



Review Article

Therapeutic peptides and their delivery using lipid-based nanoparticles

Jui-Hung Yen^{a†}, Chun-Chun Chang^{b,c†}, Tien-Yuan Wu^d, Chin-Hao Yang^e, Hao-Jen Hsu^{f*}, Je-Wen Liou^{c,e,f*}

^aDepartment of Molecular Biology and Human Genetics, Tzu Chi University, Hualien, Taiwan, ^bDepartment of Laboratory Medicine, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan, ^cDepartment of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan, ^dDepartment of Clinical Pharmacy, School of Pharmacy, Taipei Medical University, Taipei, Taiwan, ^eDepartment of Biochemistry, School of Medicine, Tzu Chi University, Hualien, Taiwan, ^fDepartment of Biomedical Sciences and Engineering, Tzu Chi University, Hualien Taiwan

[†]Both authors contributed equally to this work.

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ABSTRACT

Therapeutic peptides have become an intensively anticipated research field for novel drug discovery and design owing to their high specificity, efficacy, and biocompatibility. The advances in computer technology and structural biology, together with the invention of chemical peptide synthesis methods, have led to tremendous progress in this research field. Over the years, more than 100 peptide-based therapeutics have been approved for clinical use, and many others are currently under clinical trials. However, the *in vivo* application of therapeutic peptides is hindered by intrinsic disadvantages of peptides, such as poor stability against enzymatic degradations, short *in vivo* half-life, and low oral bioavailability. Therefore, strategies for efficiently protecting the peptides inside the body and facilitating the delivery of peptides to their targets are required. Lipid-based nanoparticles are considered a versatile class of carriers for drug delivery. Their biocompatibility, biodegradability, and ability to interact with biological membranes make them ideal platforms for *in vivo* delivery of peptides. Here, by leveraging examples, we aim to provide a comprehensive review of the current status of therapeutic peptide developments and lipid-based nanoparticles as drug carriers. Recent attempts to utilize lipid-based nanoparticles as platforms for the oral delivery of therapeutic peptides are also discussed.

KEYWORDS: *Computer-aided peptide design, Drug delivery, Lipid-based nanoparticles, Liposomes, Peptide therapeutics*

INTRODUCTION

Bioactive peptides are biomolecules with short amino acid sequences produced by all living organisms, regulating a range of biological/physiological functions, including neurochemical, endocrine, cardiovascular, gastrointestinal (GI), renal, respiratory, antimicrobial, venoms, oncological, and immune/inflammatory. Because of the fact that they can be metabolized into nontoxic metabolites, as well as their high potency and selectivity, in recent decades, bioactive peptides have become one of the popular choices in therapeutic developments [1]. The Nobel prize-winning invention of solid-phase peptide synthesis by Merrifield [2] and the following development of automatic peptide synthesizers further boosted the popularity of the research on applications of peptides for therapeutic purposes. Thus far, there have been more than 100 therapeutic peptides being approved for medical uses by the U.S. Food and Drug Administration (FDA) and official health agencies around the world for various indications,

including treating infection, cardiovascular diseases, diabetes, cancers, and other diseases, or for diagnostic purposes [1,3,4]. Meanwhile, an even greater number of lead therapeutic peptides are currently under investigations and clinical trials for pharmaceutical applications.

However, although peptide-based therapeutics have gained attention for their specificity, efficacy, and biocompatibility, they also have intrinsic disadvantages, in particular, the poor *in vivo* stability against enzymatic degradations [5] and short half-life resulting from fast clearance by kidneys [6]. These major drawbacks are reoccurring problems plaguing research scientists in peptide drug developments and applications.

***Address for correspondence:** Prof. Hao-Jen Hsu,

Department of Biomedical Sciences and Engineering, Tzu Chi University,
701, Zhongyang Road, Section 3, Hualien, Taiwan.
E-mail: hjhsu32@mail.tcu.edu.tw

Prof. Je-Wen Liou,
Department of Biochemistry, School of Medicine, Tzu Chi University, 701,
Zhongyang Road, Section 3, Hualien, Taiwan.
E-mail: jwliou@mail.tcu.edu.tw

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Amino acids and structure modifications of peptides have been used to tackle issues caused by *in vivo* enzymatic degradations. Replacing L-amino acids with their D-enantiomers in peptide sequences is a popular strategy to increase resistance to proteolytic degradation [7,8], whereas peptide cyclization is also used in the design to improve *in vivo* stability and half-life of peptides [9,10].

In addition, there are ongoing issues that need to be considered. In many cases, peptides have weak membrane permeability and thus might not reach and interact with their intracellular targets. Therefore, developments and applications of peptide delivery systems, which are able to provide protection to the peptides and increase peptide bioavailability, are also hot topics in the research of peptide therapeutics. As lipid-based nanoparticles are biocompatible and biodegradable, and their lipophilic nature may help increase the transcellular transport of loaded drugs, lipid-based nanoparticles are emerging as promising platforms for *in vivo* delivering peptide drugs [11,12].

This article aims to provide a comprehensive review and introduction to therapeutic peptides and lipid-based nanoparticles as delivery systems for therapeutic peptides.

BIOACTIVE PEPTIDES AS THERAPEUTICS

Research into therapeutic peptides began with studies on the distinct physiological roles of natural human hormones, such as insulin, oxytocin, vasopressin, and gonadotropin-releasing hormones [13]. Since the market introduction of the first therapeutic peptide, insulin, in medical practice, substantial progress has been achieved in this field, leading to numerous peptides being developed into pharmaceutical drugs and approved for clinical use. Currently, more than 100 peptide-based drugs have been granted approval for medical use [1,3,4]. In many cases, natural peptides need to be artificially modified to achieve better specificity, activity, and stability. These modifications are well demonstrated by the peptide-based drug desmopressin (DDAVP) currently in clinical use. An example of DDAVP tablets and the DDAVP

chemical structure is shown in Figure 1. DDAVP is a 9-amino-acid synthetic peptide used to treat central diabetes insipidus caused by impaired hypothalamus secretion of the natural peptide hormone arginine vasopressin (AVP). There are two major differences between DDAVP and the natural AVP: the first amino acid of DDAVP is deaminated, and the L-arginine at the eighth position is replaced with a D-arginine. The modification makes the DDAVP degrade slower in the body than the natural AVP. DDAVP targets the AVP receptor type 2 expressed on the cell surface in the renal distal convoluted tubule and collecting ducts more specifically, triggering renal water reabsorption, whereas the natural AVP, apart from the type 2 receptor, also binds to type 1 receptor in blood vessels, causing hypertension. The DDAVP peptide is also the recommended first-line treatment of nocturnal enuresis [14] and remains the only FDA-approved pharmacologic treatment for this condition [15], indicating the importance of this therapeutic peptide. This example clearly illustrates how artificial modification strategies on natural peptides can significantly improve the specificity and stability of peptide therapeutics and avoid side effects when used in the body.

