Protein-based nanocarriers and nanotherapeutics for infection and inflammation

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Abbreviations: IgG– immunoglobin G; FDA- US food and drug administration; PAMP- pathogen associated molecular patterns; PRR- pattern recognition receptors; CRP- C-reactive protein; IL- interleukins, MBL- mannose-binding lectins; APC- antigen presenting cells; IFN- interferons; GM-CSF- granulocyte macrophage colony stimulating factor; TNF- tumour necrosis factor; DC- dendritic cells; ROS- reactive oxygen species; RNS- reactive nitrogen species; COX2- cyclooxygenase 2 enzyme; TGFβ- transforming growth factor β; MCP- monocyte chemoattractant protein; LPS- lipopolysaccharide; MOF- metal organic framework; FITC- fluorescein isothiocyanate; RGD motif- arginine-glycine-aspartate motif; HIV- human immunodeficiency virus

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Abstract

Infectious and inflammatory diseases are one of the leading causes of death globally. The status quo has become more prominent with the onset of Covid-19 pandemic. In order to combat these potential crises, proteins have been proven as highly efficacious drugs, drug targets and biomarkers. On the other hand, advancements in nanotechnology have aided efficient and sustained drug delivery due to their nano-dimension-acquired advantages. Combining both the strategies together, the protein nanoplatforms are equipped with the advantageous intrinsic properties of proteins as well as nanoformulations, eloquently changing the field of nanomedicine. Proteins can act as carriers, therapeutics, diagnostics and theranostic in their nanoform as fusion proteins or as composites with other organic/inorganic materials. Protein-based nanoplatforms have been extensively explored in order to target the major infectious and inflammatory diseases of clinical concern. The current review comprehensively deliberated proteins as nanocarriers for drugs and nanotherapeutics for inflammatory and infectious agents with special emphasis on cancer and viral diseases. Plethora of proteins from diverse organisms have aided in the synthesis of protein based nanoformulations. The current study specifically presented the proteins of human and pathogenic origin, to dwell upon the field of protein nanotechnology emphasizing on their pharmacological advantages. Further, the successful clinical translation and current bottlenecks of the protein-based nanoformulations associated with infection-inflammation paradigm has also been discussed comprehensively.
Significance statement

This review discusses the plethora of promising protein-based nanocarriers and nanotherapeutics explored for infectious and inflammatory ailments with particular emphasis on proteins nanoparticles of human and pathogenic origin with reference to the advantages, ADME parameters and current bottlenecks in development of protein-based nanotherapeutic interventions.
1. Introduction

Nanotheranostics is a unique approach that has been deemed valuable for disease prognosis and treatment. The longevity and targeted delivery of nano-based formulations have proven advantageous in managing acute and chronic diseases such as infections, cancer, diabetes, and arthritis etc (Bhatia et al. 2022; Durymanov et al. 2017). Compared to classical therapeutics, their altered size enable drug delivery through various tissue barriers such as the blood-brain barrier, microbial biofilms, tumoral epithelial/endothelial barriers, and mucus layer (Carton and Malatesta 2022). Moreover, the encapsulation of drug molecules inside a polymeric nanoparticle aids in sustained release, and precise transfer of drug to the site of injury/inflammation (Moritz and Geszke-Moritz 2015). Over the years, several inorganic and organic materials have been exploited to develop drug delivery systems. Apart from significant toxicity and physiological clearance issues, these delivery vehicles/formulations require additional molecules to be biocompatible and bioavailable (Grigore 2017; Paul and Sharma 2020; Sanità et al. 2020). Various smart nanomaterials such as polymeric micelles, liposomes, dendrimers, hydrogels, mesoporous silica, metal nanoparticles, carbon, and quantum dots have also been synthesized and tested for their bench to bedside translation as potent next-generation nanoformulations (Lombardo et al. 2019). However, the unintended heterogeneity and variability in nanoparticle size and distribution of traditional nanomaterials leads to undesirable side effects, low bioavailability, and faster degradation (Park 2020). This resulted in the expedition of bio-based/inspired alternatives comprising of carbohydrates, lipids, proteins, and nucleic acids as nanocarriers and nanotherapeutics. Among these, lipid based nanoformulations have been proven as efficacious, due to their low toxicity, better biocompatibility and bioavailability, high thermo-temporal stability, loading capacity, cost effective and effortless large scalability (B García-Pinel, 2019). Apart from lipid-based formulations, the other class of nanoformulations that
are emerging for the last decade are protein based nanocarriers and nanotherapeutics (Aloss and Hamar 2023; Raza et al. 2022).

Proteins are indispensable for cellular functionality, self/cellular in origin, biocompatible, and easily distributed and amenable to several enzymatic actions, can be easily metabolised and excreted out following the regular physiological pathways (Delfi et al. 2021; Hong et al. 2020). Further, proteins display the natural tendency to self-assemble-disassemble depending on the physiological conditions, and contain diverse functional groups with high interaction potential (McManus et al. 2016; Bai et al. 2016). The charged protein surfaces and nanosize (<200 nm) enhance the drug binding capacity and proper cellular uptake enabling easy tissue permeability to the target site (Singh et al. 2014). Indeed, these nanosized biomacromolecules are a perfect fit to be applied for vast pharmacological advancements beyond classical therapeutics considering their natural characteristics of LADMET (Liberation, Adsorption, Distribution, Metabolism, Excretion, and Toxicity) even if proteins are being used as an auxiliary component of the composition (such as drug carriers) (Ruiz-Garcia et al. 2008). With the wide variety of intrinsically biodegradable and tuneable protein polymers to choose from, along with the cost-effective production, scalability, and improved efficacy, protein-based nanomaterials are receiving considerable attention of researchers and health practitioners. For instance, the intra-tumoral uptake of Paclitaxel, an anticancer drug via vascular endothelial cells, is known to effectively increase as albumin-based nanoparticles (Sofias et al. 2017). Moreover, in many cases, protein nanoparticles have been proven better in improving the immunogenic response to antigens as nanovaccines compared to their nucleic acid/lipid-based counterparts (Nguyen and Tolia 2021). As vaccines, the protein nanoparticles elicit a higher immunogenic and concurrent long-lasting IgG antibody response. For instance, subunit-based protein nanovaccines developed against influenza have an excellent safety profile due to the presence of antigens...
that are specifically required to establish an immune response (Lu et al. 2021). In addition, the ability of protein corona formation, is now being utilized explicitly to improve the stealth effects and stability, in addition with lowering the risk of immunogenic response against traditional nanomaterials (Xiao et al. 2022). These advantages over other organic/inorganic/synthetic materials make proteins a potential platform to exploit as nanocarriers and nanotherapeutics.

Although employing proteins as nanotherapeutics and nanocarrier is relatively at a nascent stage, expedition of these pharmacologically promising bionanomaterials as next-generation “nanoplatforms” is quintessential considering their structural and functional versatility. (Aloss and Hamar 2023; Raza et al. 2022). Hence, appreciating the potential and significance of these platforms, this review aims to jot down the plethora of promising protein-based nanocarriers and nanotherapeutics already explored and can be explored for infectious and inflammatory ailments with particular emphasis on albumin, collagen, ferritin, and lysozyme-based nanoparticles. The challenges and advantages with specific reference to the ADME characteristics of protein nanoparticles, disadvantages, and the future milieu of using proteins as nanotherapeutic interventions have also been discussed in detail.

2. Physiology of inflammation in infectious and inflammatory diseases

The human immune system is a complex network of diverse cellular and molecular events, which is often influenced by pathological instigators such as pathogenic infection, tissue injury, and molecular/cellular crosstalk pattern variation. Although the development of immune response is methodical, irrespective of the type of pathological process, its ultimate fate is inflammation. The phenomena of inflammation is typically stimulated by extrinsic (pathogen, toxins) or intrinsic (chronic inflammation, stress metabolites/proteins like...
cytokines released due to tissue malfunction) signals (Gusev and Zhuravleva 2022). The inflammatory response is assisted by a wide range of cells (such as epithelial/endothelial cells, and innate immunity-associated leukocytes) and plasma proteins (such as cytokines, chemokines, antibodies, and other serum proteins) that govern the critical immune-responsive molecular signalling cascades. In a broader prospect, the immune response is a two-step process. First, stimulating factors such as, antigens and cytokines mediate the primary signals by recruiting, activating, and programming the neutrophils. Whereby, in the second step, these primary signals then further activate T cells and macrophages that marks the initiation of chronic inflammation in the immune response during pathological conditions (Zaheer et al. 2022).

Once the primary tissue barriers like skin, tears, and mucus layers are damaged, a highly complex cytokine-mediated crosstalk between neutrophils and tissue-resident macrophages initiate the acute inflammation. The neutrophils are the front-line soldiers of the acute inflammation that assist in recruiting other blood leukocytes (B cell, antigen presenting cells or APCs, dendritic cells or DCs, and monocytes). Moreover, they are also involved in eradicating infectious agents by employing cytokines, hydrolytic enzymes (like proteinase 3, cathepsin), and reactive oxygen/nitrogen species (ROS/RNS) (Nathan 2006). Under normal circumstances, neutrophil infiltration is sufficient for the resolution of infectious agents. However, if the acute inflammatory response fails to eliminate antigens, the neutrophils recruit macrophages and T cells and ensure the state of chronic inflammation. Besides leukocyte recruitment, the neutrophil-secreted cytokines also govern the differentiation of macrophages into pro-inflammatory (M1), or anti-inflammatory (M2) states (Navegantes et al. 2017). The presence of LPS, toxins, and neutrophil-associated cytokines such as tumour necrosis factors (TNFs) and interferons (IFNs) drive the differentiation of M0 macrophages into M1 macrophages. M1 macrophages are pivotal in triggering chronic inflammation by
producing ROS/RNS, COX-2 enzyme, and pro-inflammatory cytokines such as IL8, IL18, MCP2, and IFNs that aggravate inflammation. The M1 macrophage-mediated uncontrolled levels of cytokines are primarily responsible for tissue damage and exhibit antimicrobial activity (Ross et al. 2021). On the other hand, the M2 macrophage differentiation is stimulated by the presence of complements, apoptotic factors, and cytokines (IL4, IL13, IL10). The M2 macrophage promotes cell proliferation and tissue repair with the help of anti-inflammatory cytokines (such as IL10, CCL24, IL1ra, and TGFβ) (Yunna et al. 2020) (Fig.1). Both the macrophagic states have a crucial role in resolution, repair, and rejuvenation of the physiological homeostasis. Moreover, the destiny of the immune response is essentially driven by the balance between the levels of M1/M2 macrophages (Yunna et al. 2020). For example, high concentrations of M2 macrophages (tumour-associated macrophages or TAMs) are a predominant characteristic of tumours, while M1 macrophages work predominantly as the first line of defence against intracellular pathogens (Etzerodt et al. 2020; Morrison 2016).

