applied sciences

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Review

Nanoformulation of Peptides for Pharmaceutical Applications: In Vitro and In Vivo Perspectives

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Abstract: Peptides are short sequences of proteins consisting of two or more amino acids that are linked by peptide bonds. Peptide-based designs and drug deliveries can offer several advantages, such as antioxidant, antimicrobial, antihypertensive activities, along with immunomodulatory and antithrombotic properties, with hormone or drug-like potential. Peptide-based therapeutic formulations are used as drug candidates for the treatment of various diseases. However, there are several concerns associated with the efficacy of peptides in pharmaceutical design and delivery, including rapid degradation, limited solubility, and poor permeability. The nanoformulation of peptides has been identified as a promising approach for improving the stability of peptides and providing metabolic stability and bioavailability. This article provides an overview of the advances in the development of peptides for drug design and formulation applications. It discusses various peptide nanoformulation approaches as well as recent developments in the in vitro and in vivo analyses of nanoformulated peptides for pharmaceutical applications.

Keywords: peptides; nanoformulation; drug design; pharmaceutical delivery; nanomedicines

Citation: Thimmiah, B.R.; Chien, B.T.C.; Fui, K.S.; Yon, L.S.; Nallathambi, G.; Jeevanandam, J.; Danquah, M.K. Nanoformulation of Peptides for Pharmaceutical Applications: In Vitro and In Vivo Perspectives. *Appl. Sci.* **2022**, *12*, 12777. https://doi.org/10.3390/app122412777

Academic Editor: Natália Cruz-Martins

Received: 12 November 2022
Accepted: 9 December 2022
Published: 13 December 2022

1. Introduction

Peptides are essential for the general functioning and health of higher living organisms [1]. They play major roles in living organisms (especially in humans) as peptide hormones, such as vasopressin and defensins [2]. Peptide-based drugs have been widely used in pharmaceutical applications for the treatment of various diseases [3]. Further, peptides are easily synthesized with good scalability compared to other biomolecules [4]. They offer several benefits, such as drug delivery agents, due to their high cellular circulation, accumulation/penetration for cell internalization, and cytosolic drug release for improved disease treatment [5]. There are increasing applications of peptides in drug designs and deliveries that explore the in vitro and in vivo properties of peptides [6]. However, naked peptide-based drugs are reported to have challenges, such as premature disintegration before reaching the target site, low stability, and poor membrane permeability [7].

The advancements in the field of nanotechnology have led to several novel nanoparticles and nanomaterials that are able to overcome the limitations of naked peptides in disease treatment [8]. Nanostructured materials have allowed for the controlled release of drugs for drug delivery, i.e., toward target cells for efficient disease therapy [9]. Further, the encapsulation of peptides in nanoparticles has the potential to address challenges associated with peptide stability during circulation, providing better membrane permeability,

and promoting tumor targeting effects with minimal adverse impacts [10]. Peptides can also be modified and self-assembled into nanosized particles, such as nanomedicine, to improve their pharmacokinetic profiles [11]. This article provides an overview of peptides, recent trends in their synthesis, purification, and characterization, as well as their nanoformulation mechanisms. In addition, recent developments in the in vitro and in vivo analyses of nanoformulated peptides for pharmaceutical applications are discussed.

2. Overview of Peptides

Peptides are specific protein fragments that possess beneficial effects for the functions of living organisms [12]. They can be synthesized via amino acids linked by covalent bonds called peptide or amide bonds [13]. Proteins and peptides play vital roles in the metabolic activities of live cells. They can exhibit antihypertensive, mineral binding, antimicrobial, immunomodulatory, antioxidant, and antithrombotic activities [13]. The amino acid constituents and arrangements are responsible for the activities of the peptides. The interplay of amino acid sequences regulates biological processes in the cells of living organisms [14]. Proteins obtained from animals and plant sources are utilized as precursors to synthesize peptides with distinct structures [13,15,16]. Several peptides display structural and functional characteristics similar to proteins, particularly peptide residues of 2 to 20 amino acid lengths with arginine, proline, or lysine amino acid groups as well as hydrophobic properties [17,18]. Recently, peptides were employed in organic transformation that occurred in the storing and processing of foods and significantly contributed to their sensory features. They are documented as a group of bioactive molecules with a wide range of biological properties in addition to their nutritional functions. Lately, nutraceuticals and functional foods with peptides have gained attention, specifically for their influence on the health of humans and their actions in the prevention of certain ailments [13,19,20].

Peptides are present in bovine milk [21], cheese, and dairy foods [21–23] as proteins. Moreover, they exist in the blood of bovine [24], meat, gelatin, fish, and eggs [13]. Peptides obtained from the proteins of animals are shown to exhibit several beneficial functionalities [25]. Recently, albumin obtained from animal blood was hydrolyzed via several trypsin concentrations and the sequence of peptides from the hydrolysates showed several properties, such as blood glucose control (inhibition of dipeptidyl peptidase-4), attenuation of hypertension (angiotensin-converting enzyme inhibition), and abrogation of oxidative stress [26]. Similarly, peptides derived from milk were reported to display antioxidant actions by preventing essential fatty acids peroxidation. Consistently, peptides liberated via hydrolysis of casein possess lipophilic and hydrophilic antioxidant activities against reactive oxygen species (ROS) and sequestering of metal ions [13,27]. Protein hydrolysates obtained from fish are treated to enzymes and improve the value of underutilized fish species and by-products of fish products. These protein hydrolysates contain peptides with distinct sequences [28]. Extracts of dry-cured ham from Spain were reported to exhibit antioxidant activities against the 1,1-diphenyl-2-picrylhydrazyl radical and superoxide ion, depending on their peptide compositions. Consistently, peptides such as Leu-Ala-Arg-Leu, Gly-Ala-Leu-Ala-Ala-His, Leu-His-Tyr, Pro-His-Tyr-Leu, GlyGly-Glu, and Gly-Ala-His isolated from *Sardinella* showed antioxidant activities against 2,2-diphenyl-1-picrylhydrazyl radical [29]. In addition, α- and β-lactoglobulin, as well as enzymatic hydrolysate, displayed enhanced free radical scavenging activities. Further, peptides and proteins isolated from eggs, gelatins, and potatoes were reported to exhibit antioxidant activities [30–32]. Leu-Lys-Gln-Glu-Leu-Glu-Asp-Leu-LeuGlu-Lys-Gln-Glu peptides (1.6 kDa) obtained from the hydrolysis of *Crassostrea gigas* (oyster) were identified to nullify lipid peroxidation, scavenged superoxide, and hydroxyl radical [33]. Moreover, the hydrolysis of bivalve mollusks (*Mactra veneriformis*) produced 21 peptides, which displayed antioxidant activities [34]. Another study showed that whey proteins, caseins, and protein fractions obtained from the digestion of goat milk by pepsin contain peptides with allergenic properties. These peptides also possess antioxidant activities against superoxide anion radicals [35].

Plant sources of peptides and proteins include rice, wheat, mushrooms, maize, pumpkin, soy, amaranth, and sorghum [36–40]. In addition, digestion of soymilk or soybean seeds via in vitro models resulted in large quantities of peptides with remarkable biological activities [40]. In another study, soy foods exposed to fermentation and soy hydrolysate produced oligopeptides after digestion with endoproteases, such as Glu C protease, kidney membrane protease, pronase, plasma protease, and trypsin. Moreover, the action of pronase on natto forms peptides with inhibitory action against the acetylcholinesterase enzyme. On the other hand, the digestion of natto with a kidney membrane protease was demonstrated to form peptides with anti-thrombotic activities [41]. Cereals, such as rice, oat, wheat, sorghum, barley, and millet, are rich sources of peptides and are reported to show activities against disorders, such as cancer, thrombosis, diabetes, hyperglycemia, and cardiovascular diseases [13,42]. Moreover, protein hydrolysates obtained from casein, potatoes, and whey were reported to inhibit oxidation of lipids [43].

Several studies have reported that peptides from both plant and animal sources have broad pharmaceutical applications. Animals, plants, and invertebrates are identified to be sources of antimicrobial peptides. These peptides are incorporated into lipid bilayers of membranes and their antimicrobial properties are attributed to their cationic sizes and charges, compositions of amino acids, and amphiphilicity [17]. Further, these antimicrobial peptides possess the ability to reduce the growth of fungal and bacterial cells [44]. Additionally, the literature shows that over 140 peptides are being investigated in human studies [6]. Further, peptides with immunomodulatory activities derived from hydrolysates of soybean and rice were reported to stimulate non-specific immunity [13]. Furthermore, an antibacterial peptide called ovotransferrin, present in egg whites, was reported to exhibit anti-inflammatory activities [45]. Thus, peptides from invertebrate, animals, and plant sources contribute positively to the health of human beings.

2.1. Recent Methods for Peptide Extraction and Formation

The extraction of peptides from several unique sources has increased in recent times. Peptides can be extracted from the proteins of milk and other sources via fermentation and other methods, as shown in Figure 1. In addition, there is a need for techniques to maximize peptide activities in food systems as well as optimize their use in pharmaceutical applications [46]. The isolation and purification of peptides involve the formation of continuous and automated systems. Thus, extensive research is required for designing specific column chromatography methods that can substitute earlier methods, such as salting out or solvent extraction. The advanced column chromatographic methods will provide peptides with optimized parameters and low degradations to enable their integration in certain nutraceutical and pharmaceutical applications [47].

