsulin, ferritin, and encapsulin are some of the enzyme-derived PNPs used as biosensing PNPs. These are possible by chemical conjugations, genetic fusions, enzymatic ligation, and cargo encapsulation. It is reported that engineered ferritin PNPs showed a 5-fold enhancement in the image resolution of magnetic resonance imaging (MRI).[58] Bioluminescent *Escherichia coli* strains have assessed mercury contamination in water by the production of protein luciferase (reporter protein) in a particular manner as the detecting signal.[59] BSA-RuO_2NPs are considered as sensitive biosensors to monitor the H_2O_2 secreted from living cells.[60] Also, glucose sensing done by a protein−metal ion system called Con A and GO_x was reported to have high sensitivity and adaptability.[2] A novel wearable SF-based SERS biosensor has been introduced in a recent study, which

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allows non-invasive and label-free monitoring of biomarkers like uric acid and creatinine in sweat, which can be regarded as a significant work in this field.[61] Also, colorimetric sensor BSA−$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflowers have been used to quantify acetylcholine (ACh) in human serum.[62]

5.5.1. Advantages. Immobilization of PNPs increases specificity and sensitivity toward target analytes. In the case of controlled protein conjugation in TMV nanorods, the attached R-phycoerythrin (R-PE), human apoferritin (AFTN) nanoparticles had modularity of the input and output signals, thereby helping the binding of both antibodies as well as Ni-NTA nanoparticles to the PNP surface.[58] In another work, BSA-RuO_2NPs showed enhanced stability, peroxidase-like activity, and salt resistance than RuO_2NPs.[60] An important PNP, ferritin nanocages, has been reported to possess multiple modifiable interfaces, a spacious internal cavity, high cargo-loading ability, and enzymatic ferroxidase activity to function as a biosensor for detecting reductants or oxidants.[63]

5.5.2. Limitations. The desolvation process of proteins and enzymes often leads to a protein structure change and loss of enzymatic activity.[18] In a recent study, glycan−lectin interaction powered biosensors were developed for virus detection, but challenges like unidentified selectivity of lectins, sensitivity issues with clinical samples, fouling, stability issues, and limited functionality need to be solved for better improvement.[64]

5.6. Bioimaging. To provide acceptable medical attention to a disease, it should be properly identified, and the stage of progression needs to be detected. This is possible only by visualizing the cellular and molecular levels of different biological mechanisms. Bioimaging has attained a high demand in this field for properly diagnosing a disease state, and it must not damage the existing cells or tissues. The wide application of bioimaging in disease management includes cancer cell labeling, detection of viruses or pathogens, bone degeneration and metastasis, gene expression studies, cell structure imaging, angiography, etc. PNPs are employed for multimodal bioimaging by use of various features like surface functionalization, genetic fusion, surface modification, delivery, bioconjugation, and synthesis of shell and optical core,[65] which enhance specific targeting. With the help of various rays or waves, these are detected by different imaging techniques like magnetic resonance imaging (MRI), computed tomography (CT), ultrasonography (US), positron emission tomography (PET), etc.[66] Fusing protein nanoparticles with this technology will have tremendous output in cancer theranostics and imaging of brain and cardiovascular diseases with the least level of toxicity. Dox@AFBS with a Bi_2S_3 radiation sensitizer, utilized for nanodiagnostic purposes, diminished the volume of subcutaneous Hela tumor.[7] It is reported that Hsp 16.5 is used for delivery of the MRI contrast agent gadolinium for pancreatic cancer detection. Engineered TMV has been used for near-infrared fluorescence (NIRF) bimodal imaging by delivering dysprosium (Dy3+) and the NIR fluorophore (Cy7.5). Copper sulfide-ferritin nanocages and ZnF16Pc-loaded scFv ferritin have wide applications in photothermal therapy and photodynamic therapy, respectively.[67] It has various advantages like high absorbency, dispersibility, nontoxicity, photobleaching resistance, and high stability over traditional imaging agents.[68] An NIR Au-HFt nanocomposite was reported for kidney theranostics. The Apo-CUR-Gd complex was administrated for liver imaging, and PNC_{SV40}-encapsulated Ag_2S quantum dots were utilized for deep tissue penetration imaging.[68] Apart from these, PNPs also have wide applications in imaging other cells and molecules by acting as fluorescent probes. A new type of collagen-based magnetic nanoparticle (CFeAb*D) produced good in vitro MRI images of gastric cancer cells through receptor-mediated endocytosis.[69] Protein-mediated probes are fabricated by various modifications to form single-molecule forms, nanocage states, nanofluorophores, etc., for NIR imaging, phototheranostics, dynamic bioimaging, TP imaging, PA imaging, FA imaging, in vivo imaging, etc.[70]

5.6.1. Advantages. Protein nanoparticles show great CT imaging performance, better biocompatibility both in vitro and in vivo, natural multivalency, good solubility, and are efficient carriers for photosensitizers (arginylglycylaspartic acid (RGD) peptide-modified apoferritins). By using the targeted property of ferritin, chronic diseases such as cancer cells, tumor tissues, atherosclerotic plaques, etc., can be visualized using magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), positron emission tomography (PET), near-infrared fluorescence (NIRF), computed tomography (CT), or multimodal imaging by labeling ferritin with signaling molecules appropriately.[63] A recent work demonstrated the discovery of the ferritin core's enzyme-mimic activity which can also serve as a native sensitive label, to enable the fabrication of nanobiosensors without redundant labeling procedures.[71] For NIRF imaging, different protein combinations like VLP-QDs and HspG41C-CTT PNPs have been reported to be used.[68]

5.6.2. Limitations. In a study, it was reported that the low purification efficiency and inner negative charge of ferritins limit the encapsulation of negatively charged biomacromolecules such as siRNA and miRNA.[72] In another study, HoS-ferritin loaded with iron for magnetic resonance imaging-based cancer theranostics caused oxidative stress and cell death for imaging and therapy.[68]