Similar to other physiological processes of human body, the immune response is managed by protein and protein-protein complexes as well. From the initial contact of pathogens with the anatomical barriers to affirming the cytokine-driven macrophagic modalities (presence of M1/M2 macrophages), proteins such as lysozymes, lactoferrin, defensins, and cytokines engage in immune-responsive activities. Immune proteins such as cytokines, immunoglobins, and antibodies drive the intensity of the immune response and are extensively used as diagnostic, prognostic, and therapeutic markers for life-threatening diseases (Tapia et al. 2023). The antigenic/pathogenic proteins are already heavily exploited as optimistic sources of therapeutics; likewise, the immune-associated human proteins are promising targets for developing nano-level formulations against infection and inflammation. Beyond the above-mentioned factors, homeostatic proteins such as serum proteins (albumin,
iron-containing proteins etc.), transporters, and structural proteins are also involved in the immune-associated molecular signalling cascade having potential to be opted as stabilizers and carriers in pharmaceuticals/nanoformulations.

3. Pharmacological advantages of protein-based nanoplatforms

Since the dawn of modern medicine, clinical translation of functional drugs has been a challenging account. Optimal parameters related to pharmacokinetics (i.e. drug response) and pharmacodynamics (i.e. drug activity) are crucial constraints for approval of drugs and their usage worldwide (Liu et al. 2021). On similar lines, pharmacological parameters such as high drug loading and release efficiency, low toxicity, facile elimination process, and targeted delivery are the other prerequisites for the clinical translation of nanomedicine as well. These parameters are governed by the physical and chemical characteristics of the drug and the nanoparticles used in the nanoformulations, which have been elaborately deliberated by Hong et al. and Cao et al. (Hong et al. 2020; Cao et al. 2019). In this regard, the pharmacological characteristics of protein-based nanoplatforms which includes ADME (absorption, distribution, metabolism, and elimination) characteristics along with the efficiency of drug loading and drug release with respect to their physicochemical characteristics (size, polydispersity index, surface properties, and charge etc.) have been eloquently discussed in this section (Hong et al. 2020; Kianfar 2021).

3.1. ADME characteristics of protein-based nanoformulations

The essence of the vitality of pharmaceutical drugs is highly dependent on ADME characteristics. The ease of degradability, compatibility and versatile nature of proteins make them preferable for developing economical drug delivery systems (Habibi et al. 2022). Several traditional drug delivery systems that showed promising results until the initial phases of clinical trials have been aborted due to their ADME score, which is a decisive
factor in the approval of drugs clinically. In the case of traditional drugs, absorption and distribution through the epithelial cells or other tissue barriers to the site of injury have been a serious concern for a long time. Both orally and intravenously administered drugs face these issues due to the difference in their polarity, size, and recognition as immunogenic agents (Cao et al. 2019).

Protein nanoparticles answer these concerns greatly due to their small size, functionalization capacity, ubiquitous presence of their cognate receptors throughout the body, and the endogenous trafficking mechanisms associated with the proteins. Several formulations have been developed, where the protein nanoparticles or their functionalized versions have been highly useful in the absorption and precise distribution of these nanomedicines at oncogenic sites. Ferritin nanoparticles have been observed to show effective absorption via transcytosis, and targeted delivery in the case of cancer treatment due to increased expression of their cognate receptor—transferrin receptor 1 (Khoshnejad et al. 2018). Moreover, significant number of proteins including collagen, albumin, zein etc. has been reported to have better cellular absorption, thus potentiating them as superior nanomaterials for designing nanoplatforms with enhanced biosorption of their loaded cargo (Kianfa 2021). For instance, soy protein nanoparticles (SPNs) enhance the absorption of the loaded Vitamin B12 2-3 times following clathrin-coated and caveolae-mediated endocytosis and macropinocytosis mediated effective internalization (Zhang 2015).

The rate of metabolism and elimination also affects the efficacy of drug delivery systems; the drug release and diffusion rate are highly dependent on the degradation of polymeric nanomaterials (Ravindran et al. 2018; Smith et al. 2017). It is expected of a nanoplateform to withstand degradation within the body until its function is accomplished and degrade with ease afterwards. The stability-degradation paradigm of protein nanoparticles
might differ from protein to protein depending on their intrinsic mechanical strength and preparation method, and often require chemical modifications/crosslinking to enhance their in vivo stability (Jain et al. 2018). Hence, utilization of a manipulatable nanoparticle that exhibits excellent stability inside the human body is a necessity that can be efficiently fulfilled by protein-based nanoparticles. Although, comprehensive reports on the clearance mechanism of protein nanoparticle are yet scarce, studies on Cowpea chlorotic mottle virus (CCMV), and heat shock protein (HSP) nanocages display rapid clearance through renal and faecal routes in a time span of 24 hours without overt retention in any particular organ/cell type (Kaiser 2007). Therefore, it can be deduced that, the metabolism and elimination processes of protein-based nanoparticles are fulfilled either by renal, faecal or hepatobiliary clearance. While small protein therapeutics (up to ~ 60 kDa) are easily removed through the renal clearance pathway after their catabolic breakdown in the body (Glassman and Muzykantov 2019). The larger protein drug delivery systems are generally eliminated via the reticuloendothelial system (RES), which consists of the liver, spleen, bone marrow, and lungs. The RES systems are large repositories of phagocytic cells, such as macrophages, which recognize and eliminate nanoparticles via opsonization and proteolytic degradation (Alexis et al. 2008).

3.2. Drug loading features of protein-based nanoformulations

Drug loading is a critical assessment of the efficiency of drug delivery systems. An intelligent drug delivery system should have a high volume to loading capacity. The drug loading depends on various factors such as drug solubility, nanoparticle size, type of nanomaterial used, and the chemistry of formulation. Moreover, the drug-polymer interaction also affects the drug loading property of the polymer used to prepare nanoparticles (Hong et al. 2020). Three key strategies are followed to enhance drug loading efficiency: (a) post-
loading, (b) co-loading, and (c) pre-loading. These strategies are applied based on the physicochemical properties (structure, surface property, size, and self-assembly) of the base material used to synthesize the nanoparticles (Liu et al. 2020). The post-loading process is facilitated by various mechanisms such as adsorption, non-covalent forces, diffusion, and entrapment (Liu et al. 2020). In the case of protein-based nanocarriers, due to their elastic nature and ability to form polymeric structures, drug loaded gelatine nanoparticles (GNPs) are ideally formulated following post-loading strategy. The loading efficiency of protein nanoparticles varies for each drug molecule and depends on the size of the drug. For instance, GNPs exhibit high loading efficiency for small molecules such as cyclosporine (90%), Paclitaxel (33-78%), and rosiglitazone (90%), while in the case of high molecular weight drugs such as FITC-dextran, amphotericin B exhibit drug loading efficiency lower than 50%. Interestingly, the technique and parameters of nanoparticle preparation also affect the drug loading efficiency of gelatine nanoparticles. Reportedly, the drug loading efficiency of FITC-dextran into GNPs may vary from 10-80% based on the varying parameters used during the one-step desolvation technique to prepare GNPs (Yasmin et al. 2017).

The second strategy is the co-loading method that is often used for nanoparticles with self-assembly properties. In this method, the drug conjugates with nanomaterial, which then self-assembles in a micelle-like structure that leads to the formation of drug-loaded nanoparticles (Bteich et al. 2017). The ability of oligomer formation provides an edge to protein-based nanoparticles to be utilized as efficient drug-loading systems. Moreover, due to the inherent self-oligomerizing characteristics, preparing protein-based drug loading systems is both time and cost-efficient. Abraxane (albumin-bound Paclitaxel) is one of the prime examples of an efficient co-loading strategy utilized as a treatment for metastatic breast cancer (Bertrand et al. 2014). Meanwhile, the third strategy, the pre-loading method, is a process where nanocarriers are fabricated, following the drug loading. This method usually
utilizes highly porous structures such as silica, carbon, and hydrogels with high surface area and tuneable porosity and interact with the pre-loaded drug via non-covalent interactions such as hydrophobic interactions, hydrogen bond, and π–π stacking (Liu et al. 2020). In general, the pre-loading strategy is not a favoured strategy for protein-based nanoplatforms considering that serum proteins can form protein corona/aggregates over the drug loaded nanoparticles, often reducing their biodistribution and bioavailability, thus making post- and co-loading as much preferred strategies for drug loading.

3.3. Drug release properties of protein-based nanoformulations

Besides drug loading, drug release is another significant parameter for efficient drug delivery systems (Couvreur and Puisieux 1993). The two critical factors affecting the drug release pattern are: (a) rate of diffusion, and (b) rate of polymer dispersion/degradation, which work independently or synergistically. Due to the biodegradable nature of proteins, the release of drugs from protein-based nanoparticles is an effective process, as it enhances the drug release and lowers the toxicity of nanoparticles (Saxena et al. 2005; Zwiorek et al. 2004). Besides diffusion and dispersion rate, the drug solubility, charge, and strength of drug-polymer interaction also affect the release rate. It has been reported that, hydrophobic drugs are released at a slower rate than hydrophilic drugs from gliadin nanoparticles due to the strong interaction between the gliadin protein and the hydrophobic drugs (Duclairoir et al. 1998). Additionally, the size and surface of nanoparticles are inversely proportional to the drug release rate. This process has been well studied in the case of GNPs, and three probable mechanisms, namely, (a) proteolytic degradation, (b) self-diffusion, and (c) release via surface erosion of polymer, have been observed (Yasmin et al. 2017; Khan and Schneider 2013). Apart from the mechanisms, the drug release process follows a biphasic pattern wherein, primarily a sudden burst release of weakly interacting drug molecules, followed by
the slow diffusion of the tightly or covalently bound drugs from the polymer material occurs \cite{Kamaraj2017}. The kinetics of biphasic release is a crucial factor defining the prolonged or sustained release of the embedded drug molecules \cite{Gaihre2009}. Mzoughi et al. studied the biphasic drug release from the rolled-up gelatine capsule with a hollow cylindrical shape with the help of model fluorescent drugs. The study revealed a steady diffusion of drug molecules present within the rolled-up layers with a lag time dependent on the radial position of drug reservoir. This steady release is often necessary for pain management and inflammatory pathologies such as migraines and hypertension \cite{Mzoughi2021}. Similarly, collagen peptide-chitosan nanoparticles exhibit a biphasic pattern of doxorubicin release for the first 20 hours followed by a sustained release over seven days for anticancer therapy \cite{Wu2016}. These instances prove protein nanoplatforms as a suitable choice for sustained drug release and owing to the properties of protein nanoparticles discussed till now, it can be inferred that protein nanoparticles are an indelible choice compared to their traditional counterparts.