Enzymatic hydrolysis, microbial fermentation, and chemical synthesis are the major methods for the production of peptides. Enzymatic hydrolysis is considered the most viable and dependable method for peptide production with intact functions, such as antioxidant properties. Hence, this method is utilized for the formation of a wide spectrum of peptides as well as peptide hydrolysates from potatoes, whey, and corn. This method has been used over the decades and it is still commonly used [49,50]. Some examples of enzymes used in this method are alcalase, neutrase, pepsin, and others [51–53]. The antioxidant abilities of peptides and peptide mixtures depend on the method of hydrolysis used, among other factors (the type of proteases used, protein source, and pretreatment of protein substrate). Both crude and pure enzymes can be applied for the extraction of peptides with antioxidant activities. Nevertheless, crude protein mixtures are used to minimize the cost of production [13,54]. Further, peptides can also be extracted via fermented milk protein products using protease. Enzymes from the bacterial strain named *Streptococcus thermophiles* were identified to be beneficial for the extraction of peptides from bovine caseins [55]. The actions of proteases from *Bacillus amyloliquefaciens* and *Bacillus subtilis* A26 on Thornback ray skin gelatin were also utilized for the extraction of peptides with antioxidant and acetylcholinesterase inhibitory activities, respectively [56]. In another study, proteases from

fungal-digested extracts of meat protein were demonstrated to have potential for extracting peptide mixtures with remarkable acetylcholinesterase and antioxidant activities in an in vitro model [57].

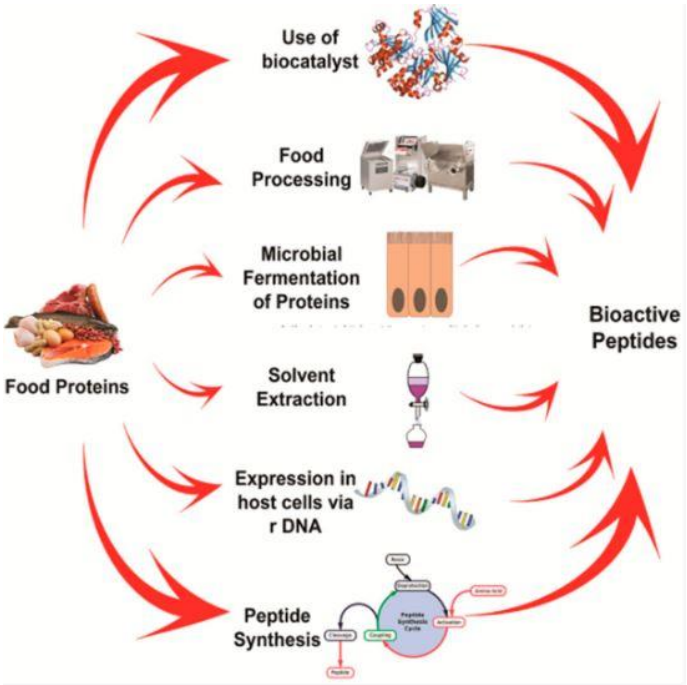

Figure 1. Various methods used to extract and form proteins/peptides. Reproduced with permission from Chelliah et al. (2021), ©MDPI, 2021 [48].

Microbial fermentation is another method for the production of peptides. The development of peptides with antioxidant activities via the fermentation process by microbes is commonly used by the food industry. Fermented soybean substances, such as tempeh and natto, were reported to contain peptides with antioxidant properties [58]. Peptides with antioxidant activities can also be produced from douchi (soybean product) via fermentation using fungal cultures [13,58]. Foods, such as yogurt with vital peptides, can be developed from milk protein through the actions of proteolytic systems [59]. The fermentation of navy bean milk with *Lactobacillus helveticus* MB2-1, *Lactobacillus plantarum* 70810, *Lactobacillus bulgaricus*, and *Lactobacillus plantarum* B1-6 showed greater acetylcholinesterase activity, compared to unfermented mil [60]. Similarly, the fermentation of milk with *Bifidobacterium bifidum* MF 20/5 has led to the production of milk products with higher acetylcholinesterase inhibitory activity. Other peptides, such as LVYPFP (inhibits acetylcholinesterase), LPLP (inhibits acetylcholinesterase), and VLPVPQK (scavenges free radical) have also been isolated from fermented milk [61].

Chemical synthesis is an essential method used for developing peptides. This method is relatively simple, inexpensive, and it involves the use of an alkaline or acidic solution. However, the demerits of this procedure include difficulties in parameter control, the formation of modified amino acids, the formation of products with distinct functional features, and chemical constituents [50,62]. Acid digestion is a vital chemical process that can alter the functional and structural features of peptides and it is recommended due to its high efficacy and low cost [63]. Examples of common acids used for the extraction of peptides are hydrochloride and nitric acid. A peptide named Lys-Arg-Glu-Ser, formed via a chemical method, has been identified to reduce the peroxidation of low-density lipoproteins and atherosclerosis as well as the attenuation of inflammation in experimental mice [64]. Intriguingly, the biological action of the peptide was reduced when its sequence was altered to Lys-Glu-Arg-Ser, which reflects the relationship with the structure. In another study, the peptide formed via a chemical method with Pro-His-His units displayed remarkable

abrogation of lipid peroxidation and lipoproteins oxidation [65]. Another advanced method for peptide extraction is via the use of ultrasound technology, which is a new, rapid, and robust technology that can be beneficial for large-scale peptide production. It has been extensively used in the extraction of peptides and proteins from natural sources with larger yields. Moreover, peptide-based drugs encapsulated with the help of ultrasounds have been identified to elevate their bioavailability and stability [66]. The advantages and limitations of the common protein/peptide extraction and formation methods are listed in Table 1.

Table 1. Advantages and limitations of protein/peptide extractions and formation methods.

Method	Advantages	Limitation	References
Enzymatic hydrolysis	Formation of specific chemical bonds, suitable for reverse-phase chromatography	Long sample treatment time (5–12 h) and low analyte recovery	[67]
Microbial fermentation	Few unexpected metabolites, lower energy consumption, avoids degradation by endogenous proteases, improves productivity	Fails to induce biological effects in the consumer and poor reproducibility, highly dependent on purification and identification	[68]
Chemical synthesis	Less expensive; requires being non-expensive	Production of intermediates, lengthy process, insolubility of the resultant peptides	[69]

2.2. Purification and Characterization of Peptides

2.2.1. Purification of Peptides

Peptides are usually encrypted in proteins and studies have shown that their release through digestion results in the biological activity of the peptide. Identification of the released peptide structure is essential; therefore, the investigation into peptide purification is necessary. Before the purification, peptides must be isolated from specific biological sources. The extract from the biological organism is initially investigated for biological activity, followed by a bioassay-guided fractionation procedure to produce peptide fractions. Then, the purification process is carried out to produce a single type of peptide with bioactivity. The purification methods that are widely used to purify peptides are membrane filtration methods (e.g., ultrafiltration (UF) and nanofiltration (NF)) and chromatography methods (e.g., cation exchange chromatography, size exclusion chromatography (SEC)/gel filtration chromatography, and reverse phase high performance liquid chromatography (RP-HPLC)).

Membrane Filtration

Membrane filtration is one of the prevalent purification methods. UF is an efficient and low cost purification technique to separate peptides with distinct molecular weight from crude hydrolysates [70]. Most studies identified that peptides with less than 3 kDa molecular weights exhibited the greatest antioxidant activities. For instance, Jang et al. (2016) compared sandfish protein hydrolysates (SPH) with four different degrees of molecular weights (>10 kDa, 5–10 kDa, 5–5 kDa, and <3 kDa), which are separated using UF. The study showed that the protein hydrolysate with a molecular weight of <3 kDa showed a higher degree of hydrolysis (DH) and the highest DPPH radical scavenging activity [71]. Further, Vandanjon et al. (2007) compared the performance of UF with two different molecular weight cut-offs (MWCO) and NF. UF with high MWCO (20 kDa) is suitable for the separation of a peptide and a non-hydrolyzed protein, while UF with intermediate MWCO (4 and 8 kDa) is more befitting for peptide fractionation to the satisfactory flux and retention. The NF membrane is efficient for concentrated peptides for both the flux and recovery rate. The disadvantage of NF is a possible risk of a high salt concentration and, hence, an additional desalination step is required [72]. Furthermore, Pouliot et al. (2000) demonstrated the effect of fractionation using NF membranes on the peptide distribution by comparing tryptic (TH) and chymotryptic (CH) hydrolysates with whey protein isolates. The study revealed that the peptides from TH with a lower negative surface charge is permeable, compared to the positive and neutral charged peptides. In addition, no significant changes in the CH

fractionation due to the lower content in each peptide sequence and, hence, permeation or retention of CH, were always missing, and the sign of the charge was not in accordance with the peptide transmission. Surface charges and properties of different short peptides may affect the intensities of both the convective and electro-migrative fluxes across NF membranes, which can later lead to poorer separation between the cation and counterion. Thus, the study emphasized that an inaccurate result and high purification cost is due to the fouling issues in the filtration membrane [73].

High Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is preparative and extremely flexible for the purification of a wide range of peptides. The three separation modes of HPLC are size exclusion HPLC (SEC), ion exchange HPLC (IEX), and reverse phase HPLC (RP-HPLC). SEC separates peptides based on the size differences of the peptides and IEX allows fractionation to occur based on the net surface charges of the peptides. The RP-HPLC isolates the peptides according to their hydrophobicity [74].

SEC is also known as gel permeation chromatography/gel filtration chromatography, where fractionation is based on the molecular size or molecular weight [75]. Sephadex is a common material used in the stationary phase; it is available on the market. The main concern of using SEC techniques is the scale-up of the chromatographic method and it has low pressure resistance, which results in a shorter life period for the bulk packing application. Peptides with low MW are not suitable for separation via SEC due to the minimal sensitivity to separate the molecule of interest with a peak from the synthetic peptide crude mixture, when the impurities are lower than the desired product [74].

IEX resolves the peptides based on their surface charges by binding the molecules with opposite charges and separating them accordingly. It can be used to separate protein ionizable chemical moieties by manipulating the pH and ionic concentration of IEX media [76]. Further, IEX is useful for purifying natural peptides from complex tissue extracts, as well as synthetic peptides [77]. There are two types of IEX, namely positive and negative chromatography, where the target is concentrated and the contaminant remains unretained in positive chromatography, while negative chromatography maximizes the volume of the adsorbent [78]. All IEX media have their own specifications in the pH, buffer, and capacity and, thus, are used for the purification of distinct samples. The IEX media, which are commonly available on the market, include Capto, MacroCap, MiniBeads, Monobeads, Sephadex, Sepharose, and SOURCE. The disadvantage of IEX is the dilemma in selecting the IEX media and column hardware as they have great influence on the lateral outcomes [79].

The RP-HPLC method is a rapid and efficient purification method for short peptides and bioactive substances (<1 kDa). Its high resolution, fast analysis speed, extreme sensitivity, and good reproducibility makes it a preferred purification method. Moreover, RP-HPLC provides the greatest scope for the manipulation of mobile and stationary phases to improve the peptide separation [74]. Moreover, Lee et al. (2009) have separated the angiotensin I converting enzyme (ACE) inhibitory peptides from rotifers named *Branchionus rotundiformis* into five short fractions, after pre-purification via RP-HPLC, which allows them to select the most potent ACE inhibitory activity [80].

The above-mentioned methods are conventional purification approaches that are currently used by the researchers to purify peptides. However, most researchers prefer to do multistep purification methods or repeated purification techniques to obtain better results, as a single peptide purification is no longer satisfactory. Normally, ultrafiltration will be used as the pre-purification step followed by IEX or SEC, prior to a final RP-HPLC. Yang et al. (2020) and Xia et al. (2020) employed such an approach to isolate antioxidant peptides from duck plasma protein and the enzyme hydrolysate of mung bean protein, respectively [81,82]. In other recent studies, the researchers attempted to exclude the use of IEX or SEC purification steps. For instance, Jang et al. (2016) used UF as an initial screening approach and conducted the RP-HPLC process twice to derive the antioxidant peptide from sand fish [71].

2.2.2. Characterization of Peptides

Most researchers perform peptide sequencing or amino acid sequence for structural characterization to obtain the database of the targeted protein. The peptide sequencing measures the peptide length, which is essential to determine the bioactivity of a specific peptide [83]. The main tools for protein identification and characterization are mass spectrometry (MS), electrospray ionization (ESI), and matrix-assisted laser desorption ionizations (MALDI) [84]. Liquid chromatography equipped with tandem mass spectrometry detection (LC-MS/MS) is also useful in peptide sequence detection, while matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) helps in generating peptide profiles [85].

Using the protein sequencer and LC/MSD, Jang et al. (2016) identified that the sequence of the purified peptide from sandfish was Ala-Thr-Ser-His-His. The high antioxidant activity of the peptide was due to the two histidine residues and the presence of alanine residues might act as inhibitors in the radical-mediated peroxiding chain reaction [71]. Further, Xia et al. (2020) used UPLC-Q-TOF-MS and identified that the molecular weight(s) (MW) of those eight antioxidant peptides from mung beans were in the range of 300 to 800 Da [86]. An antioxidant peptide usually contains 2 to 10 amino acid residues with a MW less than 1500 Da [87].

Baindara et al. (2013) isolated and purified antimicrobial peptides from bacteria (SK.DU.4) using a combination of chemical extraction and chromatographic techniques, SEC, and RP-HPLC. However, UF was not used as the pre-purification step [88]. Characterization of the antimicrobial peptide was performed using MALDI-TOF-MS to determine the MW, GC-MS to identify the fatty acid, SDS PAGE to detect the bacteriocin activity, and the agar/broth dilution method to determine the minimum inhibitory (MIC) concentration. The results revealed that 5.3 kDa of isolated antimicrobial peptides are similar to the lipoproteins *of Methylomicrobium album*, which is an enzyme exhibiting antimicrobial activity via degradation of the structural cell wall component; the narrow spectrum antimicrobial activity against Gram-positive bacteria, low molecular weight, and resistance to high temperature suggested the bacteriocin-like antimicrobial activity of the peptide [89].

It is noteworthy that the selection of a suitable and proper purification method is extremely significant as it is the rudiment of peptide characterization. As mentioned earlier, good characterization is critical as the results provide information within the isolated peptide. The purification and characterization choices depend on the original source of the peptide type. The majority of researchers have combined distinct purification methods or repeated the same purification steps to obtain better results. Thus, the best combination of a purification method is UF and RP-HPLC, as both are proven to be efficient in purification. Further, they are affordable and require less time for high quality purification. UF can be used as the first step of the peptide purification before further purification via RP-HPLC. Hence, the selection of the characterization result is highly based on the desire of the researchers to explore the interior components of the purified peptides.

3. Nanoformulation of Peptides

Several polymeric nanoparticles have been utilized as potential carriers for peptides and are used for the peptide formulation in controlled and targeted delivery applications. Nanoformulated peptides are reported to improve drug administration, where the drugs are either dissolved, entrapped, encapsulated, or attached to drug carriers [90]. In this section, three of the most commonly approaches for peptides nanoformulation, i.e., lipid-based nanoformulations (LNFs), silk fibroin nanoparticles (SF-NPs), and cavitands, are discussed.

3.1. Lipid-Based Nanoformulations (LNFs)

LNFs have been focused on in the scientific arena due to their great biocompatibility and versatility. They are great candidates for peptide carriers due to their submicron sizes, encapsulation capabilities (in protecting peptides against degradation), biodegradability, and their ability to maintain the drug activity's effectiveness [91]. Table 2 summarizes different types of LNFs that are used as peptide carriers.

Table 2. Summary of different LNF types as peptide carrier.

LNFs	Descriptions	Advantages	Disadvantages	Applications	References
Nanoemulsions (NEs)	- Oil, water, and surfactant. - Long- and medium-chain glycerides, and fatty acids. - Peptide is solubilized within the dispersed nanoparticles.	1. Excellent dispersity. 2. Prolonged stability. 3. Good penetration abilities.	1. Require high concentrations of surfactant. 2. Choices of biocompatible surfactants are limited.	1. Shah et al. (2014) discovered polyunsaturated fatty acid NEs able to encapsulate the analgesic peptide; demonstrated efficacy in the capsaicin. 2. Pattani et al. (2006) developed NEs of polymyxin B and found NEs capable of producing potent effects in short times.	[92,93]
Liposomes	- Bilayer vesicles; aqueous compartment is enclosed entirely by the membranous lipid bilayer. - Involve phospholipids, phosphatyl, glycerol derivates, and saturated and unsaturated fatty acids.	1. Consistent release of proteins and peptides. 2. Easy modification of surface attachment. 3. Increase membrane permeability, drugs with proteins 4. Toxic-free. 5. Amphiphilic character, enables self-assembly.	1. Limited stability can cause possible drug leakage and lead to aggregation. 2. Extra steps are commonly needed to modify their sizes and structures.	Omri et al. (2002) formulated a DPPC/Chol liposomal polymyxin B against *Pseudomonas aeruginosa* and discovered that it can decrease the pulmonary bacterial counts along with a higher level of polymyxin B in the lungs, compared with those treated with free drugs after administered in a rat model for 3 days.	[94,95]
Solid Lipid nanoparticles (SLNs)	- Consist of lipids in a solid state at both room and body temperature. - Solid hydrophobic core and single layer of the phospholipid coating.	1. Better drug encapsulation efficiency. 2. Encapsulate both lipophilic and hydrophilic drugs. 3. Improve drug stability. 4. Control release drug formulation as drug mobility is low in a solid state and triglycerides are slower than other glycerides. 5. Suitable for large-scale production.	1. Poor drug loading capacity. 2. Drug expulsion (burst release after intravenous injection). 3. Require stabilizer to prevent drug portioning to the outer aqueous phase.	Yuan et al. (2008) reported that PEG-SA and conjugate FA-SA that are inserted into SLN become potential applications for tumor therapy as they show efficient cellular updates and cytotoxicity by endocytosis. 2. Garcia-Fuentes et al. (2005) developed new surface-modified SLNs coated with chitosan (CS) for peptide delivery and discovered the ability of the ready release of the peptide; provided continuous delivery of the associated peptide.	[96–99]
Nanostructure lipid carriers (NLCs)	- Next generation of SLNs by improving the stability and capacity loading, and preventing drug expulsion. - Blend of solid lipids and liquid lipids (oil) in certain proportions.	1. Simple preparation. 2. Larger drug loading capacity compare with SLN. 3. Sustained drug release properties. 4. Low water content in final particle suspension.	1. Susceptible to degradation by gastrointestinal lipases.	-	[100]