5.7. Nanoparticle-Based Diagnostic Assays. Assays like proximity ligation assay (PLA) have been reported to benefit from the utilization of PNPs for the detection of ultralow levels of protein biomarkers. The use of protein nanoparticles, particularly avidin nanoparticles as the reporter, made PLA capable of high yield of ligating oligomers or PCR template. The feasibility of human chorionic gonadotropin (hCG) detection by NP-PLA for pregnancy and ovarian cancer confirmation was made possible by tagging hCG α-subunits with ANANAS nanoparticles by biotin−streptavidin linkage.[73] In another study, luciferase-displaying protein nanoparticles were engineered as detection probes by fusing ELP with a polyaspartic acid chain, NanoLuc luciferase, and a biotin acceptor peptide (ELP-D-Nluc-BAP). This technique enabled multivalent luciferase display on surfaces, leading to enhanced detection.[74] A single-stranded DNA binding protein (RPA70A) conjugated with AuNPs was used for improved sensitivity and specificity in biomarker detection in the field of point-of-care testing (POCT) for early disease diagnosis.[75] In another work, HBV capsid-derived chimeric nanoparticles, nickel nanohair, and anti-troponin I antibodies were conjugated to generate a diagnostic assay for capturing troponin markers in patients with acute myocardial infarction.[76] For early diagnosis of infectious diseases like AIDS and hepatitis C, a novel ultrasensitive system was made to detect antibody markers using supramolecular protein nanoparticles by the self-assembled hFTN-H molecules toward two targets like autoantibodies of type I diabetes and anti-S-HBsAg monoclonal antibodies.[77] Photothermal and photodynamic therapies utilized apoferritin (AFN) to carry paclitaxel (PTX) with IR1061 and folic acid. Similarly, fibroin is used as a biotemplate and reductant for the production of SMID

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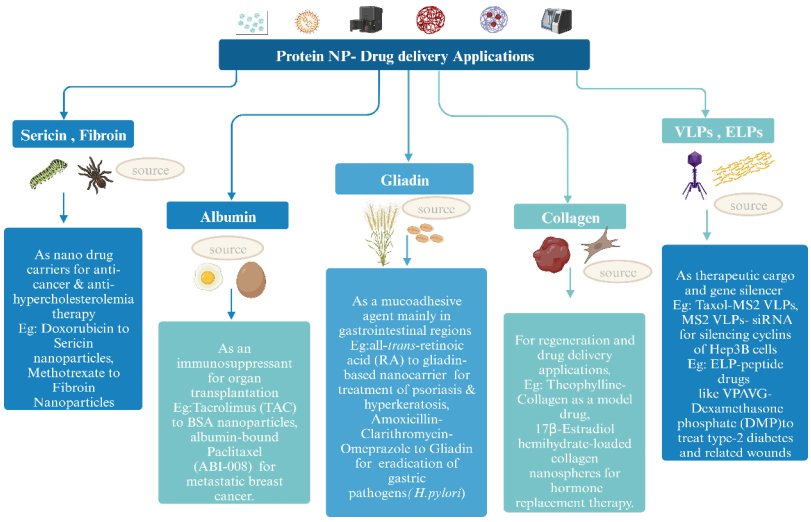

Figure 3. Flowchart showing the drug delivery applications of protein nanoparticles (created with BioRender.com).

(SF@MnO_2 loaded with ICG and DOX) to achieve combined PTT/PDT/chemotherapy with the direction of MR/fluorescence dual-model imaging.[7]

5.7.1. Advantages. Protein nanobiosensors improve the sensitivity of protein assays by assay miniaturization.[78] In a study, Mn-loaded apoferritin (Mn-Apo) was reported to be a good candidate for early stage diagnosis and evaluating the stages of melanoma, as it senses melanin formation by L-DOPA oxidation, which caused Mn reduction. Also, H-Fn nanocages showed high cellular uptake, improved fluorescence properties, and improved targeting of the tumor mass in vivo for their use in image-guided cancer surgery in another study.[68]

5.7.2. Limitations. SV40 virus-based nanoparticles assembled from multiple copies of viral capsid proteins as a nanoreactor for mineralization of inorganic AuNPs have the limitation of protein impairment during seed synthesis inside viral protein cages. In order to override this limitation, only gentle reaction conditions for synthesis of inorganic seeds should be followed.[79]

5.8. Drug Delivery. While considering drug delivery systems in the biomedical field, it is important to understand their pharmacokinetics, mode of release, delivery time, rate, amount, etc. Vaccines, drugs, growth factors, stem cells, nucleic acids, etc., are different molecules used for treatment procedures to boost immunity. So proper delivery of these molecules at the right time, rate, and amount is very essential for effective treatment.[23] Modification of these delivery systems with nanoparticles caused tremendous advantage in this area. Engineered nanoparticle delivery systems are used for chemotherapy, immunotherapy, genome editing, and precision medicine, including examples like paclitaxel from Abraxane, pegloticase from Krystexxa, factor VIII from Adynovate, etc. These are the FDA-approved protein conjugated drugs prescribed for pancreatic cancer, Gout disease, and hemophilia, respectively.[6] Resveratrol-loaded fibroin nanoparticles in the rat colitis model showed an enhanced anti-inflammatory effect when compared with pure resveratrol administration. Albumin nanoparticles were used as an antibody delivery carrier and also as a chemotherapeutic carrier for bevacizumab. DOX-loaded HFn (natural ferritin) is used as an antitumor agent, whereas 30Kc19 protein from silkworms is being used as drug and β-gal delivery agents. Zein microspheres conjugated with a chemo-immunotherapy agent polysaccharide-Kureha (PS-K) are used for chemotherapy. In spite of all these advancements, there are several viral and nonviral capsids for effective delivery systems. These can deliver all of the therapeutic molecules by self-assembly. This will overcome limitations like deactivation of enzymes, nonspecific delivery, etc.[8] Apart from this, recombinant proteins like virus-like particles (VLPs) and elastin-like polypeptides (ELPs) emerged as a nascent area of chemotherapeutic drug-loaded research. VLPs are non-infectious nanoparticles, and ELPs are derived from tissue matrices, particularly proteins, that are used for the delivery of drugs as well as biomarkers. Drug-loaded VLPs, nucleic acid-loaded VLPs, siRNA-loaded VLPs, and peptide drug-loaded ELPs are recent discoveries that point out next-generation drug delivery systems[23] (Figure 3). A DTX−HSA conjugate formulation showed enhanced solubility and higher cytotoxicity against metastatic breast cancer cells,[80] while DOX-loaded collagen-PAPBA showed effectiveness towards ovarian cancer cells.[7]