### 3.4. Targeted delivery of protein-based nanoformulations via tailored functionalization

The functionalization of protein nanoparticles aids in elevating the formulation half-life, stealthiness, and effectiveness of the protein-drug nanoformulations. The functional groups usually conjugate via covalent bonding and disulfide linkage with polar residues such as lysine (Lys), glutamic acid (Glu), aspartic acid (Asp), tyrosine (Tyr), and cysteine (Cys) present on the protein surface. Such amino acids are highly amenable for linkage with chemical and biological groups such as drugs, metal ions, peptides (RGD and M2pep), fatty acids, lipids, antibodies (mAb), and aptamers \cite{Tian2021}. The addition of these functional groups can be achieved by two methods namely, (a) modification by chemical reaction, and (b) modification by genetic engineering approaches.
**Protein nanocarrier/nanoparticle modification by chemical reaction**

Chemically induced functionalization of protein nanoparticles includes addition of a functional group to the protein surface with the help of chemical reactions such as, esterification, sulfation, phosphorylation, and carbodiimide linkage etc. These functional groups can either be chemical groups such as phosphate, amine, carboxyl, and sulphate groups or biological entities such as cell-surface targeting peptides, nucleic acid, and saccharide/fatty acid units. Several studies have reviewed the stability and drug delivery enhancing effect of chemical-based functionalization on protein-drug nanoformulations. Functionalization of ferritin-based nanoparticle systems with β-cyclodextrins, M2 macrophage-targeting peptide (M2pep) and arginine-glycine-glutamic acid motif (RGD) have been assessed and reported for their efficient and sustained drug release together with site-specific deliverable characteristics in different types of cancer (Khoshnejad et al. 2018; Abdelhamid et al. 2023; Xu et al. 2022). SP94 peptide-functionalized heat shock protein nanoparticles (HSP NPs) are known to target hepatocellular carcinoma cells (HCC). The SP94 peptide is a hydrocarbon stapled peptide that exclusively interacts with the HCC cells; hence it is used to elevate the localization of HSP NPs in the HCC tumour microenvironment (Sandra et al. 2019). Further, functionalization of the most popular protein nanocarrier *i.e.*, albumin nanocarrier/nanoparticles has also been extensively studied. Albumins have been functionalized with the help of several methods such as carbodiimide coupling, thiol/maleimide coupling, and general covalent linking of cysteines and lysine side chain with drugs and peptides (Loureiro et al. 2016). The alteration of charged surface amino acids via covalent conjugation with vitamin B9 (FA), and albumin-drug conjugates such as albumin-methotrexate and albumin-vancomycin formulation are known for their improved pharmacokinetic and targeted delivery of anticancer (Vitamin B9 and methotrexate) and antimicrobial (vancomycin) drugs (Ebrahimnejad et al. 2022; Spada et al. 2021; Jacob et
Several FDA-approved glucagon/insulin peptide functionalized albumin-based therapies such as Levemir, Tresiba, and Victoza are widely used in treatment of diabetes (Loureiro et al. 2016).

**Protein nanocarrier/nanoparticle modification by genetic engineering**

In addition to the chemical modification of protein nanoparticles, genetic engineering approaches are utilized in developing post-translationally modified proteins (such as sialylation, nitration, and glycosylation), chimeric proteins, chimeric antibodies, and virus-like nanoparticles (VLPs) (Krishnan et al. 2023). In general, the genetic engineering techniques are widely used in preparation of vaccines against infectious diseases. The most common implementation of such techniques is known in the case of pulmonary infections like Influenza, Covid-19, and MERS. For example, Cervarix and Gardasil are two highly successful VLPs used as HPV vaccines made up of self-assembling HPV capsid protein (Yllescas et al. 2022; Haghshenas et al. 2017). Likewise, SpyTag/SpyCatcher ligation system is used to display therapeutic proteins on encapsulin (Encaps) with the help of SpyCatcher protein such as EGFR, cancer cell-specific antibodies etc (Sutherland et al. 2019). Like the aforementioned method, both chemical and biological groups can be added to the protein-nanoparticle which mainly results in the formation of protein-protein nanoparticles. For generation of chemically modified proteins, the biological phenomena of post-translational modifications are exploited for which the protein nanoparticles are expressed and isolated from eukaryotic system such as yeast and insect cell lines. While in case of chimeric proteins and VLPs, genetically modified clones which express fusion proteins and self-assembling/oligomerizing proteins are used and purified from both prokaryotic and eukaryotic systems (Iravani and Varma 2019). These functionalized protein-protein nanoformulations are further loaded with drug molecules or utilized as
vaccines for inflammatory and infectious diseases. A detailed information of such nanoformulations has been provided in the following section(s).

4. Protein-based nanoplatforms for targeting infectious and inflammatory diseases

Protein-based nanomedicines have been formulated through various bioengineering and self-assembling platforms such as virus like nanoparticles (VLPs), nanocages, micelle like structures, and engineered recombinant nanoarchitectures etc. Considering the versatility of proteins, they can be either applied as therapeutic molecule or drug delivery system; or a combination of both (Diaz et al. 2018). The protein-based nanoplatforms can be classified into two broad categories: (a) protein-based nanocarriers, and (b) protein-based nanotherapeutics. Nanocarriers here are the protein nanoparticles primarily used for transporting drugs (small molecules, proteins, lipids) to the site of inflammation. On the contrary, protein nanotherapeutics are the protein-based nanoparticle or nanoplatforms loaded with protein components having therapeutic activity in clinical conditions. The following sections specifically describes the potential of protein-based nanocarriers and nanotherapeutics for targeting infectious and inflammatory disorders.

4.1. Proteins as nanocarriers against infection and inflammation

Protein-based nanocarriers are of three types — adjuvants, carriers, and presentation platforms (Zhao et al. 2014). The adjuvant platforms are emulsions of drug and protein nanoparticles, while carriers and presentation platforms carry drug molecules by encapsulation or surface attachment. Protein nanocarriers are extensively used for a wide range of functions, such as targeted drug delivery, stability, improved ADME characteristics, stealth effects, and sustained release of drugs (Nguyen and Tolia 2021). Moreover, due to their local abundance in the human body, proteins positively drive drug efficacy and bioavailability. In general, protein-based nanocarriers have been developed from various
plant, serum and immune proteins such as legumins, albumins, ferritin, gelatine, lysozyme, and lipoproteins etc., and has been elaborately reviewed recently by several literatures (Hong et al. 2020; Habibi et al. 2022). The current review specifically emphasizes the role of protein based nanocarriers developed using albumin, gelatine/collagen, ferritin, and lysozyme; and have entered into the clinical/preclinical trial phase for treating inflammatory/infectious disorders during last 5 years, as these classes of proteins have a human origin and also presumed to be less toxic and less immunogenic (Table 1) (DeFrates et al. 2018).

4.1.1. Human serum albumin nanocarriers

Human serum albumin (HSA, MW: ~ 66 kDa) is one of the most abundant plasma proteins extensively used to study the toxicological effects of drugs and protein-drug interactions. Moreover, in recent years due to its efficacy of interaction with drug molecules and long half-life, albumin has been utilized as protein nanocarriers for therapeutics and theranostic applications (Langer et al. 2003). Albumins are classically utilized as the structural unit of nanoparticles/nanocarriers due to their biocompatibility, presence of different functional groups (high binding capacity), and stability under various environmental conditions (Elzoghby et al. 2012). Similar to other protein-based platforms, the HSA-drug delivery systems are mainly available in three kinds of formulations which are drug-HSA complex/conjugate (adjuvant), drug-HSA nanoparticle (nanocarrier), and HSA-antibody binding complexes (presentation platforms) (Kianfar 2021). The drug/peptide/antibody fragments bind effectively with albumins through non-covalent and covalent bonds, or indirectly with the help of an intermediate ligand. The acidic nature of albumins is essential in their interaction with several molecules, such as vitamins, eicosanoids, and metal ions (such as calcium, copper, and zinc). Due to its high drug-binding capacity, it can easily
interact with drugs such as sulphonamides and benzodiazepines and deliver them to their site of action (Spada et al. 2021).

Several albumin-based nanoformulations are commercially available and are popularly used for the treatment of cancer (Patel et al. 2021; Rahimizadeh et al. 2020). One of the classic examples of an albumin-based nanocarrier is Abraxane which consists of an anticancer drug paclitaxel encapsulated inside the albumin nanoparticle (Ishima et al. 2022) (Fig 2A). Paclitaxel is drug originally administered as a chemical formulation solubilized in cremophor EL (non-ionic surfactant oil) or polysorbate 80, which are responsible for hypersensitivity reactions and decreased drug clearance and distribution. The paclitaxel bound albumin nanoparticles are devoid of the toxic solvents and confer greater efficiency to paclitaxel for its antitumor activity (Miele et al. 2009; Bhattacharyya et al. 2015; Green et al. 2006). Owing to its effective anti-tumour activity, Abraxane is a widely accepted protein-based nanodrug administered in the treatment of metastatic breast cancer, adenocarcinoma, and non-small cell lung cancer. The invention of abraxane have led to development of various other albumin-bound (NAb) anti-cancer drug combinations such as NAb-rapamycin/temozolomide/irinotecan hydrochloride (childhood neoplasm), NAb-rapamycin/pazopanib (soft tissue sarcoma), NAb-gemcitabine/paclitaxel (adenocarcinoma), which are under initial phases of clinical trials and tabulated in Table 1.

Similarly, albumin-based nanocarriers have been tested against viral diseases such as hepatitis C, and inflammatory diseases such as rheumatoid arthritis (Karami et al. 2020; Keam 2022; De Felice et al. 2019; Patel et al. 2021; Rahimizadeh et al. 2020). Tacrolimus (Tac) and methotrexate (Met) are anti-inflammatory drugs, which lowers the expression of inflammatory cytokines and are used in treatment of rheumatoid arthritis. The encapsulated albumin nanoparticles formulations of these drugs have been developed, and tested for the
enhanced therapeutic index due to specific targeted delivery of Tac and Met at the site of inflammation (Udalova et al. 2016; Takakura et al. 1990). Ozoralizumab is another nanodrug comprising of two anti-TNF nanobodies and one anti-HSA nanobody that is used for the treatment of rheumatoid arthritis (Ishiwatari-Ogata et al. 2022). Further, formulations such as albuferon, a fusion protein comprising of HSA and human interferon is an albumin-based drug for treatment of hepatitis C, which has shown remarkable pharmacokinetic characteristics and is currently under the third phase of clinical trials (Pilati and Howard 2020). Apart from these, albumin based nanocarrier have been exploited for cancer diagnostics as well. 99mTc Nanocoll is an albumin nanocolloid that is popularly used in nuclear medicine imaging (Aleksyniene et al. 2022).