Among the peptides investigated for medical applications, perhaps the most studied therapeutic peptides are a group of antibiotic agents called antimicrobial peptides (AMPs) [16]. Natural AMPs are discovered in almost all kinds of organisms, playing roles in their innate and adaptive immunity to fight against viral, bacterial, and fungal infections. As AMPs adopted different mechanisms from conventional antibiotics in bacterial killing, they have been considered promising candidates for treating infections by bacteria that resist conventional antibiotics. While conventional antibiotics prevent bacterial growth by inhibiting the cell wall, nucleic acid, or protein synthesis of the bacteria, AMPs typically exhibit their bactericidal effects by interacting with the membranes of bacteria. In bacterial cell membranes, AMPs can either form ion channels or pores, causing changes in ion gradient or leakages of cellular substances, subsequently resulting in the death of bacteria. Gramicidin is a marketed AMP drug containing 15 amino acids. Figure 2 shows an

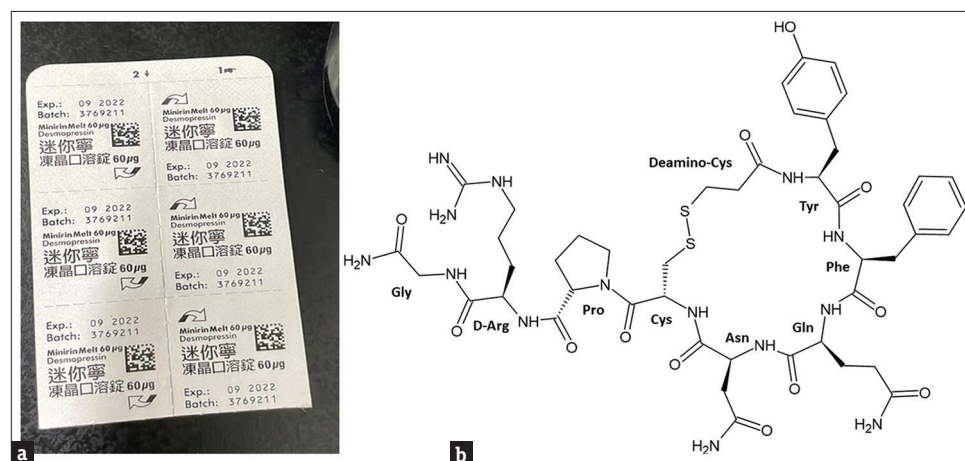


Figure 1: Desmopressin is a marketed 9-amino-acid peptide drug for treating diabetes insipidus. It is a synthetic peptide with its sequence modified from the peptide hormone arginine vasopressin naturally secreted by the hypothalamus. (a) An example of marketed desmopressin tablets. (b) Chemical structure and amino acid sequence of desmopressin

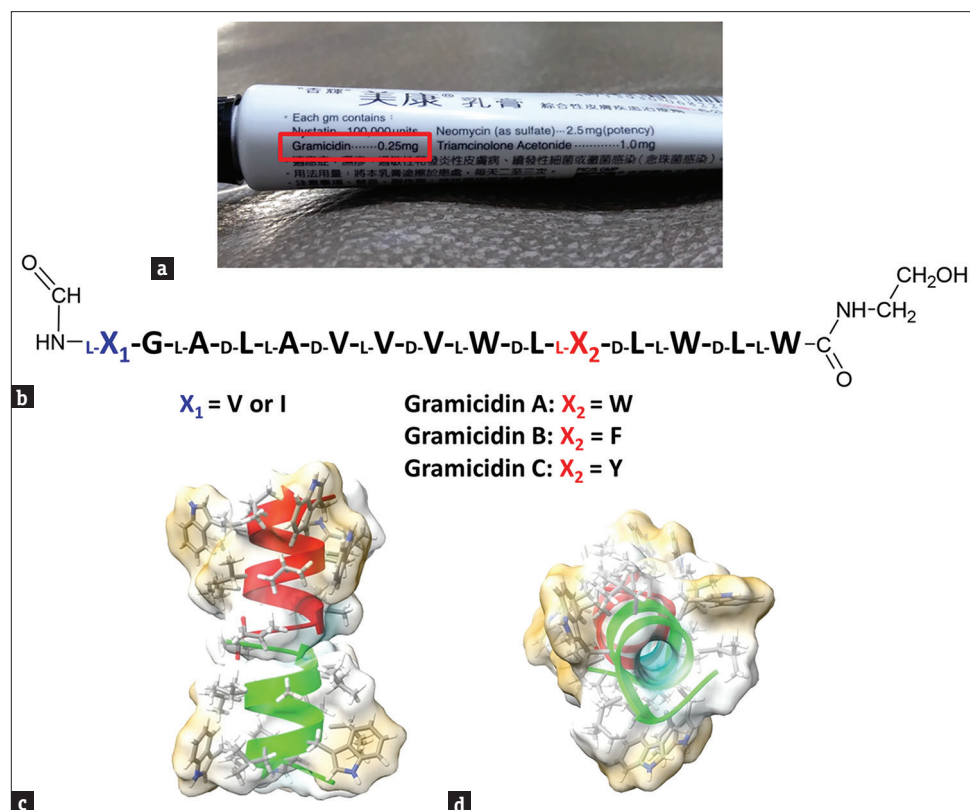


Figure 2: Gramicidin is a mixture of gramicidins A, B, and C, all of which consist of 15 amino acids. (a) An example of gramicidin as an ingredient in marketed anti-infection cream. (b) The amino acid sequence of gramicidin. (c) The gramicidin A formed a helical dimer structure when interacting with hydrated phospholipid bilayers, as revealed by NMR analysis (PDB: 1MAG [17]). The peptides are in ribbon presentation (indicated in red and green colors) with the amino acid side chains shown. The surface presentation indicates the hydrophobic (in light brown color) and hydrophilic (in light blue color) regions of the molecules. It is indicated in this structure that the outer surface of the dimer is largely hydrophobic. (d) Observation of the structure from a tilt angle of the bottom. It is observed that the helical dimer formed a hydrophilic channel (in light blue color) inside the helices, allowing monovalent ions to pass across the membrane