Table 2. *Cont.*

LNFs	Descriptions	Advantages	Disadvantages	Applications	References
Lipid nanocapsules (LNCs)	- Made of solvent-free process with biocompatible excipients. - Contain oily a core surrounded by hydrophilic surfactants. - Involve medium-chain mono-, di-, and triglycerides, and long-chain fatty acids. - Combination of polymeric nanoparticles and liposomes.	Provide considerable drug encapsulation capacity and sustain release properties.	1. Non-specificity. 2. Unable to cross the weakly permeable endothelia.	Nada et al. (2019) demonstrated the ability of antimicrobial peptide loads in LNCs using different strategies and discovered that both absorption and encapsulation methods can protect the peptide from proteolytic degradation. However, the LNC encapsulation method is not suitable for peptides with great amphipathic abilities.	[101]

3.2. Silk Fibroin Nanoparticles

Silk fibroin nanoparticles (SF-NPs) have been identified to possess excellent biocompatibility and degradability as well as conjugating abilities with other active molecules. The enhanced binding capacity toward distinct drugs, controlled release capability, and mild preparation approach make SF-NPs effective drug delivery carriers [102]. Recently, Hassanzadeh et al. (2021) successfully developed biomimetic SF-NPs against breast cancer by coating SF-NPs with polydopamine (PDA), paclitaxel (PTX), peptides (iRGD), and transformed them into iRGD-PDA-PTX-SF-NPs. This new biomimetic peptide-based nanoformulation acted as an effective drug nanocarrier to target cancer cells overexpressing integrin (selective targeting of tumor). The SF-NPs showed improved intra-tumoral penetration and accumulation. The better drug entrapment also helped to reduce the dose frequency and drug intake time into the human body for complete recovery, thus, lessening the burden to the patient. Further, Li et al. (2022) formulated SF-NPs, where doxorubicin and atovaquone were encapsulated with Arg-Gly-Asp-SF-polylactic acid (RSA) to improve the chemotherapy treatment. The treated mice (injected with breast 4T1 cancer cells) showed higher inhibition rates, compared to phosphate buffer saline (PBS) and RSA alone, with minimal changes to the weights exhibited in the treated mice. This SF-based targeted drug carrier can alleviate the hypoxia microenvironment by suppressing mitochondrial respiration, which means it may suppress tumor development [103].

3.3. Cavitands

Cavitands are synthetic macromolecules, which can act as stabilizers for peptides and protein formulations by binding to amino acids and preventing the recognition of peptidase. Cyclodextrins (CDs) and cucurbiturils (CBs) are the best example of cavitands. CDs are cyclic non-reducing oligosaccharides that are made up of glucopyranose units [104]. CDs have amphiphilic structures that allow them to form in the inclusion complex with the protein and peptide. Moreover, CDs are safe for medical administration with less side effects even after chemical modifications of their exterior parts. They possess increased bioavailability and have enhanced local tolerability to peptide formulations. Further, Jóhannsdóttir et al. (2017) formulated CD-based aqueous cyclosporin A (CyA) as an eyedrop formulation. CD was selected as a solubilizer due to its great solubility effect on CyA, with its ability to form a CyA/CD complex aggregation and nanoparticles for better ocular bioavailability of drugs [105]. However, there are adverse possible consequences of cyclodextrin intake. One study reported that the administration of 2-hydroxypropyl-β-CD, i.e., 200 mg/kg every day for the long-term, could cause bone loss [106]. Further, Li et al. (2016) compared the toxicity and solubilizing capacity of hydroxypropyl–β-CD with different degree(s) of substitution (DS). The study revealed that hydroxypropyl–β-CD with a high DS not only exhibited weak solubilizing capacity for steroids, but also low hemolytic activity, while hydroxypropyl–β-CD with a medium DS demonstrated slightly higher nephrotoxicity. On the other hand, CBs are macrocyclic co-polymers of formaldehyde and glycoluril [107]. Similarly, CBs have hydrophobic voids, whereas hydrophilic parts are located in the portal area. The CB[n] family, where n is the different number of glycoluril units, possesses high affinity, high selectivity, and constrictive binding interactions, which make them great choices for the nanoformulation of peptides. Thus, CBs can bind better together to cationic guests, compared with CDs [108].

The most ideal system among all nanoformulation is difficult to select as every system has specific advantages and limitations. For instances, SLN and NLC have the ability to improve the delivery of drugs in various ways, but the choice of lipids and surfactants can affect both the particle size and stability in the long term. Toxicity is a significant factor that needs to be considered when selecting the surfactants to prevent any adverse impacts to be included in biomedical applications. Therefore, the above-mentioned limitations must be considered when designing a novel nanoformulated peptide with enhanced stability and the drug release time for effective circulation, low toxicity, high biocompatibility, and improved biological response.

4. In Vitro Analysis of Nanoformulated Peptides

Recently, various peptide encapsulated nanoparticles were developed for anti-tumor applications with less/no toxicity toward normal or noncancerous cells, as listed in Table 3. Yu et al. (2007) synthesized nucleolin-encapsulated florescent silver nano clusters. Nucleolin acted as a protective layer to prevent silver clusters from rapid oxidization. In the study, the fibroblast cell line was utilized to observe the delivery of the encapsulant. The results revealed that the nucleolin can enter the cells via the nuclear membrane for a strong nuclear staining application [109]. Likewise, Jagani et al. (2013) developed chitosan nanoparticles, which are encapsulated by siRNA for anticancer applications. The resultant siRNA was identified to silence the overexpression of the anti-apoptotic Bcl-2 gene. The size ranges of the siRNA-encapsulated chitosan nanoparticles were identified to be 190–340 nm. The study showed that the encapsulation efficiency of the chitosan nanoparticles was ~80%. The cytotoxicity results showed that the cell viability was 95% after 48 h. After 72 h, 85% of cells were viable, which revealed that the encapsulated nanoparticles do not exhibit toxic reactions toward noncancerous cells [110]. Further, Bawa et al. (2012) compared the anticancer activities of the nano- and micro-sized formulations of a peptide drug named ellipticine, which is an anticancer drug. The particle size distributions of the peptide formulations were in the range of 40–300 nm due to the agglomeration of particles. The cytotoxicity results demonstrated that nanoformulated peptides possess enhanced cancer cell inhibition properties toward the human carcinoma cell line (A549) after 24 h via improved endocytosis-mediated cellular uptake [111]. Furthermore, Gomes et al. (2013) prepared a novel nanoemulsion with a peptide containing essential oil extracted from *Dodonaea angustifolia* Miers (DA) and *D. brasiliensis* Miers (DB). Transmission electron microscope (TEM) results showed homogeneous droplets of nanoemulsions with sizes less than 200 nm. The cytotoxicity analysis was performed toward human glioma (U-138 MG) and human bladder carcinoma (T24) cell lines. The results revealed that the essential oil from both DA and DB reduced the cell viability at a 500 $\mu g\ mL^{-1}$ concentration, while the nanoemulsion containing 4% essential oil possesses the ability to reduce the cell viability at 250 $\mu g\ mL^{-1}$. Thus, nanoemulsion has been identified to act as a promising candidate for cancer treatment at lower concentrations, compared to free essential oils [112]. Moreover, Kulsharova et al. (2013) synthesized doxorubicin (DOX)-coated gelatin nanoparticles, which are encapsulated with a specific peptide for targeting breast cancer cells. DOX is a chemotherapy drug that leads to certain side effects, such as vomiting, diarrhea, eye redness, and darkening of the skin. The goal of the study is to minimize the side effects of DOX via controlled drug delivery into cancer cells. Three cell lines were used for the in vitro analysis of the novel formulation, namely MCF7 mammary adenocarcinoma cells, 4T1 mouse mammary carcinoma cells, and 3T3 mouse fibroblasts. The results showed that the viability of tumor cells decreased to 50% after 5 h due to the targeted delivery of DOX. Meanwhile, the increase of the 3T3 mouse fibroblasts cell growth indicate that the peptide drug possesses the ability to selectively inhibit the tumor cells [113].