4.1.2. Ferritin-based nanocarriers

Ferritin is a globular iron-containing metalloprotein that is ubiquitously present in all organisms. It is a multimeric protein that consists of 24 self-assembled subunits that form a hollow spherical shell (apoferritin) surrounding an inorganic core of hydrated iron oxide ferrihydrite (Plays et al. 2021). Due to its ability to self-assembling and unique structural interfaces, ferritin exhibits excellent potential as a drug delivery system (Han et al. 2014). The outer surface of the protein shell can be easily modified chemically, and functional motifs such as RGD motifs, antibody fragments, and small molecules can be added. Similarly, the inner surface is highly compatible with metal and small molecule binding, enabling efficient loading of metal oxide nanoparticles and small drug molecules (Khoshnejad et al. 2018; Rodrigues et al. 2021). Moreover, the ferritin and fusion/chimeric ferritin (chemically modified) nanoparticles can be easily expressed in bacterial systems due to their conserved nature, making their production and availability cost-efficient (Hong et al. 2020). Due to its versatile binding capability towards multiple ligands, ferritin-based
nanoparticles are broadly utilized in producing vaccines, targeted delivery systems, and diagnostic purposes for cancer and viral diseases.

Over the years, several ferritin-based nanocarriers have been developed, clinically tested, and are commercially available for treating infectious and inflammatory diseases as summarized in Table 1 (Song et al. 2021). For instance, doxorubicin (Dox) delivery systems using H-ferritin nanocages (Dox-HFn) have been studied by Liang et al. for their efficiency for targeted delivery in comparison with a clinically approved liposomal-Dox (Doxil) nanocarrier against tumor growth. The study inferred an increase in the drug release efficiency of dox-HFn and ten times higher intratumoral drug concentration compared to free Dox and Doxil. The efficient drug release capacity of HFn nanocages can be attributed to their specific binding with the transferrin receptors on the tumor cells, which enhanced the internalization of Dox-loaded nanocages inside the tumor and exhibited a higher survival rate and lower toxicity profile (Yang et al. 2019; Liang et al. 2014). Other than cancer, ferritin-based nanoparticles have been used for vaccine development against infectious diseases such as SARS-CoV-2, influenza, and HIV-1. A FDA-approved ferritin-based nanovaccine “Novavax” was hugely successful with its broad range protection against different strains of SARS-CoV-2 (Vu et al. 2021) (Fig. 2B). Similarly, ferritin-based nanoparticles conjugated with hemagglutinin and multiple HIV-specific epitopes have been tested pre-clinically and are currently under clinical trials, which show favourable responses against influenza and HIV-1, respectively (Nguyen and Tolia 2021).

4.1.3. Collagen/gelatine nanocarriers

Like albumins, collagen/gelatin is one of the most abundant structural proteins in vertebrates, accounting for 20-30% of the body's total protein. Due to its high availability, collagen's immune response against collagen nanoparticles is meagre (Arun et al. 2021).
Additionally, the triple-helical structure (with the Gly-Pro-Ala repeat sequence) of collagen increases its surface area and absorption capacity, thus making collagen a suitable drug carrier system for antimicrobial and steroid drugs. Moreover, as the collagen/gelatine can easily escape the reticuloendothelial system, it is an efficient carrier for anti-HIV and neuroprotective drugs which must surpass the plasma membrane and blood brain barrier respectively to perform its activity (Alarcon et al. 2012). Although collagen can be used as a nanocarrier, gelatines are the most common form of collagens in nano formulations (Kianfar 2021). Gelatines are of two types: Type A gelatine is cationic and produced by partial acid hydrolysis of pig skin collagen, while type B gelatine is extracted from bovine collagen by alkaline hydrolysis.

The polymeric properties of gelatine/collagen have been exploited to form drug-embedded matrigels and nanoparticles to treat external injuries and severe infections. Considering the high absorption capacity and scaffold formation, several collagen-based nanocarriers and presentation platforms have been developed that mediate wound healing, tissue regeneration, and skin grafting other than the delivery of drugs (Rafieian et al. 2019). Recently, Tian et al. demonstrated the wound healing characteristics of elastin/gelatine-based hydrogels via promotion of innate immune cell recruitment and angiogenesis process (Fig 2C). The elastin present in the hydrogels was found to stimulate the promotion of both M1 and M2 markers M1 macrophages in vitro and attracted neutrophils and tissue regenerative M2 macrophages in the murine wound model (Tian et al. 2022). Similarly, Patel et al. showed the potential of targeted drug delivery of lupeol, an anti-inflammatory terpenoid via gelatine-chitosan which promoted the oxygen supply for proper wound healing with a biphasic drug release pattern till 24 h of application (Patel et al. 2018). Likewise, collagen encapsulation of doxorubicin-gold-hydroxyapatite nanoparticles (DoAuHAp NPs) and doxycycline-silver nanoparticles (DdAg NPs) have demonstrated enhanced drug loading,
drug release, and antibacterial action compared to the normal DoAuHAp and DdAg NPs (Mondal et al. 2019; Kurakhmaeva et al. 2009). Other than scaffolds, gelatine nanoparticles are utilized as ophthalmic solutions for treatment of eye-related disorders such as dry eye syndrome. Epigallo-catechin gallate encapsulated gelatine nanoparticles and gelatin-based mucoadhesive are two examples of gelatine-based nanoformulations that help in lowering the cytokine expression (IL-1B, IL-6, IL-8, and TNF-α) and thus inflammation by enhanced absorption and sustained release of hydrophilic drugs such as EGCG into the ocular compartments by crossing the lipophilic corneal epithelium barrier (Luo and Lai 2017; Fangueiro et al. 2016; Zaheer et al. 2022).

4.1.4. Lysozyme-based nanocarriers

Lysozymes are universal antimicrobial innate immune defence proteins consisting of a single polypeptide chain comprising four disulfide bridges. This essential protein cleaves the peptidoglycan layer of bacterial cell wall through hydrolysis of the β-1,4 linkages between N-acetyl-d-glucosamine (NAG) and N-acetylmuramic acid (NAM) residues (Ferraboschi et al. 2021). Due to their potent antimicrobial effect, lysozymes have been actively incorporated into medicines for wound healing, skin infections, tuberculosis, and ocular infection (Rananaware et al. 2022). Comparable to the proteins discussed above, lysozyme has a highly stable structure that can be readily synthesized using recombinant DNA technology and stored in the crystallized form for long-term use (Sarkar et al. 2020). Moreover, lysozymes contain a metal ion binding cavity essential for efficient antimicrobial activity (Das et al. 2020). Due to such versatile characteristics, several attempts have been made to develop lysozyme-based nanoformulations in recent years (Das et al. 2020).

Lysozymes are reported to cure otorhinolaryngological inflammatory diseases (such as acute pharyngitis) that are caused by bacterial infections (Wu et al. 2017b). For instance,
the synergistic effect of ampicillin functionalized lysozyme capped gold nanoclusters has been tested for their antimicrobial effect against MRSA infections (methicillin-resistant *S. aureus*). The functionalized lysozyme nanoparticles exhibited enhanced antibacterial activity in *in vitro* experiments and enhanced drug loading capacity of ampicillin (*Kalita et al. 2018*). Further, lysozymes exhibits anti-tumoral activity by increasing the ROS production and hence lysozyme nanoparticles loaded with drugs like gold nanoparticles, curcumin have been explored for their tumour-proliferative, anti-cancer, as well as anti-bacterial activity (*Somu and Paul 2021; Hameed et al. 2020*). Lysozymes also provide a potential platform for the delivery of hydrophobic and poorly soluble drugs. In this line, quercetin (Que) loaded self-assembled micelle like lysosome nanoparticles (LNPs) are worth mentioning. These positively charged Que/LNPs were made pH responsive by absorbing into oxidized starch microgels (OSM) via electrostatic interaction finally synthesizing Que/LNPs/OSM nanocomposites. Lysozyme nanoparticle-in-OSM microgel delivery system provided great mucoadhesive properties along with prominent biphasic pattern of drug release. These nanocomposites have prospective for application as antitumor, anti-inflammatory regimens owing to their Que cargo (*Fig. 2D*) (*Li et al. 2020*).

### 4.2. Proteins as nanotherapeutics for infectious and inflammatory diseases

The first protein-based therapeutic that has been approved by FDA was the recombinant human insulin in the 1982 by FDA, but the history of protein therapeutics dates back way before that in the form of viral particle by Edward Jenner (*Riedel 2005*). The potential of protein nanotherapeutics has been explored vividly in diverse infectious and inflammatory diseases since the beginning of this century. However, considering the range of inflammatory and infectious diseases, implication of protein nanoformulations as treatment regimen is quite lagging, and are limited mostly to cancer, and viral infections, such as
SARS, MERS, influenza, RSV, and HIV. The protein formulations developed as nanotherapeutics can categorically be divided into two types: (a) either the protein itself functions as an effector and can directly be introduced in the body as a therapeutic or, (b) the active protein component is conjugated to/encapsulated using other nanoplatforms for enhanced efficiency. Although these nanoplatforms range from metal organic framework, lipid nanocarriers, polymers, other organic/inorganic/bioinspired nanoparticles, considering the well accounted documentation of these nanotherapeutic systems in the extant literature, this section kerbed the focus to the protein nanotherapeutic formulations with proteins exclusively or active protein core with proteinaceous carrier (Iyer et al. 2015; Zheng et al. 2018). The current section deals with the advances of such protein nanotherapeutics based on their disease-oriented applicability, and has been charted in Table 2.

4.2.1. Protein nanotherapeutics for infections

Bacteria, viruses, and protozoa are the major sources of infection and inflammation in human body and are a challenge to the pharmaceutical research. Their antigenic proteins have been a proficient target to develop potential anti-infectious formulas. Unfortunately, bacterial and protozoan infections have not been much explored to develop protein therapeutics. There are a few scattered instances of protein nanotherapeutics against bacterial pathogens: *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli* (Shiga toxin) (de Souza Morais et al. 2018; El Bissati et al. 2017; Kaba et al. 2012). While malaria and toxoplasmosis are two protozoal infections for which protein nanotherapeutics have been tested (Karch et al. 2017). A few recent literatures have systemically accounted these protein nanotherapeutics based on the type of causal pathogens (Butkovich et al. 2021; Tapia et al. 2023) enlightening the diverse applicability of proteins as nanomedicine/nonvaccine platform in varied infectious and inflammatory diseases. However, viral infectious diseases, especially
lower respiratory tract infections such as influenza, SARS, MERS, RSV are the best studied systems opting protein as nanotherapeutic preventive measure. Scarce studies on protein nanotherapeutics against some other viruses viz. dengue, Hepatitis B, Epstein–Barr virus etc. are also accounted (Clark et al. 2011; Hiriart et al. 2017; Kanekiyo et al. 2015; Silva et al. 2012).