5.8.1. Advantages. The FDA-approved nanodrugs Abraxane (Celgene) and Ontak (Eisai), which are used to treat breast cancer and cutaneous T-cell lymphoma, have the advantages of improved solubility, targeted delivery to tumors, targeted T-cell specificity, and lysosomal escape properties. This is due to the own endocytosis route mediated by albumin receptor gp60 of albumin protein.[8] Protein gelatin-based nanoparticles were found to increase the bioavailability of topical ophthalmic drugs and were used in the delivery system for peptide drugs.[81] Another advantage of PNPs in this field is their nonallergic and nontoxic nature, particularly for silk fibroin, and hence, they have been utilized as injectable as well as implantable drug delivery systems.[32]

5.8.2. Limitations. It has been reported that due to the deactivation of enzyme cargo during preparation processes, there is a possibility of delaying the release of drugs, as well as the nonspecific delivery of therapeutics to nontargeted cells that have the desired receptor. Also, the release of polar drugs from gliadin nanoparticles was found to be slower due to a high affinity issue between gliadin and drugs; additionally, hydrophilic drugs are reported to have slow spread with a burst release.[8]

5.9. Immunological Adjuvants. The main purpose of adjuvants is that they are added to vaccines to improve their immunogenicity. This category can be divided into two main areas: delivery systems and immunostimulants. Polymers, nanoparticles, emulsions, microbial products, oligonucleotides,

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Table 2. Examples for Adjuvant-Targeted PRRs, Their Descriptions, Specific Examples, and Unique Applications[84,85]

adjuvant-targeted PRRs	description	examples	applications
C-type lectin receptors	recognize carbohydrate structures of pathogens	Dectin-1, Dectin-2, DC-SIGN, Mincle	• mannose anchored liposomes bind with CLR and present OVA antigens on MHC molecules • TLR and CLR agonist pairing adjuvant used along with Pneumovax vaccine • miR-511-3p in human MRC1 gene and CLRs trigger IL-10, IL-4 secretion and help in controlled immune response modulation
toll-like receptors	recognize various PAMPs (including LPS) and DAMPs	monophosphoryl lipid A, CpG oligodeoxynucleotides, AS04, AS02A	• MPLA helps in reducing toxicity and act as immunostimulant • TLR8-PEG-bl-PPS combination used for dendritic cell activation • TLR7-Alum combination used in glycoconjugate vaccines • CpG ODN-protein combinations widely used in nanomedicines • MelQbG10-CpG-ODN used in treatment of melanoma • nasal vaccine developed by the combination of recombinant PcrV adjuvanted with CpG-ODN