Nishikawa et al. (2009) successfully coated the (AG)-30 angiogenic peptide on gelatin nanoparticles (AG-30/gelatin NPs) to be utilized for the treatment of ischemic diseases. The cytotoxicity analysis of AG-30/gelatin NPs using HAECs (human aortic endothelial cells) and HASMCs (human aortic smooth muscle cells) revealed that the AG-30/gelatin NPs possess dose-dependent toxic effects [114]. Further, Imanparast et al. (2017) prepared the mZD7349 peptide, which is encapsulated with poly(lactic-co-glycolic acid) nanoparticles (of 200 nm), and loaded with simvastatin. HUVEC (human umbilical cord vascular endothelial cells) were used for the cytotoxicity analysis, which showed that an increment in the drug concentration decreased the viability of the cells. Thus, it is evident that the peptide-encapsulated nanoparticles possess concentration-dependent cell viability, compared to standalone nanoparticles [115]. Furthermore, Campos et al. (2004) prepared mucin-encapsulated chitosan fluorescein nanoparticles with the size range of 384.6 ± 8.5 nm. The encapsulated nanoparticles did not exhibit cytotoxicity at higher concentrations. Moreover, certain minor damages were identified in cells due to the usage of

the acetate buffer as observed in the scanning electron micrograph (SEM) [116]. Moreover, Narayanan et al. (2012) encapsulated human parathyroid hormone 1-34 (PTH1-34) into chitosan nanoparticles with an average particle size of 40 nm. The chitosan nanoformulation is shown to possess negligible effect toward the NIH3T3 fibroblast cells [117].

The standalone peptide has certain limitations, such as being unable to reach intracellular targets, poor stability, and insufficient drug delivery into the cell. Thus, peptide ligands were decorated on the surface of double emulsion (mPEG-b-PCL) nanoparticles via co-encapsulation for targeted drug delivery. The release rate of the peptide was slow from the nanoparticles when the pH of the peptide solution was altered. Later, the mixed peptide ligand with TGN, COG133, and RXR on the surface of the nanoparticles increased the drug release rate. The cytotoxicity analysis results showed that the double-encapsulated nanoparticles at 1 µM have the ability to suppress cell death and achieve significant peptide ligand release into the cerebellum [118]. Further, Silva et al. (2013) synthesized long peptides (OVA24) and encapsulated them within poly(lactic-co-glycolic acid) (PLGA) nanoparticles via a solvent evaporation technique for cancer immunotherapy. OVA24-loaded nanoparticles resulted in a high burst release due to low encapsulation efficiency. The burst release rate decreased from 90% to less than 10%, when there was an increase in the inner emulsion volume. Peptide encapsulated nanoparticles with a low burst release enhanced the activation of B3Z CD8+ T cells and the dendritic cell uptake due to T cell immunity generation, and played a vital role in cancer immunotherapy [119].

Table 3. In vitro analysis to evaluate the efficacy of nanoformulated peptides.

Encapsulant	Nano Delivery System	Cell Line	Applications	Reference
Nucleolin	Silver Nano clusters	NIH3T3 (Fibroblast cells)	Cancer	[109]
siRNA	Chitosan nanoparticles	HEp-2 (human epithelial laryngeal carcinoma), HeLa (human cervical carcinoma)	Anti-tumor	[110]
Self-assembling peptide (EAK16-II)	Nanoformulation	A549 (lung carcinoma)	Anticancer	[111]
DA and DB oils	Nanoformulation	U-138 MG (human glioblastoma) and T24 (human bladder carcinoma)	Cancer	[112]
PEO and PPO blended	Poly (β-amino ester) nanoparticles	MDA-MB-231 (human breast adenocarcinoma cells)	Breast cancer	[120]
Cathepsin D	Gelatin	MCF7 (Human breast cancer cell) and HeLa cells	Breast cancer	[113]
(AG)-30 angiogenic peptide	Gelatin	HAECs (human aortic endothelial cells) and HASMCs (human aortic smooth muscle cells)	Ischemic diseases	[114]
Simvastatin	Poly (lactic-co-glycolic acid) (PLGA) nanoparticles	HUVEC (human umbilical cord vascular endothelial cells)	Cardiovascular disorders and cancer	[115]
Mucin	Chitosan nanoparticles	Conjunctival epithelial cells	Cornea	[116]
PTH 1-34	Chitosan nanoparticles	NIH3T3 (Fibroblast cells)	Osteoporosis	[117]

5. In Vivo Analysis of Nanoformulated Peptides

Recently, Fu and coworkers developed RGD peptide encapsulated doxorubicin-loaded selenium nanoparticles (146 nm) to target the tumor vasculature. In this study, human breast cancer cells were injected into male nude mice. The in vivo experimental result showed that MCF-7 tumor growth was inhibited in the mice model by the RGD peptide selenium nanoparticles and the volume of the tumor decreased. However, there was no significant weight reduction in the body weights of the mice. Moreover, the RGD-

selenium nanoparticles induced apoptosis and inhibited angiogenesis. Later, the mice were sacrificed, and their blood samples were obtained from different organs. The hematological analysis showed that the DOX could increase the levels of lactate dehydrogenase, aspartate aminotransferase, creatine kinase, and creatine, while peptide nanoparticles did not show any toxic effects. The in vivo results demonstrated that the peptide-decorated selenium nanoparticles were highly efficient for antitumor applications [121]. Further, Kaliaperumal et al. (2014) designed pACC1 peptide-encapsulated chitosan nanoparticles for controlled drug delivery toward breast cancer cells. Furthermore, 7,12-Dimethylbenz[α]anthracene (DMBA) carcinoma was induced in 6 groups of female rats for in vivo analysis. The results showed that the encapsulated nanoparticles could increase the level of antioxidant enzymes for the neutralization of the free radicals. ACC1 was shown to significantly increase the production of adenosine triphosphate (ATP) via mitochondria during glycolysis and the Krebs cycle. pACC1 has been shown to have enhanced abilities at controlling the membrane receptors of HER2 EGFR, where its over-expression can lead to breast cancer. Later, the pACC1-encapsulated chitosan nanoparticles were designed for dual functions, such as blocking lipogenesis and control membrane receptors for effective breast cancer treatment [122]. Furthermore, Wei et al. (2017) prepared doxorubicin-encapsulated mesoporous silica nanoparticles, which were surface-modified by polydopamine, and the CSNRDARRC peptide was decorated on the DOX-loaded MSNs@PDA for bladder cancer treatment. In this study, the HT-1376 cells were injected into male mice. It has been identified via histological studies that the DOX-loaded MSNs@ PDA and DOX-loaded MSNs@ PDA-PEP injected mice gained weight, where mice injected with DOX in the MSNs@ PDA-PEP sample remained healthy. After scarification of the mice, there were no morphological changes in the heart, liver, spleen, lung, and kidney. On the other hand, the mice injected with DOX-loaded MSNs@ PDA were weak and noticeable damages were identified in the organs. The results suggest that DOX-loaded MSNs@ PDA-PEP possesses antitumor effects and the peptide has the ability to control drug release toward the tumor cells [123]. Moreover, Sarangthem et al. (2020) incorporated the AP1 peptide with increased molecular weights of A38, A60, A86, and A100 on an elastin-like polypeptide polymer with a particle diameter of ~38–40 nm to exhibit antitumor activity. In vivo analysis results revealed that the peptide with a higher molecular weight retained for a longer period (up to 48 h) in the tumor cells; the peptide with a lower molecular weight retained for up to 12 h, where A86 and A100 reduced the volume of the tumor cells with low accumulation in the kidney, spleen, lungs, and heart of the mice model [124].

Zhao et al. (2014) constructed nano-lipid formulations of monomeric and trimeric peptides for the treatment of atherosclerosis. The drug was administrated through oral and intraperitoneal (IP) injection toward the low-density lipoprotein receptor (LDLr)-containing mice model. The mice were fed with chow diet for 10 weeks to increase their blood cholesterol levels and to induce atherosclerotic plaque in the aorta. The plasma cholesterol level study revealed that the administration of the nano-lipid formulation has led to a 50% decrease in the total cholesterol level for reducing the atherosclerotic plaque. Ip-administrated trimeric nanoparticles reduced the area of atherosclerotic lesions more than the monomeric lipid nanoparticles. It is evident from the in vivo studies that both administration routes helped to improve the effectiveness of the nano-lipid formulation in reducing the total cholesterol level [125]. Similarly, Wang et al. (2016) studied the PEG-PLA nanoparticles, which were decorated by the iRGD peptide with a particle size distribution of 39 nm. In vivo studies showed that the peptide-decorated polymer nanoparticles possess the ability to enhance the antitumor activity by ~60% by inhibiting the cancer cell proliferation via the binding of αv integrins with tumor endothelial cells, compared to normal cells [126]. Likewise, Wang et al. (2014) conjugated iRGD-PPCD and prepared the CRGDKGPDC cyclopeptide, which was encapsulated in a PEGylated polyamidoamine (PAMAM) dendrimer. The in vivo studies revealed that the peptide-conjugated sample was released in the tumor blood vessels and their accumulation in the tumor cells were higher compared to nonconjugated samples. The peptide-conjugated drug induced the highest inhibition

of tumor vascular growth and effectively reduced the volume of vascular tumor [127]. Further, Kang and co-workers (2014) developed iNGR peptide-coated PEGylated PLGA nanoparticles for glioma treatment. In vivo studies showed that peptide-coated polymer nanoparticles possess high florescence intensity and anti-glioma efficacy, compared to uncoated nanoparticles. The nanoformulated samples were identified to be accumulated in the tumor sites and they penetrated deeply into the parenchyma of cancer cells due to their proteolytic cleavage into CRNGR and binding with NRP-1 [128].