**Respiratory Syncytial Virus (RSV)**

RSVs are the foundational pathogen of lower respiratory tract infection and are the reason for common cold and pneumonia in children. In Sf9 insect cell derived Respiratory Syncytial Virus (RSV) fusion protein based multimeric nanoparticle vaccine with aluminium phosphate (AdjuPhos) is under trial, which supports its usage strategy in maternal immunisation to protect infants from RSV associated diseases (Glenn et al. 2013; Muñoz et al. 2019). Similar structure based self-assembling multimeric vaccine featuring a ferritin core attached to eight trimeric pre-F spikes (DS-Cav1) of RSV bearing engineered glycans and DS-Cav1 attached to trimeric glycan (two component) nanoparticles are under preclinical studies for their neutralising antibody (NAb) production capacity (Marcandalli et al. 2019; Swanson et al. 2020). Palivizumab (Synagis®, Medimmune) is a monoclonal neutralizing antibody licensed as a preventive treatment for RSV which targets a conformational epitope of the fusion (F) protein specified as FsII. The antibody itself cannot prevent infection, rather it reduces the burden of bronchiolitis. The N (nucleoprotein) protein of RSV is a major target of antigenic specificity to towards cytotoxic T cell response. The other component, palivizumab-targeted FsII epitope modified chimeric N protomer self-assembled nanorings effectively reduce the viral load in treated mice. It presents an attractive vaccine candidate which combines N specific cellular immunity as well as F specific antibody protection (Hervé et al. 2017). Further, a de novo multivalent viral antigen presenting scaffolding
platform tailored engaging trimeric ectodomains of influenza, HIV, and RSV, able to adapt geometrical nanostructures have been developed to investigate the robust influence of immune response to vaccination (Ueda et al. 2020). These kinds of engineered platforms can act as potential one stop protection regime against multiple viral infections.

**Influenza**

Influenza is a common infectious disease caused by influenza viruses, which on severe cases may lead to pneumonia and acute respiratory distress syndrome. There are multiple regimens clinically approved or under clinical trial which utilises the trivalent inactivated subunit of influenza with hemagglutinins (HA) to circumvent influenza infection (Table2) (Giezeman et al. 2009). Still owing to the continuous evolution of the virus, there remains the need of novel and more potent formulations to combat the disease. HA and matrix protein 2 (M2e) are the antigenic determinants of influenza virus, which shell around the influenza N protein core forming bilayered protein nanoparticle eliciting robust/potent and long-lasting T cell specific immune responses, thus bestowing influenza viral cross-protection (Ma et al. 2022). A ferritin-H2HA based nanovaccine to tackle influenza is under phase 1 clinical trials (NCT03186781) (Houser et al. 2022). Another recent strategy targets influenza by the development of homologous influenza A virus (PR8)-specific P22 vaccine where the globular head of the HA proteins were fused with the Salmonella P22 viral capsid and subjected to PR8 influenza A infected mice trial (Sharma et al. 2020). Indeed, the M2e protein self-assembled nanostructures are under trial for their anti-influenza activity. Fusogenically engineered immunogenic adjuvant (such as Brucella outer membrane protein BP26 containing) was attached to M2e constructed nanobarrels comprising of four/eight tandem repeats for providing better protection to influenza infected mice (Kang et al. 2021). Further, another synthetic recombinant vaccine against influenza has been reported, where a
mosaic hypervariable receptor-binding domain (RBD) of viral hemagglutinin displaying ferritin nanoparticle provided sufficient avidity to the cross-reactive B cells by eliciting neutralizing antibody response against H1N1 influenza variants that were identified almost over the last century (Kanekiyo et al. 2019).

**Middle East Respiratory Syndrome (MERS)**

Currently, there are no approved therapeutic or vaccination regimen available for MERS. The spike protein of MERS has been reported to be the primary target, as antigenic determinants engaged in the receptor binding and virion entry to the host cells. Simple MERS spike protein nanoparticle in combination with Matrix-M1 adjuvant is potent enough to establish Th1 and Th2-biased and anti-S neutralizing antibody mediated protective effect against MERS (Jung et al. 2018; Coleman et al. 2017). The recognition and interaction between the receptor binding domain of the Middle East Respiratory Syndrome (MERS-CoV) with the human receptor DPP4 (hDPP4) is essential for MERS-CoV mediated infection initiation thus it aids as a potent antigenic target for inducing antigenic responses against infection. MERS trimeric spike proteins and the receptor binding domain (RBD) fusion foldon trimerization has also been effective in binding with DPP4 receptor inducing proactive neutralizing antibody response in vivo (Tai et al. 2016). Moreover, RNA binding controls the antigen folding kinetics of nanochaperons and nanoparticle assembly. Molecular insights into bacterioferritin-RNA interaction domain (RID) fused MERS-CoV RBD serum immunisation evidenced for blocking of the RBD-hDPP4 interaction, and stimulation of strong IgG1, IgG2a and IgG2b mediated responses against MERS (Kim et al. 2018). On similar lines, Okba et. al. designed an RBD coupled Lumazine synthase (LS) nanoparticle utilizing antigen-SpyTag/LS-SpyCatcher mechanism, which promisingly arrayed as a multivalent high-quality
cross clade neutralisation of MERS-CoV in rabbits and proved to be a prospective platform for MERS vaccine development and clinical translation (Okba et al. 2020).

Severe acute respiratory syndrome coronavirus (SARS CoV)

SARS CoV-19 pandemic has revolutionised the vaccinology with its epidemiological impact and prompt approval of drugs under emergency response, where protein nanotherapeutics played a key role in SARS vaccination strategy. The antigenic determinant identified was the spike (S) protein that binds to the human ACE2 receptor with specific RBD in the spike 1 (S1) subunit facilitating its entry into the host lung and intestinal epithelial cells (Yong et al. 2022). After the onset of the previous outbreak of SARS CoV in 2012 vaccine development was initiated, and as a result novel nanovaccine containing full spike protein of MERS-CoV and SARS-CoV in combination with Matrix M adjuvant was developed as a potent vaccine for infected murine models (Coleman et al. 2014). However, after the second outbreak in 2019, the pace of vaccine development and clinical trial accelerated to address the dire need. RBD decorated ferritin based engineered multi-layered S2GΔHR2 I3-01v9 SAmpNP produced multidimensional immune responses (Fig.3A). They include: (a) high antibody titre, (b) better CD4⁺ T cell and cytolytic CD4⁺ T cell responses, and (c) GM-CSF induced production of CD8⁺ effector T cells. These responses promote the generation of macrophages and functional dendritic cells (DCs) further facilitating the clearance of infected cells (He et al. 2021). Virus like nanoparticles with spike protein are the key players in this field, and six such potential formulations are currently under clinical trials as enlisted by the World Health Organization (WHO), which includes: two self-assembled VLPs, one MLV-based chimeric VLPs containing ectodomain and spike protein and other three containing Covid-19 Spike RBD. HBsAg (hepatitis B surface antigen) VLPs displaying SARS-CoV-2 RBD using yeast as a production system and is under
phase1/2 clinical trials. Considering the dreadfulness of the past pandemics, apart from the protein nanoformulations in clinical trials, a range of other novel formulations are under development against SARS-CoV related infections (Hsieh and McLellan 2022; Sung et al. 2021; Wang et al. 2023a).

**Acquired Immunodeficiency syndrome (AIDS)**

AIDS, is an acquired chronic immune interference caused by the human immunodeficiency virus (HIV). *In vivo/vitro* self-assembly protein nanoparticles have been significantly employed from time-to-time to develop anti-HIV treatments. For majority cases the target antigen is a part of envelope glycoprotein (Env), a trimer of heterodimers, consisting of noncovalently interacting three gp120 and three gp41 subunits. The basic units of these self-assembling protein-nanoparticles can have one of the multiple components fused together which can self-assemble *in vitro* or *in vivo*. For instance, ENV fused ferritin *in vivo* multimeric self-assemblies have been found to induce significant antibody titres priming immunisation (He et al. 2018; Sliepen et al. 2015). However, *in vitro* assembling nanoparticles allow more control over quality by purification in native form, thus are preferred now a days over the *in vivo* counterparts. One such nanoparticle is Env-I53-50NP composed of I53-50A (20 trimeric) and I53-50B (12 pentameric) subunits fused with viral glycoproteins. Rabbit immunisation with this formulation had generated superior neutralising antibody response compared to the *in vivo* Env-ferritin assemblies (Brouwer et al. 2019). V1V2 loop of RV144 enveloped paratope is another potential target for vaccine development (Aikins et al. 2017). Another nanoplatfrom, MPER-SAPN synthesized by the incorporation of membrane proximal external region (MPER) of HIV-1 gp41 to the N terminal of a pentameric tryptophan-trimeric leucine zipper self-assembling icosahedron nanoparticle had also induced humoral anti-HIV neutralising responses (Wahome et al. 2012).
4.2.2. Protein nanotherapeutics for cancer

A significant number of peptides and protein-based cancer vaccines and treatments have been developed and tested for multiple oncogenic ailments and are in various stages of clinical trials. Tumour associated antigens (TAAs) are proteins displayed on tumour cells trigger immune response, but peptides and protein conjugates targeting TAAs produce low immunogenicity in the tumour microenvironment (Neek et al. 2019). Thus, to overcome this issue TAA-based cancer vaccines are combined with immune activating adjuvants such as, MF59 (oil in water emulsion), aluminium salt, monophosphoryl lipid A (MPL), toll like receptors of antigen presenting cells (CpG, imidazoquinolin) in preclinical and clinical trials (Higgins et al. 2007; Shirota et al. 2015; Smith et al. 2016; Temizoz et al. 2016). Despite these strategies, tumour escape is a common phenomenon and can be addressed by combining multiple heterogenous TAA peptides in a single platform. Nevertheless, identification of optimal antigens, adjuvants and delivery mode remains as issues of concern for formulating next generation vaccines. In this context, protein components caged inside protein nanostructures are preferred, as they elicit improved immune responses against cancer.

On this note, VLPs are advantageous being efficient immunogenic and stable platform lacking mammalian replicable genetic material (hence, non-infectious to the host). Diverse viral particles CPMV, PVX, TMV, bacteriophage Qβs are employed in cancer defence opting wild type or engineered antigenic peptides (Her2, Tn, p15e, Melan-A26-35 etc.) as therapeutic targets with or without adjuvants (Neek et al. 2019). In this context, it is worth mentioning that melanoma specific Melan-A/Mart-1 peptide conjugated Qβs with TLR9 adjuvant CpG is an efficient cancer nanovaccine under phase 1/2 clinical trial (Goldinger et al. 2012; Speiser et al. 2010). A slightly modified version of this formula with antibody anti-
PD1 (Pembrolizumab) is being tested for advanced stage melanoma patients (Zawit et al. 2021).