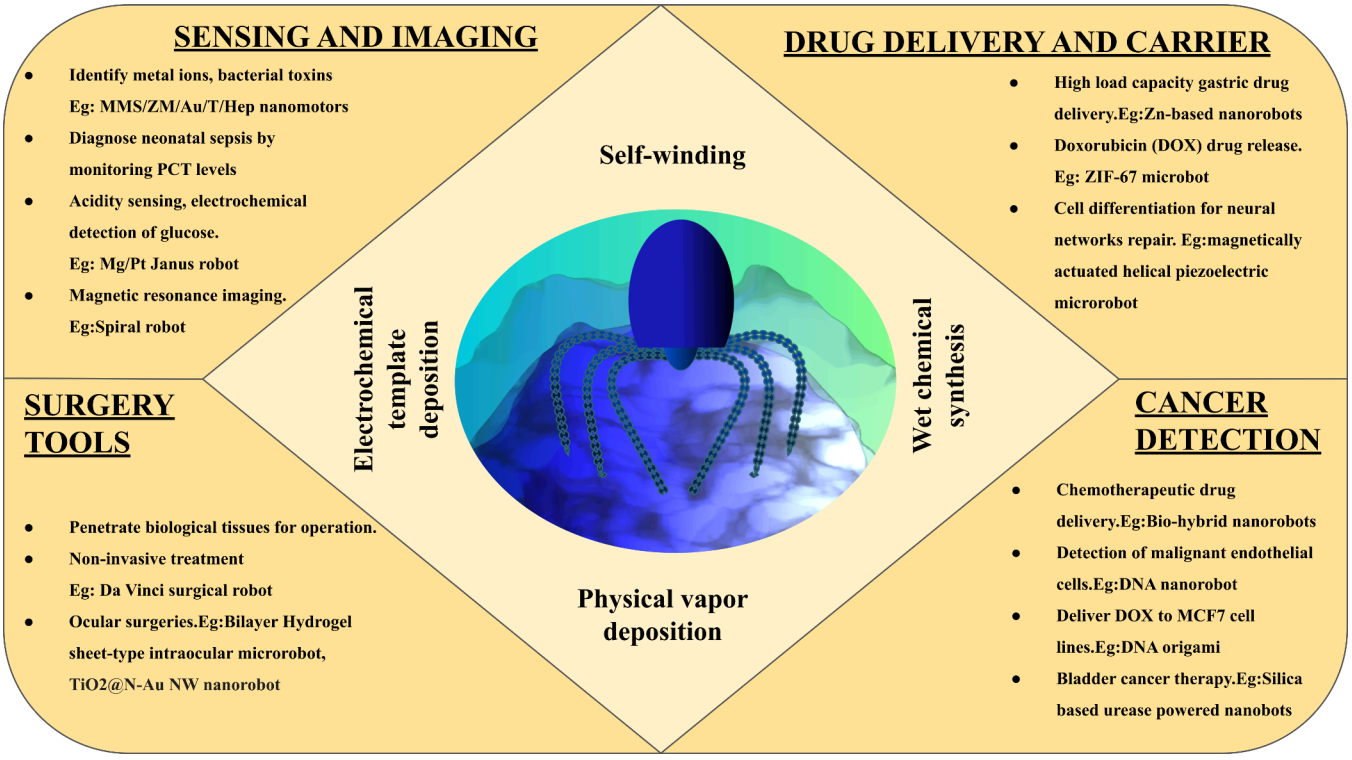

Figure 4. Representation showing different applications of nanobots in the biomedical field (created with Inkscape).

liposomes, saponins, etc., are different compounds that play an adjuvant role in the clinical field.[82] This will help to induce long-lasting immunity for the vaccinated individual. Immunostimulation is made possible by the activation of various immune receptors like TLRs (toll-like receptors), PRRs (pattern recognition receptors), and CLRs (C-type lectin receptors) and also by the induction of B cells, T cells, cytokines, chemokines, etc.[83] Delivery systems help display and present antigens to antigen-presenting cells (APCs) for immunization. This can be prolonged by combining the delivery system with immunostimulatory adjuvants like PRRs. These PRRs are specialized receptors in both immune and non-immune cells that are expressed by macrophages and dendritic cells mainly for pathogen detection. The conserved pattern sets in pathogens called PAMPs (pathogen-associated molecular patterns) are recognized by PRRs and killed. MenBioVax, a meningitis vaccine, is a heat shock protein−antigen complex (HspC) against *Neisseria meningitidis* in humans.[84] The examples for adjuvant-targeted PRRs are listed in Table 2.[84,85]

5.9.1. Advantages. In one work, lectin/ScLL was used against Neospora lysate antigen (NLA), and it showed increased IgG1, survival rate, as well as decreased parasite load.[86] Lectins and heat shock proteins have been utilized in anticoccidial vaccine formulations for inducing mucosal and systemic immunity due to the interaction and translocation efficiency of proteins.[84]

5.9.2. Limitations. It is reported that limited spatial control of ligand attachments may occur in the case of multiple ligand attachments on the same protein.[32] In one study, albumin/AlbiVax nanocomplexes were found as efficient in vaccine delivery and immunotherapy, but the possibility of modifications due to conjugation challenges, immunomodulatory effects, and nonspecific conjugation risks with other cysteine-accessible biomolecules demands its further optimization.[87]

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6. DIVERSE NANOMATERIALS, NANOPRODUCTS, AND NANODEVICES IN BIOMEDICINE

Even if nanotechnology deals within the 1−100 nm range, its wide applications and vast range of products made it an essential part in the medical field. The diverse applications of nanotechnology in the medical field encompass a vast array of nanomaterials, nanoproducts, and nanodevices, many of which are not limited to protein-based nanoparticles (PNPs). Some of these advanced nonbiological materials are discussed here (Figures 4 and 5).

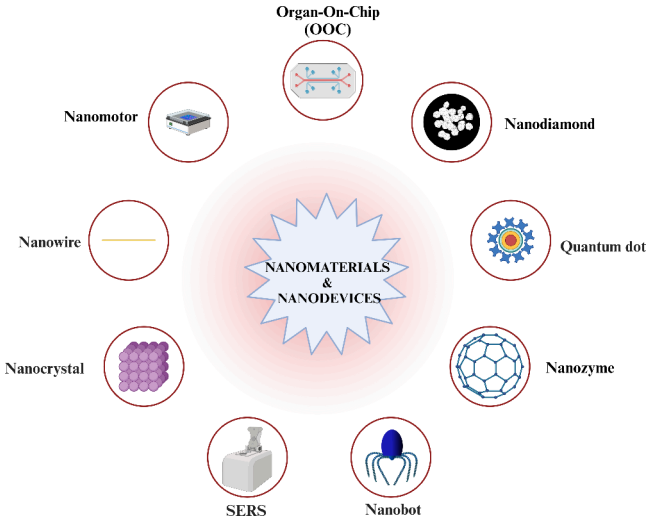

Figure 5. Graphical representation showing current trends in nanomaterials, nanoproducts, and nanodevices (created with BioRender.com).

6.1. Nanopharmaceuticals. Nanomedicine has a successful place in the healthcare area, as it covers many limitations of traditional pharmaceutical agents, like difficulty in the removal of residues, toxic effects, high initial drug release, low drug solubility, etc. Mostly, nanotechnology is used as a carrier option for different drugs. Hence, there are different types of nanocarriers used for drug/gene/vaccine delivery, biosensing, cell scaffolds, chemotherapy, etc. (Table 3).[88]

Apart from these, mesoporous silica NPs are used in the biomedical field as biosensors, antidotes, and diagnostic agents. Carbon nanotubes are being used in bone cell scaffolds and to repair spinal cord injuries.[88]

6.2. SERS. Surface-enhanced Raman spectroscopy (SERS) is an important diagnostic tool that uses nanotechnology such as gold NPs. This technique can be used for developing biosensors, SERS active nanomedicines, cellular imaging, and drug delivery. Faceted Co_3O_4 microcrystals, Raman reporters, etc., are used for their biotagging capability, parallel detection of more biomolecules, and photocatalysis. They have the ability to perform biomarker detection, pathogen detection, and blood component analysis for various disorders like cancer, infections, autoimmune diseases, cardiovascular disorders, diabetes, etc.[89]