example of gramicidin used in a pharmaceutical formulation [Figure 2a] and the gramicidin sequence [Figure 2b], as well as the three-dimensional structure of gramicidin in lipid bilayers [PDB: 1MAG [17], Figure 2c and d]. Gramicidin has long been believed to form dimers that span across the membrane and act as cation channels, allowing monovalent inorganic ions such as potassium and sodium ions to diffuse across the membranes, thus causing bacterial death by breaking the ionic balance in the bacterial cells [18,19]. Nevertheless, a study by Liou *et al.* has also indicated that in addition to ion channel formation, gramicidin can also create large pores on the bacterial surfaces and induce hydroxyl radical generation in the cells, further contributing to bacterial death [20], indicating that the actual bactericidal mechanisms adopted by AMPs might be more complicated than previously hypothesized.

Several common structural and physical properties are shared by a large number of AMPs. Bacterial membranes tend to be negatively charged because of their negatively charged contents, such as phosphatidylglycerol and lipopolysaccharides. On the other hand, the surfaces of mammalian cell membranes are typically neutrally charged owing to their net neutrally charged zwitterionic phospholipid compositions in the outer leaflets of the membranes. Because of these differences, most of the AMPs are cationic, and this property imparts AMPs with some measures of selectivity toward bacterial

cells over mammalian ones [21]. Upon targeting, AMPs need to interact with and disrupt the integrity of the amphipathic membranes. Therefore, AMPs normally adopt amphipathic structures in which residues are segregated into hydrophobic and cationic regions [22]. Although AMPs can adopt different secondary structures, the largest group of AMPs fold into an amphipathic α -helical conformation when interacting with the target membranes [23,24]. These AMP characteristics are well exemplified by the peptide magainin 2, an α -helical cationic and amphipathic AMP extracted from the skin of the African clawed frog *Xenopus laevis* [25]. The amino acid sequence gives the magainin 2, a net charge of +3. The NMR structure of magainin 2 when interacting with dodecylphosphocholine micelles (PDB: 2MAG [25]) is shown in Figure 3, in which the helical conformation and amphipathic property of the peptide can clearly be observed. Intensive and extensive researches on natural and modified AMPs have produced a large number of peptide sequences aiming to combat the crisis caused by the antibiotic resistance developed in bacteria. A number of AMPs have been approved for clinical use [26], whereas more are under clinical trials. Till date, there have been more than 3900 AMP sequences deposited in the Antimicrobial Peptide Database [27] (<https://aps.unmc.edu/>, access date: 2024/12/9).

Recently, it has been suggested that the electrostatic charge properties of cancer cell surfaces are, to a certain degree,

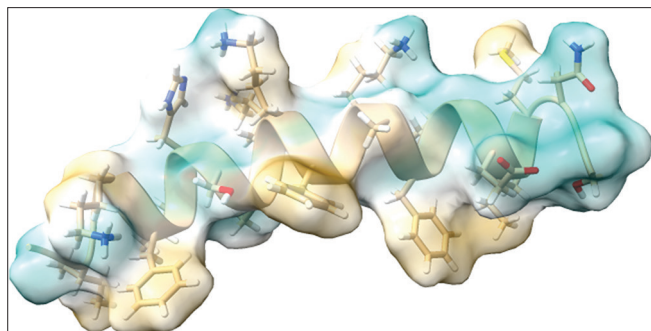


Figure 3: An NMR structure of a typical α -helical cationic AMP magainin 2 (GIGKFLHSAKKFGKAFVGEIMNS, PDB: 2MAG [25]) isolated from the skin of *Xenopus laevis*. The structure of the peptide is induced by the interaction with dodecylphosphocholine micelles. The surface display indicates the amphipathicity of the molecule. Light brown color: Hydrophobic; Light blue color: Hydrophilic

similar to those of bacterial membranes. Because of the increased levels of anionic molecules, such as phospholipid phosphatidylserine, O-glycosylated mucins, sialylated gangliosides, and heparin sulfate, are present in the outer leaflets of their membranes, the cancer cell surfaces are negatively charged [28]. Therefore, the cationic AMPs might also be able to selectively interact with cancer cells. The anticancer potentials of cationic AMPs have been investigated, and many of the AMPs have been found to exhibit anticancer activity [29,30]. Anticancer peptides are also found to adopt different secondary structures, including α -helical, β -pleated sheet, random coil, and cyclic structure, and the AMP-turned anticancer peptides exhibit their anticancer functions by disrupting the integrity of the cancer cell membrane, inducing cancer cell necrosis/apoptosis [31]. Anticancer peptides can also be developed to target specific cellular components and perform anticancer effects by inhibiting tumor angiogenesis and/or regulating the immune response of the hosts [32]. More than 20 different anticancer peptides have been approved by the U. S. FDA and/or European Medicines Agency for treating different types of cancers [33].

THERAPEUTIC PEPTIDES DESIGNED WITH COMPUTATIONAL METHODS

Structure-based computational studies for functional peptide design

Advances in computer technology and structural biology are now able to accelerate the process of investigating peptides targeting specific cell proteins and improve success rates in therapeutic peptide developments by using computer-aided design tools. These tools utilize computational models and algorithms to predict the structures, functions, and interactions of peptides before they are synthesized and tested experimentally, thus greatly reducing the development cost and time. The large quantity of experimentally resolved protein three-dimensional structures deposited in the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB, <https://www.rcsb.org/>, access date: 2024/12/10) [34] has made it possible to investigate protein-protein interactions by using computational tools such as molecular docking and molecular dynamics (MD) simulations. The knowledge obtained from the analyses of protein-protein interactions can then be used to design peptides

for therapeutic purposes. Here, we provide several examples. Interleukin-8 (IL-8, CXCL8) is a pro-inflammatory cytokine that activates neutrophils after infection and plays an important role in inducing sepsis [35]. The interactions and binding processes at submolecular level between IL-8 and its receptor CXCR1 have been successfully investigated with molecular docking and MD simulations [36]. Based on *in silico* analysis, IL-8-derived anti-inflammatory peptides were yielded, and these peptides were able to interrupt the ligand-receptor interactions and inhibit cellular inflammation response upon activation by bacterial endotoxin LPS [37]. Severe inflammation and sepsis have been linked to the overproductions of pro-inflammatory cytokines, such as IL-6, tumor necrosis factor-alpha (TNF- α), and IL-1 β . Computational analyses of molecular docking of pro-inflammatory cytokines IL-6, TNF- α , and IL-1 β to their receptors have helped to design a multitarget therapeutic peptide, and this peptide is able to inhibit inflammation induced by all these three cytokines [38,39], thus inhibiting the inflammation as revealed by experiments.