Recently, Feng and co-workers (2016) designed a pyropheophorbide-a-conjugated polymeric drug, decorated with the F3 peptide. The peptide was shown to possess the ability to bind with nucleolin and it is expressed in tumor cells. The in vivo pharmacodynamic analysis showed that the inhibition of a tumor by the peptide-decorated nanoparticles was high, compared to free nanoparticles (79.92%). The apoptosis of tumor cells was shown to be induced after the treatment of the peptide-decorated nanoparticles, which indicates that the peptide effectively helps in targeting the tumor cells to control their growth. The histopathological analysis results revealed that there was no toxicity on normal tissues or the organs of the mice model treated with the peptide containing the nanoparticle [129]. Further, Liang et al. (2015) introduced tLyP-1-functionalized nanoparticles into the mice model to evaluate their antitumor properties. The peptide-functionalized nanoparticle was injected though the tail vein of the animal models for equal distribution throughout their bodies. TLyP-1-functionalized tLPTS/HATS nanoparticles were identified to be completely penetrable into the tumor tissues and reduced the accumulation of nanoparticles in the liver, spleen, and kidney. The growth of the tumor was suppressed at around 74%, compared to standalone nanoparticles. Additionally, the study demonstrated that the lipid-loaded nanoparticles did not exhibit a loss of net body weight, indicating its nontoxicity. However, the cell apoptosis rate increased after the incorporation of peptide-functionalized nanoparticles [130]. Furthermore, Xiao et al. (2012) prepared the OA02 peptide encapsulated in micellar nanoparticles for cancer treatment by releasing the peptide against the a-3 integrin, which is overexpressed in ovarian cancer. In vivo bio distribution studies showed that the peptide-loaded nanoparticles possess the ability to target tumor sites faster than unloaded nanoparticles. Moreover, the peptide-coated nanoparticles were identified to penetrate deeper into the ovarian cancer cells and bind with the a-3 integrin to exhibit enhanced cancer cell inhibition [131]. Moreover, Miyano et al. (2017) synthesized a novel cyclic Arg-Gly-Asp (cRGD) peptide and coated it on the surface of cisplatin-loaded micellar nanoparticles to target the SAS-L1-Luc cells, which were inoculated onto the mice tongues. An in vivo antitumor analysis showed that there was no distinct weight loss in the peptide-encapsulated nanoparticle-treated mice and it has a high capacity to inhibit tumor growth. Moreover, the cRGD nanoparticles were identified to be rapidly accumulating on the tumor sites and interacting with $\alpha v\beta 3$ integrins to be expressed in the endothelial cancer cells [132].

Fang and co-workers (2017) developed a micelle nanoparticle (below 50 nm) using the cyclic RGD peptide cross-linked with poly(ethylene glycol)-b-poly(e-caprolactone) (PEG-PCL) to target glioma cancer. In vivo results indicated that cRGD-RCCMs had the potential to inhibit tumor growth and their efficiencies were enhanced by increasing the concentration of cRGD. No weight loss was identified in cRGD-RCCM-treated mice and their survival time prolonged. However, slight toxicities was observed in the liver, spleen, and kidney due to an increment in the cRGD concentration [133]. Moreover, Bi et al. (2016) prepared carmustine-loaded polymeric nanoparticles using the T7 peptide, encapsulated on the surfaces of loaded micelles for targeting the tumor cells in the central nervous system. The results indicate that the T7 peptide-encapsulated nanoparticles possess enhanced abilities to highly accumulate in the tumor cells and penetrate deeper into brain tumors. Moreover, T7 peptides have been identified to potentially target Tf receptors, which could be overexpressed in the blood–brain barrier. In this study, luciferin was injected into the tumor and it has been identified that the intensity of the luminescence decreased due to the inhibition of the tumor cell growth. Weight was not observed in the animal model,

compared to the uncoated T7 peptide-polymeric nanoparticles, with the longest survival time of the peptide. Histopathology results showed that there were no toxic effects on the spleen, liver, kidneys, and lungs. It is evident from the results that the T7 peptide-encapsulated polymeric nanoparticles can target the tumor cells for the controlled delivery of drug candidates without inhibiting the normal noncancerous cells [134]. Figure 2 presents the summary of the in vivo analyses, which were used to evaluate the biological applications of nanoformulated peptides.

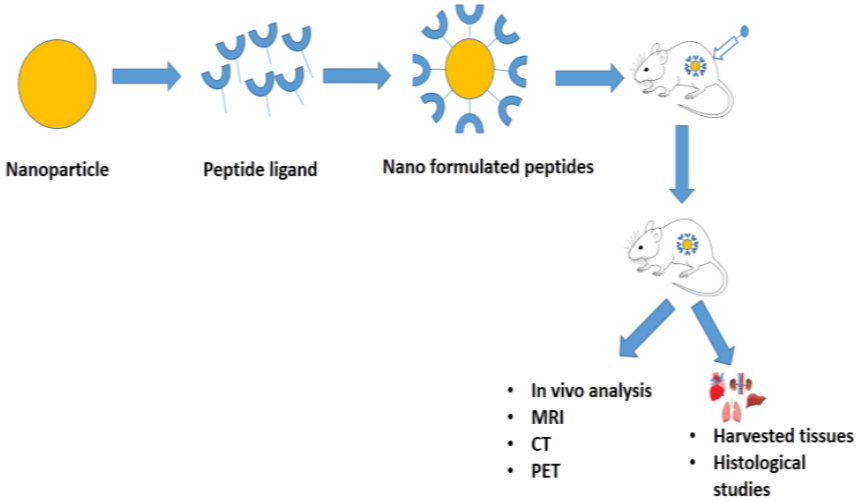

Figure 2. Types of in vivo analyses to evaluate the biological applications of nanoformulated peptides or peptide-conjugated/functionalized nanoparticles.

6. Mechanism of Nanoformulated Peptides in Disease Theranostics

Bio-physiochemical differences between the microenvironments of diseased and normal tissues allow peptides to be potential candidates for therapeutic drug delivery applications [135]. The pH differences of these two environments have gained the attention of researchers, leading them to develop pH-responsive systems toward disease-specific drug deliveries [136]. Thus, the design of a specific therapeutic nanoformulations relies greatly on the understanding of the underlying mechanism of the disease microenvironment (DME). Overexpressed cell surface receptors in DME have also become targets, i.e., leading to researchers creating peptide-based delivery systems to reduce the adverse effects toward patients affected by diseases [137]. There are several types of nanoformulated peptide-based mechanisms that have the ability to deliver therapeutic drugs, as summarized in Figure 3. Hence, certain mechanisms of nanoformulated peptides, such as the self-assembled peptide, cell targeting peptide, cell penetration peptide, and endosomal disrupting peptide are discussed in this section.

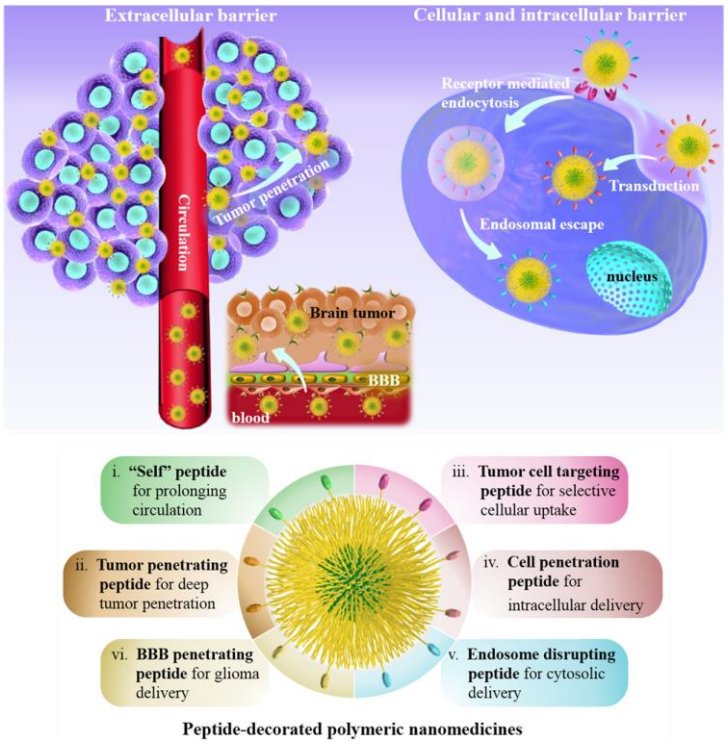

Figure 3. Mechanisms of peptide-decorated polymeric nanomedicines in the blood stream, brain tumor therapy, and cellular and intracellular environments. Reproduced with permission from Sun et al. (2018) [138], ©Elsevier, 2018.