Table 3. Types of Nanocarriers Used as Nanopharmaceuticals, Their Applications, and Related Examples[86,88]

nanocarrier	pharmaceutical application	nanomedicine examples
organic nanocrystal	chemotherapy, cholesterol control	Emend, Ostim, Rapamune
polymeric NP	nanospheres for drug/gene delivery	Cimzia, Adagen, Macugen, Renagel
liposomes	DNA/drug/hormone delivery	Doxil, Onivyde, Marqibo
magnetic NP	MRI application, contrast agent	Gadomer-17, ferumoxsil (MPIO), gadobutrol
protein NP	drug carriers, coagulant	Ontak, Rebinyn, Abraxane

6.3. Nanofibers. Nanofibers are used mainly for the dressing of wounds, implants, burned areas, etc. An interesting revolution is the creation of intelligent bandages made from nanofibers to detect infectious symptoms. They become absorbed into cells after wound healing.[90] It is reported that electrospun or self-assembled nanofibers will mimic the extracellular matrix (ECM), thereby allowing guided tissue and bone regeneration in oral surgery.[91]

6.4. Nanobots. Nanorobotics is an area having a coordinated system for repairing damaged cells and replacing entire intracellular components, DNA molecules, different organs, etc., in biomedicine. Sensors and propulsion equipment include the major components of a nanobot. The manufacturing of such robots is done by several methods, like physical vapor deposition technology, self-winding technology, electrochemical template deposition technology, and wet chemical synthesis. The application of artificial intelligence in nanotechnology has improved efficiency and circulation due to the programming options. They can be connected with different people to monitor cancerous cells, damaged cells, etc.[90] Additionally, they are exclusively used in pretreatment analyses like metal-ion detection in blood, identifying sepsis, and acidity sensing. Moreover, they are utilized in medical imaging and drug delivery, act as drug carriers, and serve as surgical tools (Figure 4).[92]

6.5. Nanocages. The interior of the cage is a matrix type made of PNPs that is filled with therapeutic agents, whereas the exterior part will have increased monodispersity and specificity.[93] They can be made of either single or multiple proteins. The PNPs will ensure equal distribution of drugs and helps to customize drug delivery.[94] The size of this nanomaterial is in the range 5−100 nm, making it highly useful as a template. This nanomaterial includes nonviral and viral protein nanocages. Common nonviral cages include ferritin, heat shock protein, encapsulin, DNA-binding protein (Dps), E2 proteins, etc. Ferritin nanocages regulate iron levels in the body, especially holo-ferritin, and will load guests like β-carotene, cyanidin-3-O-glucoside, etc.[17] Dps are used for the supplementation of mineral elements. E2 proteins are being used as a platform for doxorubicin encapsulation.[95] M13 bacteriophage, MS2 bacteriophage, adenovirus, tobacco mosaic virus, etc., have been utilized to deliver interleukin-12 (IL-12) and a TGF-β inhibitor (SB) for exerting antitumor activity, for crossing the blood−brain barrier (BBB) with an MRI contrast agent, etc.[96]

6.6. Nanosensors. Sensors, along with nanotechnology, are used to detect malignant organs, concentrations of body O_2 and CO_2, nutritional deficiency, circulatory malignant cells, etc. Nanoflares, which have the capacity of luminescence to detect cancer cells, are an example of this.[90] It is reported that nanomicrobivores as well as nanosensors are used in the detection and detoxification of chemical warfare agents like marijuana, alcohol, and banned materials in patients.[97] Graphene optical biosensors are used for identifying cancer cells during chemotherapy. Graphene-based FET nanosensors are used for detecting coronavirus from swab specimens, and investigations

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of the healing process by bioresorbable pressure sensors which highlights their noteworthy applications in healthcare.[98]

6.7. Nanobubbles. These are a drug delivery structure or aggregate formed when exposed to body temperature. Through ultrasonic stimulation, the bioactive agents are released from these bubbles. The formation occurs as a result of air gas trapping at the hydrophobic surface of the supersaturated solutions. Various types of nanobubbles like bulk, oscillating, plasmonic, and interfacial nanobubbles exhibit distinct properties. They have very good applications in the treatment of tumor cells, as they are unaffected by healthy cells. Anticancer nanobubbles for doxorubicin was a successful carrier. They can be used for clot removal and as a carrier for viral vectors.[99]

6.8. Nanofilms. Nanofilms are polymeric sheets of nanometer thickness intended for cell interaction. These sheets are assembled into thin layers, forming different biofilms by methods like spin coating, fluidic assembly, etc. They involve surface cross-linking and nanoparticle templating, thus having mechanical rigidity and bioactivity.[100] Nanofilms are used as patches to heal incisions in surgical procedures. Ubiquitous transference nanoadhesive plaster, poly(lactic acid) nanosheets, and poly(acrylic acid) sacrificial supporting films are a few examples.[101]

6.9. Nanozymes. These are a combination of enzymes with nanoparticles, mainly gold NPs. They can absorb light, which aids in photothermal therapies. They are also used as delivery vehicles, in vivo imaging agents, immunoassays, etc. The various applications include gold electrodes with aptamers for cardiac troponin I, the detection of urinary spermine and HIV, the detection of pesticides and mycotoxins for water treatment, building artificial biochemical models, etc. Apart from that, magnetite nanoparticles with peroxidase activity were another successful venture.[102] Iron oxide, cerium oxide, graphene, and manganese nanozymes were reported to exhibit great antimicrobial activity and ROS scavenging activity.[103]

6.10. Nanocrystals. These are 100% pure, stable solid drugs without drug vehicles tin the range of 1000 nm. They can be made into a nanosuspension with the addition of a surfactant. High pressure homogenization, controlled precipitation, vortex mixing techniques, etc., are used for the manufacture of nanocrystals. For pulmonary hydrophobic drug delivery, cinaciguat nanocrystals have been used.[104]