In silico methods have also been applied to antiviral developments. Hepatitis C virus (HCV) is a viral pathogen that only infects humans and chimpanzees. It is known that interactions between the HCV envelope protein E2 and host cell surface protein CD81 are crucial for HCV entry into the host cells. A study employed *in silico* molecular docking and MD simulations to compare the binding of the HCV E2 protein onto human and rat CD81s [40]. This study successfully identified an amino acid sequence in the HCV E2 protein loop domain crucial for species-specific binding to human cells. A peptide was then designed, and this peptide was tested to be able to bind to human CD81 and interrupt the ligand binding of human CD81. The outbreak of the COVID-19 pandemic has also prompted intensive research efforts in the development of SARS-CoV-2 inhibitory agents. Computational tools have played important roles in this process [41], and peptide-based therapeutic strategies for treating COVID-19 have been proposed. By computational structural analysis of the SARS-CoV-2 spike protein receptor-binding domain (RBD) and human cell surface receptor angiotensin-converting enzyme 2 (ACE2), Han and Kral designed several ACE2-derived peptides, and these peptides showed specific and stable binding to SARS-CoV-2 spike protein RBD, thus are suggested to be potential therapeutic agents for COVID-19 [42]. In addition to spike protein RBD, the main protease (M^{pro}) of SARS-CoV-2 has also been considered a therapeutic target. By using computational tools and structural analysis, Jin *et al.* identified a peptide as a potent inhibitor of SARS-CoV-2 M^{pro} [43].

As cancer is still one of the significant threats to human health and a major cause of human death, perhaps the development of anticancer peptides attracts the most attention in the field of peptide research. This is well exemplified by the design of peptide inhibiting MDM2/MDMX activity. MDM2 and MDMX are oncoproteins that negatively regulate the tumor suppressor protein p53 by binding to its N-terminus. These protein-protein interactions initiate the inhibition of transcriptional activity and promote the degradation of p53. Thus, disrupting p53-MDM2 and/or p53-MDMX interactions can lead to activation of p53 and can be used as a strategy for anticancer therapy. In 2009, Pazgier *et al.* [44] reported that by

screening peptide phage library against p53 binding domains of human MDM2 and MDMX, a potent peptide inhibitor (TSFAEYWNLSP) for the p53-MDM2/MDMX interactions was identified. The structure of this peptide in complex with human MDMX is shown in Figure 4. Comparative structural analysis between this peptide and the p53 native sequence (ETFSDLWKLPE) binding to MDM2 or MDMX was applied to understand the structure basis for high-affinity peptide inhibition of the p53-MDM2/MDMX interactions [44]. Phan *et al.* also identified a 12-residue peptide (LTFEHYWAQLTS) with inhibitory activity against MDM2 and MDMX by using a phage display method. The authors further analyzed the co-crystal structures of this peptide and N-terminal domains of human MDMX and MDM2 and designed a derivative peptide (ETFEHWWSQLLS) with greatly improved potency over the original peptide against MDM2/MDMX [45]. Computational molecular docking using p53-derived peptides to MDM2 was also applied to evaluate the contributions of important amino acids in p53 for MDM2 binding [46], providing valuable information to the future development of more stable peptides with better anticancer efficacy. Systematic computational approach has also been applied in the design of anticancer peptides targeting specific tumor markers. As reported in 2024, Naeem *et al.* [47] selected three receptors (CXCR1, DCR3, and OPG) suggested to play significant roles in cancer pathogenesis and tumor cell proliferation as their targets. By using the identified interacting residues in the natural ligands of these receptors, the researchers created a peptide library through simple permutation and predicted the structure of each peptide. Computational molecular dockings were then used to analyze the binding of peptides with their target receptors. With this approach, these researchers obtained novel short peptides for targeting each receptor with great affinities [47].

Conventionally, structure-based binding analyses require protein structures resolved with experimental methods such as X-ray crystallography, NMR, and cryo-electron microscopy.

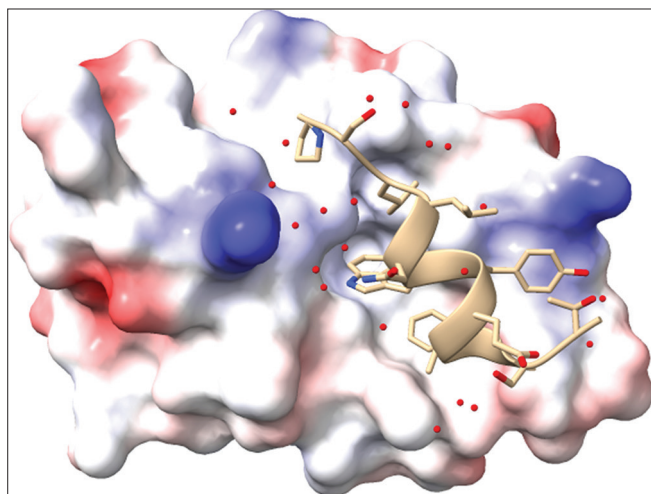


Figure 4: A crystal structure of a 12-mer peptide (TSFAEYWNLSP) inhibitor in complex with human MDMX (PDB: 3EQY [44]). The peptide is shown in a light brown colored ribbon presentation; the human MDMX is on display with the electrostatic surface, with the red color indicating the negatively charged regions and the blue color indicating the positively charged regions

However, these techniques are time- and cost-consuming, and more importantly, they are only applicable to certain proteins. Recently, the breakthrough in computational deep learning approaches has led to the development of efficient and accurate protein structure prediction tools such as AlphaFold [48,49] and RoseTTAfold [50]. These sequence-based protein structure prediction tools have made it possible to apply structure-based computational studies for functional peptide design without the need for experimentally resolved protein structures.