6.1. Self-Assembled Peptide

Surface PEGylation is widely used to improve the effectiveness of drug delivery toward targeted cells and genes, as it delays phagocytic clearance and leads to a longer circulating time [139]. However, the major challenges in these surface PEGylated particles are the loss of therapeutic efficacy and an increment in the side effects after repeated administration of the anti-PEG antibody after immunization with PEGylated proteins [140]. Several studies have identified that the self-assembled peptides were highly beneficial in inhibiting the growth of targeted cells affected by a disease, especially cancer cells. Rodriguez et al. (2013) demonstrated that self-assembled peptides designed using human CD47 have the ability to minimize the phagocytic uptake, prolong circulation time, enhance tumor accumulation, and improve the delivery of nanoparticles toward the tumor site, compared to PEGylation-modified particles in non-obese diabetic (NOD) scid gamma (NSG) mice [141]. Further, Li et al. (2019) successfully designed a tumor microenvironment-adaptable self-assembly (TMAS) of short peptides via the synthesis of a pentapeptide FF-Amp-FF (AmpF). This pentapeptide AmpF has been identified to possess the ability to assemble them into super-helics in a neutral solution and transform them into nanoparticles at low pH conditions due to the cis/trans isomerization of proline amide bonds. This adaptability of the peptide-based nanoparticles has enhanced their circulation times and improved their accumulation and retention at tumor sites [142]. Furthermore, Zhao et al. (2014) designed a TMAS nanoformulation of hybrid peptide nanoparticles via conjugation of functional 3-diethylaminopropylisothiocyanate (DEAP) molecules with short peptides, which transform based on the pH of the tumor environment [143]. In addition, several reports showed that nanoformulated self-assembled peptides can be potential therapeutic agents in the treatment of various diseases in the future [144–146].

6.2. Cell Targeting Peptide

In recent times, targeted peptides were used in disease therapeutics to target specific receptors that were overexpressed in cells affected by a disease, specifically due to their low molecular weights and abundant versatility [147]. Recently, researchers have focused on conjugating peptides with nanocarriers, such as micelles, polymers, or liposomes, to improve their therapeutic drug delivery toward cells affected by a disease [148]. In case of cancer therapy, Yu et al. (2016) designed T7 peptide-modified core–shell nanoparticles (T7-LPC/siRNA NPs) for an effective systemic delivery of siRNA, especially for the targeted breast cancer therapy. The characteristic core shell structure aids in siRNA encapsulation by preventing them from RNAse degradation. Hence, siRNA remained intact for more than 36 h of incubation for T7-LPC/siRNA NPs, compared to standalone siRNA, which were completely degraded after 3 h of incubation. An in vivo experimental analysis revealed an enhanced tumor accumulation for T7-LPC/siRNA NPs, attributed to their active targeting via receptor-mediated interactions through T7-peptide modification and are effective in tumor growth inhibition. Moreover, T7-LPC/siRNA NPs have been identified to be effective and safe systemic delivery agents for siRNA with negligible body weight changes in mice models, less hemoglobin (HGB) levels, interferon alpha (IFN-a), and interleukin-6 (IL-6) levels [149]. Similarly, Sorolla et al. (2019) successfully developed a peptide-based targeting ligand with docetaxel (DTX) nanoparticles, EN1-iPeps, and RGD peptides (EN1-RGD-iPeps NP) for the treatment of chemo-resistant triple negative breast cancer (TNBCs). This DTX-NP formulation has reduced TNBC growth in vivo, with no alteration in organ appearance, body weight, liver enzyme profile, and lack of evidence for tissue damage at the histopathological level, which proves the fewer side effects of these NPs [150]. It is noteworthy that these cell-targeting peptides were either formulated in nanoparticles or coated on nanoparticle surfaces to be utilized for the desired disease treatments in the future [151–153].

6.3. Cell-Penetrating Peptide

The cell-penetrating peptide (CPP) possesses the ability to permeate through the plasma membrane of the cells for facilitating the internalization of impermeable molecules [154]. The effectiveness of CPPs can help in decreasing unwanted side effects and the administration dosage to be utilized as an efficient drug carrier. Moreover, CPPs were incorporated into therapeutic drugs in two ways—a stand-alone delivery system and a conjugated-with-nanoparticle delivery system. Thus, the nanoformulation of CPPs has shown an enhancement in the delivery of chemotherapeutic agent [147,155]. Further, Liu et al. (2018) developed cell-penetrating α-helical polypeptide (PVBLG-8/siRNA/PLG@PLG (PSPP) NPs)-based metastable nanoparticles to deliver systemic siRNA for anticancer treatment. High siRNA encapsulation efficiency is achieved with PLG to act as a stabilizer to facilitate the complexation between positively charged PVBLG-8 and negatively charged siRNA. Additional PLG also enhances the serum stability of the NPs for their better circulation. Deeper penetration is observed in acidic conditions (the tumor microenvironment is more acidic) compared to neutral conditions due to the de-shielding of the PLG at the outer layers of NPs, which can lead to the exposure of cationic PVBLG-8 and improve tumor accumulation. The study also showed that the peptide formulation possesses an enhanced inhibition ability against tumor growth with PSPP NPs, especially to improve their in vitro and in vivo antitumor efficacies [156]. Another research study by Jing et al. reported that CPP-loaded nanobubbles (NB) synergized with ultrasound-targeted microbubble destruction (UTMD) technology were able to improve the delivery of EGFR-targeted siRNA (siEGFR) for TNBC therapy. The proliferation level of MDA-MB-231 TNBC cells with the CPP-NB (siEGFR)+ ultrasound (US) group has led to the lower proliferation of cancer cells, compared to other groups (siEGFR + US, NB + US, CPP-NB + US, NBsiEGFR + US, CPP- NBsiEGFR) after 48 and 72 h of transfection for effectively inhibiting tumor cell proliferation. Moreover, it is evident from the in vivo studies that the TNBC xenograft tumors possess significant abilities to inhibit the CPP-NB (siEGFR) + US group [157]. Cell-penetrating peptides formulated with

nanoparticles were also identified to be beneficial for the treatment of other diseases in the future [158].

6.4. Endosomal Disrupting Peptide

Endosomal escape is a major challenge for the poor nanocarrier-mediated delivery of therapeutics, due to the endocytic pathway for cellular internalization of entrapped endosomes and degradation risk via active enzymes and acidic pH [159], as shown in Figure 4. It is easy to overcome the nanomedicine's endosomal escape challenge via endosomal disrupting peptides, such as fusogenic and melittin.

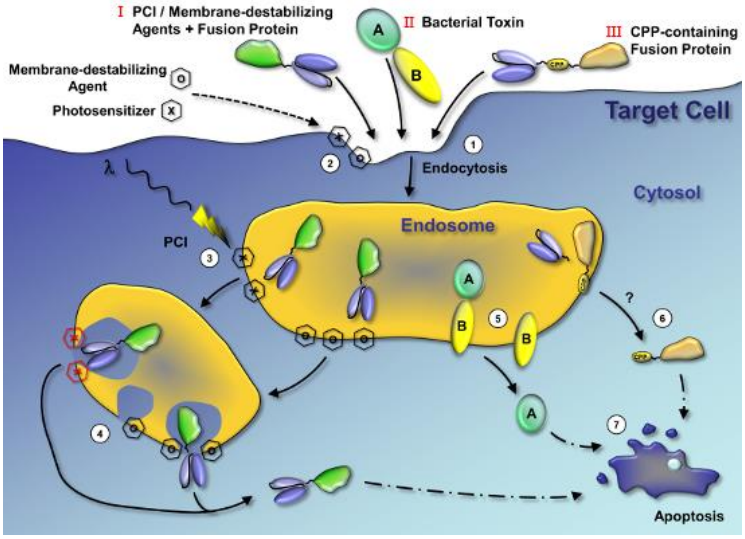

Figure 4. Mechanism of endosomal escape by drug candidates. Reproduced with permission from Fuchs et al. (2013), ©MDPI, 2013 [160].

Fusogenic peptides are pH-dependent, endosomal-disrupting peptides [155]. They induce endosomal escape with various mechanisms, such as the formation of an intermediate membrane state and membrane (endosome) disruption during the fusion process, later releasing the therapeutic drug content into the cytosol. It can be noted that the nanoformulated fusogenic peptides have improved the efficacy of this delivery system. Recently, Yao et al. (2018) developed a dual-functionalization of smart polymersomes with targeting and fusogenic peptides (cRGD/GALa-Ps) to deliver cytochrome C (CC) to A549 human lung cancer cells. They discovered cRGD/GALa-Ps has great loading efficiency for CC, which is between 60.5% and 62.5%, and better endosomal escape of FITT-CC, as shown when compared with cRGD-Ps via confocal microscopy studies. Cell apoptosis of cRGD/GALa-Ps (with 4 mol% GALA) was greater than cRGD-Ps alone, which is 36.4% versus 14.4%. This smart polymersome revealed its ability to boost the cytosolic release of CC, which helps to enhance apoptosis and anticancer activities [161]. Further, Shixian et al. (2021) designed D-melittin polymeric micelles (DMM) for anticancer treatment by inducing hemolysis via a pH-dependent approach and immunogenic cell death. The in vivo studies revealed that the nanoformulated peptide not only boosted the efficacy of the tumor growth prohibition, but also increased the survival rate, compared to PBS and D-melittin-treated mice [162]. Moreover, a nanoformulated endosomal disrupting peptide was also proposed, in recent studies, to be beneficial in the treatment of other diseases in the future [163–165].

7. Current Challenges and Future Perspective

Although the nanoformulation of peptides have proven capabilities to improve the disease therapeutic efficacies of naked peptides, there are certain limitations that must be addressed in future research endeavors to promote widespread pharmaceutical application.