Protein cages synthesised for heat shock protein (HSPs), E2, and ferritins provide an attractive platform for protein nanoparticle delivery to tumors. HSPs especially, are overexpressed in cancer cells and usually bind to cancer antigens forming HSP-tumor antigen complexes (Wu et al. 2017a). Considering these pathophysiological responses, recombinant HSP nanoparticle bearing HER2, gp100, MAGE1 cancer antigens have been developed (Ge et al. 2009; Manjili et al. 2002; Wang et al. 2003; Zhang and Zheng 2013). These formulations display generation of high level of IFNγ, TH cell activity in preclinical studies. HSP glycoprotein 96 peptide nano-complex (HSPPC-96) is another potential protein nanoformulation which shows minimal toxicity with significant efficacy in clinical trials. The phase 2 and 3 clinical trials have been promisingly shown sustained anti-tumour (against glioblastoma, non-small cell lung cancer-NSCLC, melanoma, pancreatic adenocarcinoma and colorectal cancer) activity by the activation of both innate and adaptive immune systems (Ampie et al. 2015) (Fig.3B). Epidermal growth factor receptors (EGFR) also overexpress in most cancer cells and acts as a potential target for cancer treatment. Various anti-EGFR antibody fragment loaded engineered E2 protein assembled nanoparticles specifically target EGFR overexpressing cancer cells (Buecheler et al. 2015).

As described in section 2, chemokines are ubiquitous in all inflammatory events and cancers are no different. Anti-inflammatory chemokine CCL21 loaded protein vault nanoparticles can efficiently endorse the recruitment of T lymphocytes and dendritic cells into the tumor microenvironment leading to antitumor activity towards lung cancer (Kar et al. 2011). As cancer cells are ever evolving and follow multiple alternative escape pathways to survive, it is essential to device multi-antigenic synergistic formulations and protein
nanoplatfroms with protein carriers that can act as feasible antigen based immunotherapeutic in this milieu.

5. Fate of other proteins in the development of nanotherapeutics

Besides albumins, ferritins, collagen/gelatin and lysozyme, several immune-associated and blood proteins, such as haemoglobin, mucins, antibody fragments, and cytokines, have also been used as potential protein polymers to develop protein-based therapeutics. For instance, several attempts have been made to produce semi-artificial red blood cells containing haemoglobin, and highly advanced haemoglobin-based oxygen carriers in the past decade to counter the short half-lives, circulation times, and bioavailability of haemoglobin (Funaki et al. 2019; Jansman and Hosta-Rigau 2018). Moreover, Hosaka et al. developed haemoglobin-HSA-Platinum (Pt) nanoparticles to slow down the conversion of active haemoglobin to its inactive state (methaemoglobin) and found that the HSA-Pt group of the nanoparticles enhanced the half-lives of haemoglobin together with no toxic effect on vital organs during the in vivo preclinical studies (Hosaka et al. 2014; Haruki et al. 2015; Iwasaki et al. 2018). Similarly, Transferrin, mucin, antibodies, and antibody fragments have been utilized to prepare protein-based nanoparticles and have been briefly summarized in Tables 1 and 2.

Besides antibodies and enzymes, research has been conducted in bits and pieces, where cytokines-based nanoparticles have been proposed as a source of immunostimulants (Torrealba et al. 2016). For example, Lopez-Cano et al. evaluated the inclusion bodies (IB)-based cytokine (swine IL1β, IL6, IL8, TNFα) nanoparticles (IB-cyt NPs) to assess their immunostimulant efficacy on the intestinal mucosa of piglets. These IB-cyt NPs were recombinantly expressed and isolated from Lactococcus lactis subsp. cremoris NZ9000 and tested for their immune-modulatory response with the help of in vitro and in vivo pilot studies.
Similarly, several chemokines have been tested for their potential use as nanocarriers/therapeutics. For instance, Du et al. established that chemokines such as CXCL4 can form nanoparticles with DNA that superinduces TLR-driven immune inflammation (Du et al. 2022). Similarly, erythrocyte-anchored chemokine-encapsulating nanoparticles have been evaluated by Zhao et al. for their systemic tumour suppression property against lung metastases (Zhao et al. 2021). Such studies highlight the utility of cytokines and chemokines as a potential target for developing nanoformulations with immunostimulant effects, which might drive the macrophage induced immune responses. Targeting the neutralization of the innate macrophagic responses during diseases can be a promising way to increase the mortality rates of infectious and inflammatory diseases, where the haywire macrophagic response (such as cytokine storm) causes life-or-death situations.

6. Current bottlenecks in development of protein nanocarriers and nanotherapeutics

The compatible nature and ease of chemical modification have placed protein nanoparticles at the forefront of nanotechnology-based therapy. However, despite the progress, the clinical translation of protein-based platforms is still limited due to several bottleneck listed as follows:

**Batch-to-batch variability**

In general, natural protein polymers can be present as heterogeneous mixtures, and the oligomerization process is a fairly uncontrolled process, due to which the final product obtained can have different physicochemical/functional characteristics and low purity. In addition, the mass production of recombinant proteins for therapeutic purposes is mainly achieved using genetic engineering techniques by a bacterial vector, which can lead to contaminations with miniscule bacterial product like endotoxins, bacterial DNA, and other outer wall proteins that induce immunogenic responses (Wakelin et al. 2006; Fazolin et al. 2023).
Further, the amount of protein isolated also varies from batch-to-batch and is highly dependent on environmental/experimental conditions.

**Immunogenicity profiles**

Immunogenicity is a crucial factor in assessment of safety and efficiency of drug delivery systems. Nanoformulations based on proteins with no-to-minimal homology with human proteins can encourage provocative immunogenic responses, and reduce the therapeutic effects. Although protein engineering techniques have made it possible to reduce the immunogenicity by preparation of fusion proteins, they also mark the increase in production cost (Kianfar 2021). Moreover, the variation in the three-dimensional structure of fusion proteins from their native state may also elicit B cell mediated antibody responses against the therapeutic (Sauna 2020). The immune response against protein-based therapeutics might lead to hypersensitivity responses such as anaphylaxis (Rosenberg et al. 2018). Hence, the choice of protein, fabrication method and native structure of proteins are the major influential factors that determine the protein nanoparticle immunogenicity and their fate of clinical translation.

**Ease of degradation and off-target effect/accumulation**

Proteins have been utilized to produce nanoparticle/drug delivery systems to enhance the stealthiness and viability of delivery systems. However, their biodegradable nature and precise eliminatory mechanisms via hepatobiliary/RES (endo-lysosomal, ubiquitin-proteasomal degradation, serum, and digestive proteases) and renal systems confer a high chance of low efficacy due to rapid elimination (Jao et al. 2017; Glassman et al. 2019; Feitosa et al. 2019). Although, proteins such as collagen and gelatine can overcome this effect, the usability of such nanoparticles in preparation of therapeutics is shallow. Another bottleneck of applying protein based nanoformulations is the risk of their off-target
accumulation, as a recent study evidenced the accumulation of agglutinated proteins in pulmonary neutrophils during acute inflammation (Myerson et al. 2020).

Limited oral delivery prospects

The intravenous and subcutaneous administrations are the most common routes of protein therapeutics till date. The presence of proteolytic enzymes, low pH of the stomach and mucosal layer are of great concern for the oral administration of protein therapeutics (Sadeghi et al. 2020; Bakhru et al. 2013; Muheem et al. 2016). To deal with this issue several inorganic/organic polymeric coatings such as methacrylate and PEG are used along with the proteinaceous formulations, which provide protection from the intestinal acidic condition and helps the therapeutics to surpass the mucosal layer, immunogenic responses against these nonbiological components can still be a matter of concern (Cao et al. 2019).

7. Conclusions and future perspectives

Protein-based nanoparticles have versatile applications in biomedicine as well as the industrial and food preservations. The compatible nature and ease of chemical modification have placed protein nanoparticles at the forefront in the field of nanomedicines for various pathophysiological conditions as both nanocarriers and nanotherapeutics. The current review comprehensively describes these potential protein-based nanoplatforms in major viral and oncogenic occurrences. Although the clinical translation of protein-based platforms is still limited due to a number of bottlenecks, in recent years, several techniques such as desolvation, emulsification, and recombinant techniques are being employed to overcome these issues and limit the need of toxic organic chemicals as cross-linkers. Computational biology and protein engineering concepts can be extensively applied for the development of protein-based nanoparticles with self-assembly properties, and multicompartamental nanoparticles for targeting the delivery of highly dissimilar drugs altogether at the same time.
Moreover, further exploration of the degradation and clearance pathway of protein nanoparticles will strongly establish this field in molecular medicine. Despite the classical challenges in their growth trajectory, the astonishing progress in the field of protein-based nanotherapeutics as described in the present review and other recent reviews instate their promising ramifications against inflammation, infection and other immunomodulatory disorders.

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Conflict of Interest statement:

The author declares no conflict of interest.

Author Contribution:

Performed data analysis: N. Nagar and G. Naidu; Wrote or contributed to writing manuscript: N. Nagar, G. Naidu, A. Mishra, K.M. Poluri.
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Table 1: Summarized account of novel protein-based nanocarriers developed within the last five years, together with their targeted disease, activity/role of the protein, and their current clinical status.