Therapeutic peptide design using artificial intelligence tools

In recent years, artificial intelligence (AI) tools have become indispensable in biochemical research and have been applied to aid the design of novel therapeutic peptides. For example, an ensemble artificial neural network was applied to the *de novo* design of membranolytic anticancer peptides. In this work, Grisoni *et al.* [51] applied two datasets of peptides targeting breast and lung cancer cells assembled and curated from the database CancerPPD [52]. The datasets were then used to train artificial neural networks. By using computational tools, they generated 1000 α -helical peptides and selected predicted highly active peptides for further *in vitro* tests. According to the *in vitro* experiments, six *de novo* designs showed anticancer activity, five of which were active against both breast cancer MCF7 and lung cancer A549 cell lines [51]. AI tools have also been applied to research on peptides targeting specific cancer cell markers. It has been known that interactions between the transmembrane protein PD-L1 on cancer cell surfaces and PD-1 on T-cells reduce T-cell functional signaling, thus preventing the host immune attack on tumor cells. Therefore, PD-L1 and/or PD-1 have been considered a hot target for anticancer therapy [53,54]. Since 2014, there have been 10 inhibitors approved for clinical use in cancer treatments. These agents are PD-1 inhibitors nivolumab, pembrolizumab, cemiplimab, dostarlimab, retifanlimab, and toripalimab [53,55-59] and PD-L1 inhibitors atezolizumab, avelumab, and durvalumab [60-62]. All of the above inhibitors are antibody based. As the development and production of monoclonal antibodies are complex and costly, the treatments using monoclonal antibodies are expensive for both patients and healthcare systems. It might be helpful to have therapeutic materials efficiently designed and produced at a comparatively much lower cost. Recently, Goudy *et al.* [63] applied the deep learning structure prediction tool AlphaFold2 [48] and the sequence design tool ProteinMPNN [64] to design miniproteins/peptides as PD-L1 antagonists. In this mentioned study by Goudy *et al.* [63], 4 of the 23 created PD-L1 binder peptides were tested experimentally to have equilibrium dissociation constants below 150 nM, with the lowest equal to 0.9 nM [63], indicating the efficiency of these peptides in PD-L1 binding. On the other hand, for PD-1, Guardiola *et al.* [65] exploited the power of the protein design tool Rosetta [61] and used it to design heterochiral cyclic antagonist peptides targeting PD-1. With this AI tool, they designed two novel peptides with PD-1 inhibitory functions, as proven by experimental evaluations [65]. These studies indicate that deep learning AI methods can be powerful and efficient in therapeutic peptide designs for cancer treatments.

Machine learning has also been applied in the design of antidiabetic peptides. Yue *et al.* recently developed a single-channel convolutional neural network (CNN) model and a three-channel model combining CNN, recurrent neural network (RNN), and bidirectional long-short-term memory (Bi-LSTM) [66], and used the peptides deposited in the BioDADPep database [67] for model training. When testing the models with an independent test set containing newly published antidiabetic peptides, they found that the single-channel CNN model achieved the highest accuracy of 90.48% in predictions. They then applied the SeqGAN (Sequence Generative Adversarial Nets) to generate new antidiabetic peptide candidates and screened them using the constructed CNN model. The physicochemical property and structural forecasts for pharmaceutical potential were then evaluated with *in silico* methods. This study concludes that the tools established can offer great help in the discovery of novel peptides for antidiabetic purposes. In another study, Puszkarska *et al.* [68] trained neural network models with sequences of *in vitro* validated peptides with dual agonist activity on the human glucagon receptor (GCGR) and the glucagon-like peptide-1 receptor (GLP-1R). Model-guided sequence optimization was used to design peptides with predicted dual activity. The experimental results showed that three of the designed peptides exhibited enhanced dual agonist activity on GCGR and GLP-1R, suggesting that these peptides have the potential to treat type 2 diabetes and obesity.

Abnormal NLRP3 inflammasome activity has been linked to atherosclerosis, diabetes, metabolic syndrome, cardiovascular disease, and neurodegenerative disorders. Therefore, targeting NLRP3 and modulating its immune response presents a promising avenue for the development of novel anti-inflammatory treatments. In 2023, Ahmad *et al.* reported a computational method for the *de novo* design of peptides targeting NLRP3 inflammasomes [69]. The method leverages an RNN-based LSTM network to model valuable latent spaces of molecules. The resulting classifiers are utilized to guide the selection of peptides generated by the model based on circular dichroism spectra and physicochemical features derived from high-throughput MD simulations. The experimental results showed that 60% of the selected peptides exhibited NLRP3-mediated inhibition of IL-1 β and IL-18. One of the peptides was tested to inhibit NLRP3-mediated but not NLRC4 and AIM2 inflammasome-mediated IL-1 β secretion, indicating the selectivity of the peptide toward the NLRP3 inflammasome.

For extensive generations of functional protein/peptide sequences, published in 2024, David Baker's team developed ProteinGenerator, a sequence-space diffusion model built on RoseTTAFold, capable of simultaneously generating protein sequences and structures [70]. They then used this tool to design thermostable proteins with diverse amino acid compositions, internal sequence repeats, and bioactive peptide cages. The structures of the designed proteins/peptides were validated experimentally using circular dichroism spectroscopy and NMR. ProteinGenerator design trajectories are also able to incorporate experimental sequence-activity data, enabling an integrated approach to computational and experimental

optimization of protein function. FlexPepDock [71] is a high-resolution protocol implemented within the Rosetta framework for refining and modeling peptide-protein complexes. Reported in 2024, Chen *et al.* developed a multistep algorithm that incorporates a gated recurrent unit-based variational autoencoder with Rosetta FlexPepDock for peptide sequence generations and binding affinity assessments [72]. MD simulations were then applied to narrow down the selection of peptides. With this algorithm, high-affinity inhibitory peptide binders targeting β -catenin and NF- κ B essential modulator (NEMO) were generated. Experiment results indicated that six of the 12 designed β -catenin inhibitors exhibited enhanced binding affinity as compared to that of the parent peptide. For NEMO, two of the four peptides display substantially enhanced binding compared to the parent peptide. It was demonstrated that integrating deep learning and structure-based simulations is effective for generating sequences of peptides targeting specific proteins.

With the continued advances in AI technology, it is believed that AI tools will play increasingly important roles in novel therapeutic peptide designs.

LIPID-BASED NANOPARTICLES AS DRUG DELIVERY SYSTEMS

Even though peptides exhibit high specificity and selectivity for drug targets, providing pharmacological advantages over small molecules by enhancing efficacy and reducing side effects, it is not easy to develop efficient delivery systems for peptides due to their hydrophilicity and relatively large molecular size as compared to small compounds. It is also challenging to deliver therapeutic peptides across barriers, such as the blood-brain barrier, or to target specific tissues.