6.11. Nanowires. These are specific nanomaterials, usually 1D structures, used in biosensors. The key benefits of these structures are their superior charge conduction and strong sensing characteristics.[105] Silicon nanowires are being used in the fabrication of pH sensors, the detection of biomolecular interactions like biotin or streptavidin, the detection of single-stranded DNA molecules, the detection of influenza virus A, etc.[106]

6.12. Nanodiamonds. Nanodiamonds are basically carbon nanoparticles that have a size of less than 10 nm, along with the inherent characteristics of bulk diamonds like stable fluorescence, intrinsic biocompatibility, low toxicity, apparent functionalization, surface modification, etc. The synthesis methods of nanodiamonds include detonation techniques, ball milling, laser ablation, and chemical vapor deposition. Mainly, they have bioimaging applications and biosensing applications and are being used in heat therapy, targeted therapy, etc.[107] Different nanodiamond-conjugated complexes have been discovered to enhance their applications, like albumin-conjugated fluorescent nanodiamonds (FNDs), which can be utilized as photostable labels, tracers, and biomolecular nanocarriers.[108−110]

6.13. Nanomotors. Nanomotors are developed from the inspiration of different biomotors like kinesin and dynein. These devices convert different types of energy into mechanical energy to perform various tasks efficiently. They are extensively used in assisted fertilization, microsurgeries, targeted therapies, etc. They have properties like high adaptability to the internal environment, magnetotaxis, chemotaxis, anaerobism, rapid multiplication, etc.[111]

6.14. Carbon-Based Quantum Dots. These are nanocarbon crystals with a semiconductor core and a shell that have properties like photoluminescence, multiproton excitation, low toxicity, high water solubility, and environmental friendliness. Hence, they are extensively used in bioimaging purposes, nanomedicine, catalysis, light emitting diodes, solar cells, and the investigation of targeted analytes.[4]

6.15. Organ-on-Chip (OOC). OOCs are nanodevices that can replicate the microenvironment of organs or cells, by creating the 3D models, which serves as a platform for drug discovery and screening. As these models mimic the physiological conditions of the human body, they can be used for preclinical research. These devices can create human tissue-like structures and can be used for HPT screening and survival monitoring. This technology has been applied for the construction of various nanodevices like single organ-on-chip, multiple organ-on-chip, human-on-chip, patients-on-chip, etc. Another nanodevice, cancer-on-chip (3D breast COC), has been used to treat breast tumors through the evaluation of drug delivery systems.[111]

In addition to these examples, there exists a plethora of other nanostructures like nanospheres, nanorods, nanogels, nanoemulsions, nanocapsules, nanomicelles, etc. Various hosts like β-casein, whey protein, ovalbumin, etc., help in the loading of substances like curcumin, carotenoid, folic acid, etc.[17] Nanospheres and nanorods are often utilized for hyperthermia treatment, nanostars for the treatment of malignant growth, nano core−shells for bioactive molecule delivery, carbon nanotubes for cell-level diagnosis or treatment, nanocubes for biosensing, etc.[99]

Through the various preparation technologies mentioned in Table 1, new designs of nanomaterials or devices are being invented, prompting the success in the field of nanomedicine. PNPs stands out as a competent candidate among the vast range of nanoparticles. However, designing novel nanostructures or devices incorporating PNPs whether through modifying existing PNPs or integrating them with other nanoparticles will yield encouraging quality products in the market.

7. ERA OF MULTIFUNCTIONAL PROTEIN NANOPARTICLES

The modification of PNPs through engineered assembly has led to the manufacture of multifunctional protein nanoparticles, which have made notable contributions with enhanced effectiveness and biospecificity in the field of TERM (tissue engineering and regenerative medicine) strategies. Self-assembly is a sophisticated technique used to build peptide nanomaterials with high loading capacity and prolonged circulation, which have a good future in regenerative medicine and drug delivery.[27] Other assemblies produced by the colloidal nature of nanoparticles make them attractive candidates in the field of 3D bioconstructs. But the record of such bioconstructs started from biofunctional building blocks to multiscale modular

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bioarchitectures with user-defined geometrics. The main goal of this particular category is to perform many functions synergistically, which will lead to the simultaneous attainment of therapeutics as well as diagnostics (Table 4).[112]

The remarkable discovery of biopolymer nanocomposites helped to create enhanced multifunctional supports or devices in the medical field. Biopolymer nanocomposites are the integrated nanointerface of biological materials (polysaccharides, proteins, polynucleotides) as well as synthetic materials (carbon, minerals, metals, graphene). The improved optical properties made them an important candidate for the production of electronic devices. Especially, proteins like silk, collagen, and keratin have wide applications in multifunctional bioimplants. Apart from these, they have various applications in the fields of photoluminescence, photonic crystals, fast DNA diagnosis, etc.[113] The directed assembly of cell-laden microgels to form 3D microfabricated tissue constructs with increased photolithography,[114] macrophage microtissues, coculture tissue models, etc., paved the way for tissue and organ transplantation using a nanoscale approach.[49] Their qualities like high penetration ability and tunable surface properties, made them ideal candidates in the TERM field.[29] In the dental field, the NPs are used as nanofillers for repairing mechanical properties, implant coatings, etc. Chitosan-based composite scaffolds were used for the treatment of periodontal defects and bone loss by forming interfacial bonds. Collagen-based nerve conduits were used to repair small nerve gaps, and gelatin-based scaffolds were used for neural tissue engineering via electrospinning to promote axon growth. Other PNPs such as elastin and keratin are combined with polypeptides to form suitable substrates. HemCon bandage for hemostatic dressing is a chitosan-made product that has wide application in the skincare industry for its enhanced antibacterial properties. The dendrimer-camptothecin conjugate used in PDA treatment showed high antitumor activity and is being used in the field of drug delivery.[115]