<table>
<thead>
<tr>
<th>Type of Nano-carrier</th>
<th>Disease</th>
<th>Cargo/ composition</th>
<th>Activity</th>
<th>Unique property</th>
<th>Clinical status (Trial no./Ref)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (NAb) paclitaxel NP (NeoNAB/NABptx)</td>
<td>Metastatic melanoma of the eye, breast cancer, NSCLC</td>
<td>Albumin-bound (NAb) paclitaxel (GA) NPs with various anticancer drugs</td>
<td>Drug delivery to the surgically inoperable oncogenesis</td>
<td>Non-solvent containing protein formulation</td>
<td>Phase 2 Complete d; Phase 3 Ongoing (NCT023 82263/NCT0183 0244)</td>
</tr>
<tr>
<td>CuS-BSA/lysozyme nanocomposite</td>
<td>Bactericidal</td>
<td>Loaded lysozyme</td>
<td>NIR light-induced photodynamic activity</td>
<td>Combined photothermal, photodynamic activity</td>
<td>Preclinical (Swaidan et al. 2021)</td>
</tr>
<tr>
<td>Hb/HSA/ N-succinimidyl3-maleimido-propionate</td>
<td>-</td>
<td>Haemoglobin (Hb), N-succinimidyl3-maleimido-propionate</td>
<td>Enhanced half-life of Hb</td>
<td>Artificial oxygen carrier</td>
<td>Preclinical (Funaki et al. 2019)</td>
</tr>
<tr>
<td>Hb/HSA/Pt NPs</td>
<td>-</td>
<td>Haemoglobin (Hb), Platinum (Pt)</td>
<td>Increased oxygen carrying capacity</td>
<td></td>
<td>Preclinical ((Haruki et al. 2015))</td>
</tr>
<tr>
<td>NAb rapamycin/te mozolomide/irinotecan HCl</td>
<td>Childhood neoplasm</td>
<td>rapamycin/ temozolomide / irinotecan HCl</td>
<td>Increased targeted drug delivery system</td>
<td>Enhanced permeability surgically inoperable oncogenesis/</td>
<td>Phase 1 (NCT029 75882)</td>
</tr>
<tr>
<td>NAb rapamycin/pazopanib</td>
<td>Soft tissue sarcoma</td>
<td>rapamycin/pazopanib</td>
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<td></td>
<td>Phase 1/2 (NCT036 60930)</td>
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<tr>
<td>Nanocarrier</td>
<td>Disease</td>
<td>Drug</td>
<td>Mechanism of Action</td>
<td>Stage</td>
<td>Reference</td>
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<tr>
<td>NAb</td>
<td>Adenocarcinoma</td>
<td>Paclitaxel/Gemcitabine</td>
<td>Selective obstruction of IL2 expression and lowering T cell activation</td>
<td>Phase 1 (NCT02336087)</td>
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<tr>
<td>Tacrolimus loaded NAb</td>
<td>Rheumatoid Arthritis</td>
<td>Tacrolimus</td>
<td>Enhanced hydrophilicity and targeted drug delivery system</td>
<td>Preclinical (Udalova et al. 2016)</td>
<td></td>
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<tr>
<td>Methotrexate loaded NAb</td>
<td>Rheumatoid Arthritis</td>
<td>Methotrexate</td>
<td>Inhibit multiplication of macrophage thus blocking the expression of inflammatory cytokines</td>
<td>Preclinical (Takakura et al. 1990)</td>
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<tr>
<td>Albuferon</td>
<td>Hepatitis C</td>
<td>Interferon</td>
<td>Enhanced half-life and interferon stability</td>
<td>Phase 1/2 (NCT00724776, NCT00759200)</td>
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<tr>
<td>Ozoralizumab</td>
<td>Rheumatoid arthritis</td>
<td>Anti TNF nanobody</td>
<td>Unique structure and non-immunogenic</td>
<td>Phase 3 (NCT04077567)</td>
<td></td>
</tr>
<tr>
<td>Ferritin Based Nanocarriers</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Nanocages</td>
<td>Cancer</td>
<td>Doxorubicin, Curcumin, Quercetin</td>
<td>Easy conjugation of anticancer drugs to Fe II- and rapid pH-dependent release</td>
<td>Excellent stability</td>
<td>Preclinical (Ahn et al. 2018)</td>
</tr>
<tr>
<td>Nanoparticle (H2HA-ferritin NP)</td>
<td>Influenza</td>
<td>Viral type 2 hemagglutinin (H2HA) fusion</td>
<td>Acts as a vaccine eliciting hemagglutination inhibition antibodies</td>
<td>Self-assembling nanoparticle</td>
<td>Phase 1 completed (NCT038 14720/NCT0318 6781)</td>
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</tr>
<tr>
<td>Spike-ferritin nanoparticle (SpFN)</td>
<td>SARS-CoV-2</td>
<td>SARS-CoV-2 spike protein</td>
<td>Induce robust S binding, ACE2 inhibition, and authentic and pseudo virus neutralizing antibodies</td>
<td>Potent immunogenicity and protection against SARS-CoV2 infection</td>
<td>Phase 1 Ongoing (NCT047 84767)</td>
</tr>
<tr>
<td>NanoFlu (Novavax)</td>
<td>Influenza</td>
<td>Recombinant hemagglutinin protein nanoparticle with saponin-based Matrix-M</td>
<td>Acts as a broad-spectrum vaccine</td>
<td>Quadivalent nanoparticle</td>
<td>Phase 3 Complete (NCT041 20194)</td>
</tr>
</tbody>
</table>

### Collagen/Gelatine Based Nanocarriers

<table>
<thead>
<tr>
<th>CE-loaded gelatine NPs (GNPs)</th>
<th>Glioblastoma</th>
<th>Cardamom extract-loaded GNPs</th>
<th>Effective targeting</th>
<th>Increased permeability to BBB due to gelatine</th>
<th>Preclinical (Nejat et al. 2017)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatine-β-cyclodextrin-asparaginase nanobio-composite</td>
<td>Brain cancer and cervical cancer</td>
<td>1-Asparaginase immobilised onto gelatine and β-cyclodextrin nanobio-composite</td>
<td>Anticancer activity</td>
<td>Enhance biocompatibility</td>
<td>Preclinical (Baskar et al. 2020)</td>
</tr>
<tr>
<td>DOX@BET gelatin nanoparticles (GNPs)</td>
<td>Cancer</td>
<td>Betanin and Doxorubicine</td>
<td>pH responsive smart drug delivery</td>
<td>Controlled release and stealthiness</td>
<td>Preclinical (Amjadi et al. 2019)</td>
</tr>
<tr>
<td>Nanocarriers</td>
<td>Disease</td>
<td>Drug</td>
<td>Functions</td>
<td>Publication</td>
<td></td>
</tr>
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<tr>
<td>Gelatine-Magnetic NP</td>
<td>Human colorectal cancer</td>
<td>mTOR-siRNA</td>
<td>Prevents aggregation of MNP and improves storage</td>
<td>Preclinical (Selimovic et al. 2022)</td>
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<tr>
<td>Gelatine-chitosan loaded lupeol NPs</td>
<td>Cancer</td>
<td>Lupeol</td>
<td>Anti-tumour, anti-inflammatory activity</td>
<td>Preclinical (Patel et al. 2018)</td>
<td></td>
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<tr>
<td>Gelatine loaded EGCG</td>
<td>Dry eyes syndrome</td>
<td>Epigallocatechin gallate (EGCG)</td>
<td>Surpass the corneal epithelium barrier and anti-inflammatory effect</td>
<td>Preclinical (Luo and Lai 2017)</td>
<td></td>
</tr>
<tr>
<td>Lysozyme Based Nanocarriers</td>
<td>Que/Lysozyme/OSM NPs</td>
<td>Cancer</td>
<td>Quercetin (Que)</td>
<td>Anti-tumour and anti-inflammatory effect</td>
<td>Preclinical (Li et al. 2020)</td>
</tr>
<tr>
<td>Amp loaded lysozyme NPs</td>
<td>MRSA infection</td>
<td>Ampicillin (Amp)</td>
<td>Antimicrobial effect</td>
<td>Multivalent presentation platform; enhanced permeation of drug</td>
<td>Preclinical (Kalita et al. 2018)</td>
</tr>
<tr>
<td>Other Protein Based Nanocarriers</td>
<td>Asiatic acid (AA)-loaded PLGA NPs</td>
<td>Glioblastoma</td>
<td>AA loaded PLGA NPs functionalized with transferrin</td>
<td>Enhanced targeting ability</td>
<td>Brain targeting delivery system</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>Applications</td>
<td>Properties</td>
<td>Preclinical Studies</td>
<td></td>
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<tr>
<td>UCNP@Tf-99mTc</td>
<td>Multimodal cancer bioimaging</td>
<td>Enables active targeting of transferrin receptors overexpressed in diverse cancers. Single replacement for multiple imaging agents.</td>
<td>Preclinical (Akhtar et al. 2022; Ramalho et al. 2022)</td>
<td></td>
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<tr>
<td>HTfNPs</td>
<td>Colorectal Cancer</td>
<td>Hypericin-loaded transferrin nano-formulations</td>
<td>Dual functionalized targeted delivery</td>
<td>Preclinical (Sardoiwa la et al. 2020)</td>
<td></td>
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<tr>
<td>qNIV/CoV23 73</td>
<td>SARS-CoV-2</td>
<td>Combination of quadrivalent seasonal influenza HA NPs and SARS-CoV-2 spike with matrix M adjuvant. Dual functionality against influenza A and B with hACE2 receptor-mediated anti-SARS-CoV-2 antibody response. Highly immunogenic and protective property due to quadrivalent influenza HA NPs.</td>
<td>Preclinical (Massare et al. 2021)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNLYZ-Cur</td>
<td>Anticancer, antimicrobial</td>
<td>Surface-conjugated self-assembled lysozyme nanoparticles</td>
<td>Broad spectrum applicability as anticancer, antimicrobial and antioxidant formulation. Enhance hemo- and cytocompatibility of curcumin.</td>
<td>Preclinical (Somu and Paul 2021)</td>
<td></td>
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<tr>
<td>RSV-ZN NPs</td>
<td>Human colorectal carcinoma</td>
<td>Resveratrol-loaded zein nanoparticles</td>
<td>Induce oxidative stress (ROS and endothelial nitric oxide synthase). Effective drug delivery and cellular uptake.</td>
<td>Preclinical (Khayat et al. 2022)</td>
<td></td>
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<tr>
<td>Nanoparticles</td>
<td>Disease/Condition</td>
<td>Function</td>
<td>Advantages</td>
<td>Study Reference</td>
<td></td>
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<tr>
<td>I-Zein NP-PEG</td>
<td>Diabetes</td>
<td>Induce potent hypoglycemic effect and enhanced oral bioavailability</td>
<td>Better oral bioavailability due zein-PEG NPs</td>
<td>Preclinical (Reboredo et al. 2021)</td>
<td></td>
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<tr>
<td>DM1-Zein NP</td>
<td>non-small cell lung cancer</td>
<td>Maytansine (DM1)</td>
<td>Anticancer activity</td>
<td>Preclinical (Yu et al. 2020)</td>
<td></td>
</tr>
<tr>
<td>rHBsAg loaded protamine nanocapsules</td>
<td>hepatitis B</td>
<td>Recombinant hepatitis B surface antigen (rHBsAg)</td>
<td>Enhance cytokine secretion and complement activation</td>
<td>High stability profile</td>
<td>Preclinical (González-Aramundi et al. 2018)</td>
</tr>
<tr>
<td>CU-CHPNPs</td>
<td>Breast Cancer</td>
<td>Curcumin encapsulated chitosan protamine nanoarrier</td>
<td>Down-regulate expression of the Bcl-2 anti-apoptotic gene along with inhibition of NF-κB, IL-6, and TNF-α</td>
<td>Enhanced stability and bioavailability of curcumin due to protamine NPs</td>
<td>Preclinical (Abdel-Hakeem et al. 2021)</td>
</tr>
</tbody>
</table>
Table 2: Summarized account of novel protein-based nanotherapeutics for viral infections and cancer developed within the last five years, together with their targeted disease, activity/role of the protein, and their current clinical status.