Liposomes are lipid-based vesicles extensively investigated for their drug-delivery capabilities. Since their discovery by Bangham in 1964 [73], liposomes have steadily become valuable tools for studying cell membrane function and serving as unique drug carriers that enhance drug efficacy and reduce toxicity. Drug carrier potential of liposomes in cancer chemotherapy has been proposed and tested since 1974 [74]. The approval for medical use of the first injectable liposome product Doxil[®], a liposomal formulation of doxorubicin [53], by the U.S. FDA in 1995 has made the clinical application of lipid-based drug carriers a reality. Doxil[®] has been approved for indications including breast cancer, ovarian cancer, and multiple myeloma [75]. Today, more than 20 liposomal products have been approved by the U.S. FDA and/or the European Medicines Agency for cancer treatments, infection treatments, and vaccines [76]. Among these products, perhaps the most widely known are the RNA vaccines utilized to combat the COVID-19 pandemic. These vaccine technologies, including those adopted in COMIRNATY[®] (Pfizer-BioNTech) and SPIKEVAX[®] (Moderna) COVID-19 vaccines, use PEGylated liposomes to coat the fragile mRNA for their delivery [77]. PEGylated lipids are used to enhance particle stability and circulation time within the body. Although other surfactants can be used, drug-loading lipid-based nanoparticles are typically composed of phospholipids, which are also major

components of biological membranes. Cholesterol is also very often added to formulations to assist particle formation and increase the stability of the particles. When loading negatively charged cargo, cationic or ionizable lipids can be doped into the lipid formulations to increase the loading efficiency.

Currently, there are several different types of lipid-based nanoparticles, typically with diameters ranging from 10 to 1000 nm, designed for drug delivery. The schematic structures of different types of lipid-based nanoparticles for drug delivery are illustrated in Figure 5. Liposomes are vesicles having structures of phospholipid bilayers with an aqueous internal cavity, enabling the encapsulation of hydrophilic drugs. Nanoemulsions can be prepared as oil-in-water (o/w, used to carry hydrophobic drugs) or water-in-oil (w/o, used to carry hydrophilic drugs) droplets stabilized by surfactants for dispersion in an aqueous phase or in an oil phase, respectively, to prevent aggregation [78]. Solid lipid nanoparticles contain a surfactant-coated solid lipid core matrix that is utilized for the stabilization of lipophilic drugs [79]. Nanostructured lipid carriers are derived from solid lipid nanoparticles by injecting liquid lipids into the solid core, creating a nonuniform internal core containing solid and liquid lipids [80]. This modification can increase drug capacity and facilitate better-controlled delivery of the drugs. Lipid polymer hybrid nanoparticles are advanced core-shell nanostructures with a polymer-based core enclosed by a lipidic layer [81]. Lipid polymer hybrid nanoparticles can offer the benefits of both systems and can be custom-tailored for enhanced stability and controlled drug-release properties.

As the lipid materials used to produce lipid-based nanoparticles are normally those found in the body, lipid-based nanoparticles are generally biocompatible and biodegradable. Lipid-based nanoparticles can, therefore, be suitable carriers for encapsulating peptides for delivery and protecting the

peptides from harsh *in vivo* environments. In addition, because of their lipophilic nature, lipid-based nanoparticles can facilitate absorption by enhancing peptide solubility and crossing biological barriers, such as the intestinal epithelium or the blood–brain barrier. Thus, these nanoparticles are considered excellent candidates for the oral delivery of peptides. In the next section, the applications of lipid-based nanoparticles for oral delivery of therapeutic peptides are discussed.

ORAL DELIVERY OF THERAPEUTIC PEPTIDES USING LIPID-BASED CARRIERS

Oral administration of therapeutic peptides is an extremely difficult task, as peptides are normally absorbed poorly through the GI tract due to the existence of barriers, such as the enzymatic barrier, mucosal barrier, and epithelial barrier, in the GI tract [11]. Therefore, most of the current therapeutic peptides are delivered through parenteral administration. However, compared to oral delivery, parenteral administration is less welcomed by the patients, and this method normally requires skilled medical personnel to operate. Therefore, research efforts have been made to explore the possible oral administration strategies for therapeutic peptides. For this purpose, lipid-based carriers are considered.

The initial application of oral liposomes was with the delivery of insulin [82]. However, this initial attempt was not very successful, with only 54% of the normal rats and 67% of the diabetic rabbits responding to oral liposomal insulin treatments [83]. GI instability and relatively poor permeability have hindered the applications of liposomes for oral drug delivery. Thanks to modern modification technologies to enhance liposomal stability and permeation, attempts to use liposomes as drug carriers for oral delivery have once again become popular recently [73]. In the past decade,

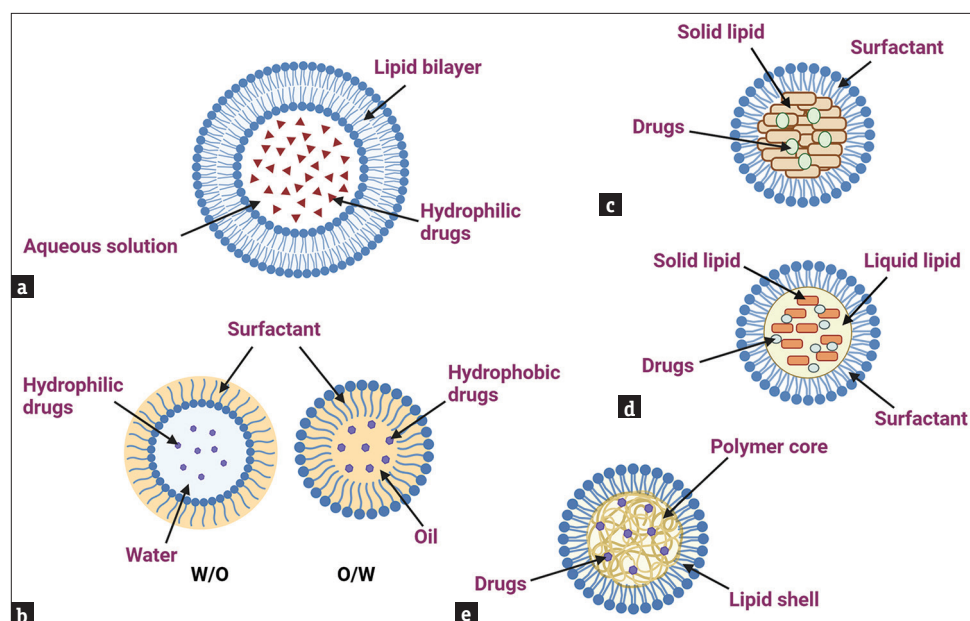


Figure 5: Schematic illustration of the structures of various lipid-based nanoparticles used for drug delivery. (a) Liposome; (b) Nanoemulsions; (c) Solid lipid nanoparticle; (d) Nanostructured lipid carrier; (e) Lipid polymer hybrid nanoparticle. Graphics were created with BioRender.com