Apart from these, a variety of proteins like hemoglobin, transferrin, mucin, and HSA were used for the development of alternate drug delivery platforms. Hemoglobin is used for the manufacture of semiartificial RBCs and Hb-based oxygen carriers. Mucins are basically glycosylated proteins that stabilized by cationic cross-linkers which can be used in the manufacture of cargo systems. Transferrin has been used in cancer treatment as a carrier, as it facilitates the transport of therapeutics across the blood−brain barrier and targets malignant tissues. Medical implants fabricated with NPs and their body acceptability have given satisfactory results in the daily lives of people. But the risk of nanotoxicity has to be evaluated, and thereby, the design has to be modified.[116]

8. CHALLENGES OF SYNTHETIC NANOPARTICLES AND THE EMERGENCE OF NATURAL PROTEIN NANOPARTICLES

Even though the biomedical field gained many advancements through the effective manipulation of materials on the nanoscale, their utilization caused many hazards to mankind in the name of cytotoxicity and waste mismanagement. The common challenges of synthetic nanoparticles are high cost, production of toxicants, immune response, nanomaterial characterization, etc.[99] After the formation of protein corona, the next interaction happens with phagocytes that may cause infections or inflammation.[117] Meanwhile, the lymphatic system directs them to various areas and leads to the suppression of NK cells, proinflammatory cytokine production, reactive oxygen

Table 4. Advancement of Nanoparticle Assembly, Related Products, and Applications[112]

assembly type	functions/applications	examples	products
cell-rich assembly	fabrication of human heterotypic cell constructs, improved healing after implantation, recapitulation of complex structures with enhanced organogenesis and morphogenesis	2.5D cell sheets, 3D multicellular microtissues, programmable cell-rich assembly	Up Cell (Okano's group), TiO_2 nanodot films, fibroblast cell sheets, 3D spheroids, human 3D organoids
cell-biomaterial assembly	3D cell culture, nonviral gene delivery, cell surface functionalization, nanoparticle-cell functionalization, microparticle cell assembly	cell-laden hydrogels	layer-by-layer (LbL) assembled L929 fibroblast cells, microtissue constructs, hydrogen bonded LbL films, TA/poly(*N*-vinylcaprolactam) (PVCL) multilayers
combined 3D assembly	promote cell spreading, cell−cell interactions, cell−particle interactions, platforms for 3D cell encapsulation	layered multicellular constructs, nanoparticle-cell clusters, multiblock microgels, aggregated 3D spheroids, dense cell−cell programmed constructs, stacked cell sheets	IKVAV-functionalized dendrimers, 20 nm carboxylated polystyrene nanostickers, gelatin norbornene microgel, collagen gel bead
multiscale modular bioarchitecture	promote osteodifferentiation of hMSCs, formation of hyaline cartilage structure	capsular liquified microenvironments, vascularized multicomponent constructs, spatially organized multiblock hydrogels	vascularized hierarchic microtissue assemblies
implantable engineered tissue mimetics	promote peripheral nerve regeneration, regulate cell adhesion, orthopedic applications	tissue engineered scaffolds	NeuraGen, Neuromaix nerve conduits, GORE-TEX vascular grafts, INFUSE bone graft
medical implants	healing joint pains, mineralization of teeth, replacement of diseased/clotted heart valves, restore normal eye vision	eye implants, shoulder implants, heart implants, dental implants	shape-memory braces, intraocular lens

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ACS Omega 2024, 9, 42613−42629

species (ROS) induction, tissue damage, organ damage, and oxidative stress. But all the interactions are not detrimental, according to nature, and the evolution of NPs in the body may be beneficial also.[118] The concepts like transportation, elimination, and accumulation of NPs are very important, as they cause changes in proper body functioning. AgNPs were reported to cause ROS-mediated leakiness of endothelial cells, which led to inflammation in the liver, lungs, and kidneys. CuO NPs caused liver tissue apoptosis and impaired liver function, while mesoporous silica nanoparticles (MSNs) triggered intestinal inflammation responses, oxidative stress and damaged the intestinal mucosal epithelial structure.[119] Another great challenge of conventional synthetic nanoparticles is their biodegradability. The emergence of protein therapeutics leads to the exploration of nanomedicines correlated with bioinspired or natural proteins. They exhibit various inherent features like high modification flexibility, specificity, improved bioavailability, stability, controlled release, etc. If natural or purified protein NPs are converted to engineered protein NPs, then they can also overcome low immunological responses. Poly(L-glutamic acid) (PGlu), poly(α,β-aspartic acid) (PAsp), or poly(L-lysine) (PLys) as hydrophilic segments and poly(γ-benzyl-L-glutamate) PBLG are a few examples of polypeptide blocks used in the biomedical field. The versatility of this can be increased by rDNA technology. Apart from these features, conjugation capability and surface modification with several drugs, active ingredients, and dyes have been utilized for antibody purification processes. PNPs can be easily cleaved by different enzymes according to their degree of cross-linking, creating a residue-free or toxin-free internal environment. The availability and affordability of natural proteins makes them a major stabilizer in the manufacture of nanomedicine.[4] Besides the advantages of bioinspired nanoparticles, particularly PNPs,[49] challenges such as the correct targeting of infection sites, accurate release of optimal drugs, stable characterization of physicochemical properties, and pharmacological profiles are still being corrected.