<table>
<thead>
<tr>
<th>Type of Nanotherapeutics</th>
<th>Disease</th>
<th>Composition</th>
<th>Activity</th>
<th>Unique property</th>
<th>Clinical status</th>
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</thead>
<tbody>
<tr>
<td>NVX-CoV2373 NP</td>
<td>SARS-CoV-2</td>
<td>SARS-CoV-2 spike protein trimeric full-length SARS-CoV-2 spike glycoproteins and Matrix-M1 adjuvant</td>
<td>Acts as a promising vaccine candidate against SARS-CoV-2; higher reactogenicity</td>
<td>Spike protein of SARS formulated as nanoparticle</td>
<td>Phase 1-2 Completed (NCT04368988)</td>
</tr>
<tr>
<td>GBP510 NP</td>
<td>SARS-CoV-2</td>
<td>Self-assembling SARS CoV-recombinant protein nanoparticle with AS03 adjuvant</td>
<td>Acts as a promising vaccine candidate against SARS-CoV-2</td>
<td>Highly immunogenic due to presence of receptor-binding domain</td>
<td>Phase 1-2 Completed; Phase 3 Ongoing (NCT04742738 / NCT04750343)</td>
</tr>
<tr>
<td>Multivalent hemagglutinin nanoparticle</td>
<td>Influenza</td>
<td>HA ectodomain from 4 seasonal influenza variants fused with the N terminus I53_dn5B, a trimeric component of two-component icosahedral nanoparticle</td>
<td>Elicit neutralizing antibodies directed at both the immunodominant head and conserved stem; induce broad spectrum immunity</td>
<td>Combination of potent receptor-blocking and cross-reactive stem-directed antibodies</td>
<td>Preclinical (Boyoglu-Barnum et al. 2020)</td>
</tr>
<tr>
<td>qNIV/CoV2373</td>
<td>SARS-CoV-2</td>
<td>Combination of quadrivalent seasonal influenza HA NPs and SARS-CoV-2</td>
<td>Elicit antibody response against influenza and SARS</td>
<td>Dual functionality against influenza A/B with hACE2 receptor-mediated anti-</td>
<td>Preclinical (Massare et al. 2021)</td>
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<tr>
<td>Product</td>
<td>Vaccine Type</td>
<td>Antigen</td>
<td>Response</td>
<td>Phase</td>
<td>Study ID</td>
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<tr>
<td>qNIV</td>
<td>A/H3N2</td>
<td>spike with matrix M adjuvant, recombinant hemagglutinin (HA) quadrivalent nanoparticle influenza vaccine with Matrix M adjuvant</td>
<td>Increased influenza HA-specific polyfunctional CD4+ T cell response</td>
<td>Phase 1 and 2 Completed; Phase 3 Ongoing</td>
<td>NCT03658629, NCT03293498</td>
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<tr>
<td>MPLA-adjuvanted double-layered nucleoprotein-neuraminidase (NP-NA)</td>
<td>Influenza</td>
<td>Double-layered protein nanoparticles incorporating two conserved influenza antigens (nucleoprotein and neuraminidase)</td>
<td>Antigen-specific humoral and cellular responses, such as neutralizing antibody and cytokine (IFN-γ and IL-4)-secreting cells, and NP147–155 tetramer-specific cytotoxic T lymphocyte (CTL) responses</td>
<td>Enhanced stability and immunogenic response due to combination of MPLA and antigens</td>
<td>Preclinical (Wang et al. 2023b)</td>
</tr>
<tr>
<td>AdjuPhos</td>
<td>RSV</td>
<td>RSV fusion protein NPs with aluminium phosphate adjuvant</td>
<td>Antigen-specific humoral and cellular responses, such as neutralizing antibody</td>
<td>Stronger immune response due to aluminium phosphate adjuvant</td>
<td>Phase 1 (NCT02296463)</td>
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<tr>
<td>Product/Approach</td>
<td>Virus/Pathogen</td>
<td>Description</td>
<td>Response</td>
<td>Stage/Reference</td>
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<tr>
<td>DS-Cav1-Ferritin NPs</td>
<td>RSV</td>
<td>Multimeric vaccine featuring a ferritin core attached to eight trimeric pre-F spikes</td>
<td>Antigen-specific humoral and cellular responses, such as neutralizing antibody</td>
<td>Structure-based vaccine Preclinical (Marcandalli et al. 2019)</td>
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<tr>
<td>Palivizumab</td>
<td>RSV</td>
<td>Palivizumab-targeted FsII epitope modified chimeric N protomer self-assembled nanorings</td>
<td>Cellular immunity as well as F specific antibody protection</td>
<td>Heterologous antigen presentation Preclinical (Hervé et al. 2017)</td>
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<tr>
<td>Ferritin-H2HA Influenza</td>
<td>Influenza</td>
<td>Ferritin bound H2HA antigen</td>
<td>Antiviral effects against influenza</td>
<td>Safe, immunogenic ferritin platform Phase 1 (NCT03186781)</td>
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<tr>
<td>PP26-M2e nanobarrel Influenza</td>
<td>Influenza</td>
<td>Self-assembled M2e nanostructure with BP26 adjuvant</td>
<td>Long lasting T cell specific immune response</td>
<td>Adjuvant free self-assembling versatile platform Preclinical (Kang et al. 2021)</td>
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<tr>
<td>RBD coupled Lumazine synthase (LS) nanoparticle MERS</td>
<td>MERS</td>
<td>RBD coupled Lumazine synthase (LS) nanoparticle</td>
<td>Multivalent high-quality cross clade neutralisation of MERS-CoV</td>
<td>Prepared utilizing antigen-SpyTag/LS-SpyCatcher mechanism Preclinical (Okba et al. 2020)</td>
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<tr>
<td>S2GΔHR2 I3-01v9 SApNP SARS-CoV</td>
<td>SARS-CoV</td>
<td>RBD decorated ferritin based engineered multi-layered S2GΔHR2 I3-01v9</td>
<td>T cell mediated cytotoxic activity</td>
<td>Preclinical (He et al. 2021)</td>
<td></td>
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<tr>
<td>Formula</td>
<td>Disease</td>
<td>Description</td>
<td>Immunogenicity</td>
<td>Phase</td>
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<tr>
<td>ENV fused ferritin</td>
<td>HIV/AIDS</td>
<td>ENV fused ferritin in vivo multimeric self-assemblies</td>
<td>Induce significant antibody titres priming immunisation</td>
<td>Preclinical (He et al. 2018)</td>
<td></td>
</tr>
<tr>
<td>Env-I53-50NP</td>
<td>HIV/AIDS</td>
<td>I53-50A (20 trimeric) and I53-50B (12 pentameric) subunits fused with viral glycoproteins</td>
<td>Neutralising antibody response Two component protein NP to enhance the immunogenicity of HIV-1 envelope proteins</td>
<td>Preclinical (Brouwer et al. 2019)</td>
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<tr>
<td>HSPPC96</td>
<td>Cancer</td>
<td>HSP glycoprotein 96 peptide nanocomplex</td>
<td>High level of IFNγ and Th cell activity</td>
<td>Phase 2/3 (NCT00098085; NCT00126178)</td>
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<tr>
<td>Engineered EGFR antibody-E2 protein NPs</td>
<td>Cancer</td>
<td>anti-EGFR antibody fragment loaded engineered E2 protein assembled nanoparticles</td>
<td>Blocks EGFR Stealthiness due to E2 protein NP</td>
<td>Preclinical (Buecheler et al. 2015)</td>
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<tr>
<td>MelQbG10</td>
<td>Melanoma</td>
<td>Melan-A/Mart-1 peptide conjugated Qβs with TLR9 adjuvant CpG</td>
<td>T cell specific response Virus-like particle for targeted delivery of Melan-A/Mart-1 peptide to lymph nodes</td>
<td>Phase 1/2/3 (NCT00651703; NCT00306566; NCT00306553; NCT00306514)</td>
<td></td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Melanoma</td>
<td>Anti-PD1 antibody conjugated Qβs with TLR9 adjuvant CpG</td>
<td>T cell specific response</td>
<td>Phase 1/2 (NCT03200847)</td>
<td></td>
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<tr>
<td>CCL21 loaded protein vault NP</td>
<td>Lung cancer</td>
<td>Recruit of T lymphocytes and dendritic cells into the tumor microenvironment</td>
<td>Elevated bioavailability of CCL21</td>
<td>Preclinical (Kar et al. 2011; Poelaert et al. 2020)</td>
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<tr>
<td>Mucin-conjugated PLGA nanoparticles</td>
<td>Diabetes</td>
<td>Insulin absorption</td>
<td>Better oral delivery system for insulin</td>
<td>Preclinical (Jaradat et al. 2020)</td>
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</tbody>
</table>
Figure Legends:

**Fig. 1: Schematic showing the role of cytokines and antigens during macrophage-mediated immune response against infection and inflammation.** Upon entry of pathogens, the antigens (such as bacterial lipopolysaccharide (LPS) and toxins) and cytokines (activated by the complement factors, tumour-associated antigens (TAAs), and apoptotic cells) recruit, reprogramme and activate antigen-presenting cells (APCs) and neutrophils, which further activate the T cells and macrophages. As primary inflammatory mediators, the activated neutrophils produce cytokines to recruit other immune cells like dendritic cells (DCs) and monocytes, which control macrophage differentiation. Depending upon the type of immune signal received from the neutrophils, the macrophages differentiate into M1 [primary signals from Tumour Necrosis factor (TNF) and interferons (IFNs) or M2 (signalling from Interleukin 4, 10 and 13 (IL4, IL10, IL13)) states. These two states are interchangeable and display reciprocal functional inhibition. M1 macrophages trigger inflammation by producing pro-inflammatory cytokines such as IL8, IL18, MCP1, and Reactive Oxygen Species (ROS), eventually producing pro-inflammatory effects, such as microbial killing and tissue injury. On the other hand, the M2 state macrophages produce anti-inflammatory factors like TGFβ and IL10, which leads to cell proliferation, tissue regeneration, and other anti-inflammatory effects.

**Fig. 2: Schematic representation of the structure and function of four types of protein-based nanocarriers in treating infection and inflammation.** (A) Abraxane (Paclitaxel loaded albumin-based NP) for cancer treatment; (B) Novavax (SARS-CoV-19 spike protein coated ferritin-based nanovaccine) against SARS-CoV-2; (C) Elastin Matrigel (collagen-based NPs) for wound healing; (D) Quercetin loaded self-assembled Lysozyme-oxidized starch microgel (OSM) NPs (lysozyme-based NPs) for tumour eradication.
Fig. 3: Schematic representation of the structure and function of protein nanotherapeutics in treating viral infections and cancer. (A) SApNPs (S1-RBD decorated ferritin-based NPs) as preventive measure against SARS-CoV-19; (B) HSP/HSPPC-tumour antigen complexes (HSP protein cages containing tumour antigen peptides) for treatment of cancer via stimulating innate and adaptive immune response.
Figure 1
Figure 2
Figure 3