there has been a growing trend in enhancing oral delivery of liposomes through modifications to either their surfaces or compositions. These modifications aim to improve both the stability and absorption of liposomes. Several examples of surface modifications of liposomes, including coating the liposomes with polysaccharides and polyethylene glycols (PEGs), are provided in Supplementary Figure S1. It has been suggested that coating the liposomes with polysaccharides is a strategy to enhance GI absorption [84]. Liposomes can be coated with a single layer of polysaccharides or multiple polysaccharide layers [85] [Supplementary Figure S1a]. Polysaccharide coating enhances the adhesion of liposomes to the epithelial mucus lining, increasing the chances of absorption of the drug [86-88]. It has also been found that coating with chitosan, a linear polysaccharide composed of linked glucosamine and N-acetylglucosamine, can disrupt tight junctions, facilitating the absorption of the drug and even the whole liposome. Coating the liposomes with chitosan was also found to greatly improve the absorption of poorly absorbable drugs in the GI environments [88]. As reported in 2018, Shalaby and El-Refaie applied chitosan-coated cationic nanoliposomes to encapsulate insulin for oral administration [89] and found that in mice with induced diabetes, a reduction in glucose levels was observed 1 h after oral administration of these insulin-containing modified liposomes, and the effects could be maintained up to 8 h. In a study published in 2019, Wu *et al.* [90] constructed a liposomal carrier coated with deoxycholic acid-conjugated chitosan and used this nanocarrier to deliver insulin. The results showed that this modified liposome carrier could promote the intestinal absorption of insulin through bile acid transporters, as visualized with fluorescently stained tissue slices of rat small intestine and a Caco-2 cell uptake experiment. Pharmacokinetic analyses in this study indicated that the insulin-loaded carrier showed a significant hypoglycemic effect with an oral bioavailability of 16.1% in rat models with type 1 diabetes.

Exendin-4 is a GLP-1R agonist for treating type 2 diabetes patients. In 2019, Suzuki *et al.* reported a study [91], in which chondroitin sulfate-glycocholic acid-coated liposomes were applied for exendin-4 oral delivery in rat models. The results showed that although a single oral dose of this administration has a relative oral bioavailability of only approximately 20% (sustained pharmacokinetics for up to 72 h) as compared to subcutaneous administration, the long-term pharmacodynamic effects of daily administration for 4 weeks were equivalent to or better than daily subcutaneous injections of exendin-4 solution.

Despite the fact that bile salts are GI factors that destabilize liposomes, it was, however, indicated that incorporating bile salts in the lipid bilayers can stabilize the liposomes and protect the cargo [92]. In addition, although the mechanisms controlling oral absorption of liposomes are still not fully understood, it has been reported that bile salt-enriched liposomes showed significantly higher oral bioavailability for a number of active ingredients [93,94]. Bilosomes are the novel modified form of liposomes and niosomes (vesicles constructed with nonionic surfactants) incorporated with

bile salts. In a study reported in 2012, Niu *et al.* compared the hypoglycemic activity and oral bioavailability of insulin-loaded bilosomes with different cholate types and particle sizes in rats [95]. All the prepared insulin-loaded bilosomes elicited certain degrees of hypoglycemic effects, with glycocholate-incorporated liposomes exhibiting the highest oral bioavailability in both diabetic and nondiabetic rats. The insulin-loaded bilosomes showed slower and sustained action for over 20 h. The hypoglycemic effect was also bilosomes size-dependent, with the highest effect observed at the sizes of 150 or 400 nm.

Alginate hydrogel coating is also a popular strategy to enhance oral bioavailability of liposomes. It has been reported that a coated calcium alginate hydrogel-entrapped liposome was able to facilitate oral colon-specific drug delivery for a bee venom model peptide [96]. It was also reported in 2023 that insulin-loaded liposomes packaged in alginate hydrogels can promote the oral bioavailability of insulin [97].

In addition to those aiming to treat diabetes, lipid-based nanoparticles were also applied to orally deliver AMPs. For instance, as demonstrated in a study published in 2024, Werner *et al.* [98] applied a surface-modified liposomal nanocarrier (tetraether lipid-stabilized liposomes with cyclic cell-penetrating peptide-modified surface) for the oral delivery of an AMP derived from vancomycin. Pharmacokinetics studies in rat models showed increased oral bioavailability of the liposomal AMP, as compared to the free drug.

The choices of phospholipids in the production of lipid-based nanoparticles for efficient oral delivery of peptides were also investigated. Cyclosporine A is a cyclized peptide containing 11 amino acids used as an immunosuppressant medication. In 2022, Minami *et al.* reported a study [99] using cyclosporine A as the model peptide to investigate and compare the liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) for oral peptide oral delivery. They analyze the peptide encapsulation mechanisms using powder X-ray diffraction and polarized light microscopy and observed peptide release profiles in fasted-state simulated intestinal fluid. They also monitor oral absorption of the peptide from liposomal formulations in rats. The results showed that DPPC liposomes quickly released the peptide in fasted-state simulated intestinal fluid due to the interaction with bile acid, whereas the effect of bile acid was negligible in DSPC liposomes. This study indicates that the choice of phospholipids for liposome production is crucial for creating liposomal carriers with better oral bioavailability.

Coating liposomes with polymers is a popular and effective strategy to stabilize liposomes. Coating with PEGs [Supplementary Figure S1b], a method also used in injectable long-circulating liposomes, assists liposomes in withstanding harsh environments in the GI tract [100] and improves absorption of loaded drugs [101]. Modifications of liposomes with PEGs can further enhance absorption by inhibiting P-glycoprotein-mediated efflux [86,102]. For peptide delivery, Iwanaga *et al.* prepared PEG-coated liposomes (PEGylated liposomes) for oral delivery of insulin [103]. The results showed that coating the surface with PEGs reduced the transit

rate of liposomes in the small intestine after oral administration to rats *in vivo*, indicating that PEGylated liposomes interact strongly with the intestinal mucous layers. Thus, PEGylated liposomes are considered desirable for oral delivery of peptide drugs.