9. CONCLUSION AND FUTURE PERSPECTIVE

The toxicity issues associated in the nanomedical field were reduced to a greater extent by the integration of organic biomolecules. Proteins isolated from plants and animals are smart candidates in therapeutics and diagnostics for hybrid nanoscaffold formation due to some unique properties like spatial distribution of functional groups at well-defined sites, inherent size uniformity, self-assembly profile, and their nontoxic degradable byproducts. Different in vivo approaches like surface functionalization, therapeutic conjugation or blending, multiple modifiable interfaces, recombination, and multiple ligand attachments have made them a revolutionizing vector in areas like regenerative medicine, antimicrobial bioagents, targeted therapies, drug and gene delivery, imaging and sensing, immunological adjuvants, and more. The effect of such protein-based nanotherapeutics highly influences their unique design and preparation methods. These effects have to be surveilled properly by checking the protein corona composition formed due to its interactions and the need to use alternative methods for better results. The challenges and gaps of particular nanoprotein innovations in each field have to be addressed for boosting the effectiveness in treatments and providing a new horizon of personalized therapeutics. PNPs can be surface-modified or conjugated with drugs or active ingredients (both organic or inorganic), making them a safer alternative to synthetic nanoparticles or to reduce their side effects. Among the different natural proteins studied, albumin, zein, and fibroin are mostly used for therapeutic and diagnostic purposes in various fields in purified form or modified or in conjugated conditions. Overall, the synergy of nanotechnology and protein nanoparticles in nanomedicine has opened new frontiers in efficient treatment strategies, contributing to an increased life expectancy.

As these technologies advance, new designs incorporating PNPs, either through modification or integration with other nanoparticles and nonbiological materials, will address existing gaps in various fields. Looking ahead, the future of nanomedicine will likely involve a synergistic approach where the best attributes of diverse nanomaterials incorporating PNPs are combined to create multifunctional, highly effective therapeutic and diagnostic solutions. This integration or conjugation will not only enhance the efficacy of existing treatments but also pave the way for breakthroughs in personalized medicine and minimally invasive diagnostic tools.

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Notes

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ABBREVIATIONS

NPs, nanoparticles; PNPs, protein nanoparticles; FDA, Food and Drug Administration; DLS, dynamic light scattering; SEC, size exclusion chromatography; DCS, differential centrifugal sedimentation; TEM, transmission electron microscopy; PAGE, polyacrylamide gel electrophoresis; LC-MS/MS, liquid chromatography−tandem mass spectrometry; DC, differential centrifugation; SPR, surface plasmon resonance; ITC, isothermal titration calorimetry; AuNPs, gold nanoparticles; NSCs, neural stem cells; PU/SPI, polyurethane/silk protein; VEGF-

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loaded PCL NPs, vascular endothelial growth factor-loaded polycaprolactone nanoparticles; AgNPs, silver nanoparticles; AuNP-ZnO, gold−zinc oxide nanoparticles; LYZ−AuNCs, lysozyme−gold nanoclusters; CuNCs, copper nanoclusters; MNCs, metal nanoclusters; R-BSA-S, reduced bovine serum albumin-silver treatment; PEG, polyethylene glycol; PLGA, poly(lactic-*co*-glycolic acid); AIDS, acquired immunodeficiency syndrome; PPI dendrimer, poly(propyleneimine) dendrimer; PTX, paclitaxel; LNP siRNA, lipid nanoparticle small interfering RNA; CZP, certolizumab pegol; SELPs, silk−elastin-like protein polymers; PAMAM dendrimers, poly(amidoamine) dendrimers; HSA, human serum albumin; Qβ, Q-beta virus; HBV, hepatitis B virus; Fd, filamentous bacteriophage; E2, estradiol; Fn PNPs, fibronectin protein nanoparticles; MRI, magnetic resonance imaging; CT, computed tomography; PET, positron emission tomography; US, ultrasound; NIR imaging, near-infrared imaging; TP imaging, two-photon imaging; PA imaging, photoacoustic imaging; FA imaging, fluorescence angiography imaging; PLA, proximity ligation assay; ANANAS nanoparticles, aptamer-nanoparticle-aptamer nanostructures; ELP-D-Nluc-BAP, elastin-like polypeptide-NanoLuc luciferase-biotin acceptor peptide; RPA70A, replication protein A 70 kDa subunit A; POCT, point-of-care testing; hFTN-H, human ferritin heavy chain; anti-S-HBsAg, antibody against surface antigen of hepatitis B virus; Hsp, heat shock protein; ZnF16Pc-loaded scFv ferritin, zinc phthalocyanine loaded with single-chain antibody fragment (scFv) ferritin; NIR Au-HFt nanocomposite, near-infrared gold−hollow ferritin nanocomposite; Apo-CUR-Gd complex, apoferritin-curcumin-gadolinium complex; PNC_{SV40}, polyomavirus SV40 capsid; Ag_2S, silver sulfide; HFn, heavy chain ferritin; VLPs, virus-like particles; ELPs, elastin-like polypeptides; TLR, toll-like receptor; PRR, pattern recognition receptor; CLR, C-type lectin receptor; APC, antigen-presenting cell; PAMPs, pathogen-associated molecular patterns; OVA antigens, ovalbumin antigens; DAMPs, damage-associated molecular patterns; MPLA, monophosphoryl lipid A; TLR8-PEG-bl-PPS, toll-like receptor 8-polyethylene glycol-*block*-poly(propylene sulfide); CpG ODN-protein, cytosine-phosphate-guanine oligodeoxynucleotide-protein; MelQbG10-CpG-ODN, melanin-binding peptide QbG10-CpG oligodeoxynucleotide; AS02A, adjuvant system 02A; SERS, surface-enhanced Raman scattering; ECM, extracellular matrix; MCF7, Michigan Cancer Foundation-7; Dps, DNA-binding protein; FET nanosensor, field-effect transistor nanosensor; ROS, reactive oxygen species; COC, cancer-on-chip; OOC, organ-on-chip; HPT, high-throughput screening; TERM, tissue engineering and regenerative medicine; PVCL, poly(*N*-vinylcaprolactam); LbL, layer-by-layer; hMSCs, human mesenchymal stem cells; PDA treatment, posterior descending artery treatment; PGlu, polyglutamate; PAsp, polyaspartic acid; PLys, polylysine; PBLG, poly(γ-benzyl-L-glutamate); rDNA, recombinant DNA

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