Although modified or coated liposomes are popular systems for the oral delivery of therapeutic peptides, the possible oral delivery of peptides with other lipid-based nanoparticles was also investigated. Zhao *et al.* [104] prepared a peptide oral delivery system by loading a four amino acid antihypertensive peptide (YGLF) into nanoparticles by a double-emulsion internal phase/organic phase/external phase (W/O/W) solvent evaporation method. The peptide-containing lipid nanoparticles were further coated by a membrane hydration-ultrasonic dispersion method. These prepared peptide-loaded lipid nanoparticles exhibited sustained release of peptide *in vitro* and a 5-day long-term antihypertensive effect in rats. In 2020, Li *et al.* [105] developed a controlled release system composed of a poly(lactic-co-glycolic) acid core loaded with a five amino acid antihypertensive peptide (VLPVP) and a folate-decorated lipid shell for the oral delivery of this antihypertensive peptide. This system successfully improved the cellular uptake of peptide both in Caco-2 and HT29 cells and enhanced *in situ* intestinal absorption in SD rats. Lipid-based nanoparticles were also applied to develop oral peptide vaccines. An example is provided by Naciute *et al.* in 2020 [106]. In this study, oral vaccines consisting of a long tumor peptide and the Toll-like receptor 2 ligand Pam₂Cys were formulated in W/O/W double emulsions. It was found that the emulsion vaccine successfully increased the numbers of activated T-cells, B-cells, and CD11c⁺F4/80⁺CD11b⁺ cells *in vivo* and significantly reduced the tumor size in colorectal cancer mouse models. With continuous efforts put into this field of research, it is believed that in the future, more novel formulations for oral delivery of therapeutic/functional peptides will be developed.

CONCLUSION

Therapeutic peptides are a class of biologically active molecules attracting attention for their potential applications in diverse medical areas such as infectious diseases, inflammatory disorders, oncology, metabolic disorders, and cardiovascular diseases. They are chains of amino acids linked with amide bonds and can be chemically synthesized. These molecules also possess advantages such as high specificity, efficacy, and biocompatibility. As a result, therapeutic peptides are considered alternatives to small molecular drugs. Intensive and extensive research efforts have led to the approval of more than 100 peptides for medical use, and an even greater number are under investigation and in clinical trials. Advances in computer technology and structural biology have provided tremendous aid in the design of more efficient and more specific peptide sequences and structures for targeting disease-related biomolecules. Lipid-based nanoparticles have emerged as promising platforms for the oral delivery of peptide therapeutics, as they have been approved for medical use to deliver a number of therapeutic agents. Various lipid-based nanoformulations, including liposomes, nanoemulsions,

solid lipid nanoparticles, nanostructured lipid carriers, and lipid polymer hybrid nanoparticles, have been prepared for the delivery of drugs, including peptide-based therapeutics. These nanoparticles are composed of biocompatible and biodegradable lipids; thus, induced toxicity is relatively low. These nanoparticles can encapsulate peptides, protecting them from enzymatic degradations and increasing their *in vivo* stability. Lipid-based nanoparticles can also aid the peptides in withstanding the harsh GI environments and facilitate the transport of peptides across the intestinal epithelium. Therefore, lipid-based nanoparticles are investigated for their ability to be used as oral delivery systems for therapeutic peptides, and some successful progress has been made in animal model studies. Continued research into the design and functionalization of lipid-based nanoparticles is expected to expand their clinical applications and potentially change the landscape of effective peptide therapy.

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Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflicts of interest

There are no conflicts of interest.

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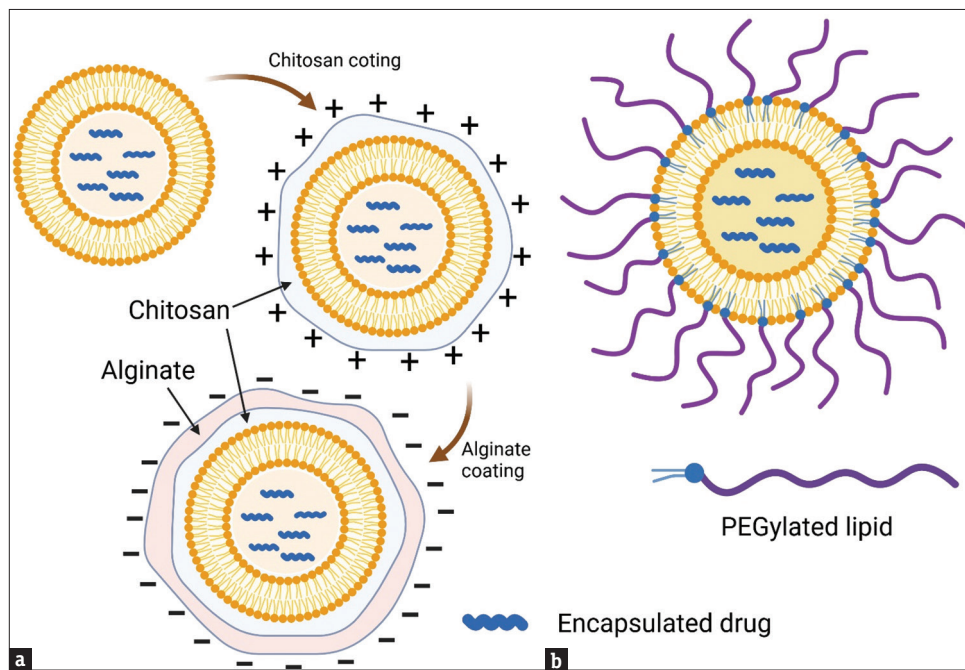
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SUPPLEMENTARY MATERIAL



Supplementary Figure S1: Examples of surface-modified liposomes. (a) Liposomes can be coated with a single layer of polysaccharides or coated with multiple polysaccharide layers. Chitosan coating is a popular surface modification of liposomes. Chitosan-coated liposomes can be further coated with negatively charged natural polysaccharides such as alginate, hyaluronate, and pectin. (b) Polyethylene glycol-coated liposomes (PEGylated liposomes) can be produced by mixing PEGylated lipids into the lipids used for liposome preparations. Graphics were created with BioRender.com