The exact mechanisms of the nanoformulated peptide in disease therapy must be explored to modify them according to the desired application. Further, extensive research is required to develop a novel self-assembled peptide as the surface of this system, and the qualitative stability in TME may transform with time. Moreover, the cytotoxicity and lack of cell specificity for CPPs will be major issues in introducing them as potential therapeutic agents in the future. Cationically charged CPPs possess the ability to enter cells, including healthy cells, which can lead to an increase in their non-specific delivery, eventually leading to non-specificity toward the target, compared to other delivery systems. This non-specificity of CPPs can be attributed to the acidic conditions created by large numbers of positively charged residues [147]. Fusogenic peptides can decrease the cell targetability and cell uptake prior to endosomal encapsulation at low doses, although they can improve the efficiency of the therapeutic drug released into the cells [166].

Therefore, the mechanism of a cell targeting peptide has high potential, creating insight into delivering specific therapeutic drugs to cells affected by disease. This eventually indicates that the possibility of adverse side effects can be reduced or even prevented. Extensive research on cell specificity, binding affinity, ligand biocompatibility, and mass production, are still required. Moreover, the in-depth understanding of the interaction between nanoformulated peptides and the body's immune system is required to address the limitations of nanoformulated peptides. Furthermore, the exploration of TME will help in designing highly efficient nano-vehicles for the effective, controlled, and targeted delivery of conventional and emerging therapeutic drugs.

8. Conclusions

Peptide-based drugs are gaining attention among researchers due to their potential efficacy in targeting specific cells affected by a disease (in the treatment of diseases). However, their specificity and stability in biological fluids are low compared to other conventional drugs. Thus, nanoformulated peptides can be beneficial in enhancing efficacy. Although they show promise in improving delivery and treatment efficacies, compared to naked peptides, there are several challenges related to cytotoxicity, biodistribution, and lack of cellular excretion, which challenge their introduction to large-scale pharmaceutical applications. Thus, research focused on investigating the mechanisms of nanoformulated peptides will promote their efficacy in the treatment of diseases.

Author Contributions: Conceptualization, writing—original draft preparation, B.R.T., B.T.C.C., K.S.F. and J.J.; writing—review and editing, L.S.Y., G.N. and M.K.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The study did not report any data.

Acknowledgments: M.K.D. acknowledges the support from the USDA-NIFA funded iNEST project at the University of Tennessee at Chattanooga. All other authors acknowledge their respective universities, departments and funding agencies for their support in the preparation of this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lorenzo, J.M.; Munekata, P.E.; Gómez, B.; Barba, F.J.; Mora, L.; Pérez-Santaescolástica, C.; Toldrá, F. Bioactive peptides as natural antioxidants in food products—A review. *Trends Food Sci. Technol.* **2018**, *79*, 136–147. [CrossRef]
2. Muttenthaler, M.; King, G.F.; Adams, D.J.; Alewood, P.F. Trends in peptide drug discovery. *Nat. Rev. Drug Discov.* **2021**, *20*, 309–325. [CrossRef]
3. Baig, M.H.; Ahmad, K.; Saeed, M.; Alharbi, A.M.; Barreto, G.E.; Ashraf, G.M.; Choi, I. Peptide based therapeutics and their use for the treatment of neurodegenerative and other diseases. *Biomed. Pharmacother.* **2018**, *103*, 574–581. [CrossRef]

4. Zhang, Y.; Song, W.; Li, S.; Kim, D.-K.; Kim, J.H.; Kim, J.R.; Kim, I. Facile and scalable synthesis of topologically nanoengineered polypeptides with excellent antimicrobial activities. *Chem. Commun.* **2020**, *56*, 356–359. [CrossRef] [PubMed]
5. Sun, X.; Wang, G.; Zhang, H.; Hu, S.; Liu, X.; Tang, J.; Shen, Y. The blood clearance kinetics and pathway of polymeric micelles in cancer drug delivery. *ACS Nano* **2018**, *12*, 6179–6192. [CrossRef] [PubMed]
6. Fosgerau, K.; Hoffmann, T. Peptide therapeutics: Current status and future directions. *Drug Discov. Today* **2015**, *20*, 122–128. [CrossRef]
7. Yang, W.; Yan, J.; Zhuang, P.; Ding, T.; Chen, Y.; Zhang, Y.; Zhang, H.; Cui, W. Progress of delivery methods for CRISPR-Cas9. *Expert Opin. Drug Deliv.* **2022**, *19*, 913–926. [CrossRef] [PubMed]
8. Rahman, M.M.; Islam, M.R.; Akash, S.; Harun-Or-Rashid, M.; Ray, T.K.; Rahaman, M.S.; Islam, M.; Anika, F.; Hosain, M.K.; Aovi, F.I. Recent advancements of nanoparticles application in cancer and neurodegenerative disorders: At a glance. *Biomed. Pharmacother.* **2022**, *153*, 113305. [CrossRef]
9. Chauhan, N.; Saxena, K.; Jain, U. Smart nanomaterials employed recently for drug delivery in cancer therapy: An intelligent approach. *BioNanoScience* **2022**, *12*, 1356–1365. [CrossRef]
10. Sarabandi, K.; Gharehbeglou, P.; Jafari, S.M. Spray-drying encapsulation of protein hydrolysates and bioactive peptides: Opportunities and challenges. *Dry. Technol.* **2020**, *38*, 577–595. [CrossRef]
11. Mamuti, M.; Zheng, R.; An, H.-W.; Wang, H. In vivo self-assembled nanomedicine. *Nano Today* **2021**, *36*, 101036. [CrossRef]
12. Korhonen, H.; Pihlanto, A. Bioactive peptides: Production and functionality. *Int. Dairy J.* **2006**, *16*, 945–960. [CrossRef]
13. Sánchez, A.; Vázquez, A. Bioactive peptides: A review. *Food Qual. Saf.* **2017**, *1*, 29–46. [CrossRef]
14. Fields, K.; Falla, T.J.; Rodan, K.; Bush, L. Bioactive peptides: Signaling the future. *J. Cosmet. Dermatol.* **2009**, *8*, 8–13. [CrossRef] [PubMed]
15. Carrasco-Castilla, J.; Hernández-Álvarez, A.J.; Jiménez-Martínez, C.; Gutiérrez-López, G.F.; Dávila-Ortiz, G. Use of proteomics and peptidomics methods in food bioactive peptide science and engineering. *Food Eng. Rev.* **2012**, *4*, 224–243. [CrossRef]
16. Bhat, Z.F.; Kumar, S.; Bhat, H.F. Bioactive peptides from egg: A review. *Nutr. Food Sci.* **2015**, *45*, 190–212. [CrossRef]
17. Brogden, K.A. Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? *Nat. Rev. Microbiol.* **2005**, *3*, 238–250. [CrossRef]
18. Vinogradov, A.A.; Yin, Y.; Suga, H. Macrocyclic peptides as drug candidates: Recent progress and remaining challenges. *J. Am. Chem. Soc.* **2019**, *141*, 4167–4181. [CrossRef]
19. Haque, E.; Chand, R.; Kapila, S. Biofunctional properties of bioactive peptides of milk origin. *Food Rev. Int.* **2008**, *25*, 28–43. [CrossRef]
20. Moldes, A.B.; Vecino, X.; Cruz, J.M. Nutraceuticals and Food Additives. In *Current Developments in Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 143–164.
21. Mohanty, D.; Jena, R.; Choudhury, P.K.; Pattnaik, R.; Mohapatra, S.; Saini, M.R. Milk derived antimicrobial bioactive peptides: A review. *Int. J. Food Prop.* **2016**, *19*, 837–846. [CrossRef]
22. Pritchard, S.R.; Phillips, M.; Kailasapathy, K. Identification of bioactive peptides in commercial Cheddar cheese. *Food Res. Int.* **2010**, *43*, 1545–1548. [CrossRef]
23. Choi, J.; Sabikhi, L.; Hassan, A.; Anand, S. Bioactive peptides in dairy products. *Int. J. Dairy Technol.* **2012**, *65*, 1–12. [CrossRef]
24. Przybylski, R.; Firdaous, L.; Châtaigné, G.; Dhulster, P.; Nedjar, N. Production of an antimicrobial peptide derived from slaughterhouse by-product and its potential application on meat as preservative. *Food Chem.* **2016**, *211*, 306–313. [CrossRef] [PubMed]
25. Bhat, Z.F.; Kumar, S.; Bhat, H.F. Bioactive peptides of animal origin: A review. *J. Food Sci. Technol.* **2015**, *52*, 5377–5392. [CrossRef] [PubMed]
26. Arrutia, F.; Puente, Á.; Riera, F.A.; Menéndez, C.; González, U.A. Influence of heat pre-treatment on BSA tryptic hydrolysis and peptide release. *Food Chem.* **2016**, *202*, 40–48. [CrossRef]
27. Clare, D.A.; Swaisgood, H.E. Bioactive milk peptides: A prospectus. *J. Dairy Sci.* **2000**, *83*, 1187–1195. [CrossRef]
28. Elias, R.J.; Kellerby, S.S.; Decker, E.A. Antioxidant activity of proteins and peptides. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 430–441. [CrossRef]
29. Bougatef, A.; Nedjar-Arroume, N.; Manni, L.; Ravallec, R.; Barkia, A.; Guillochon, D.; Nasri, M. Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinella aurita*) by-products proteins. *Food Chem.* **2010**, *118*, 559–565. [CrossRef]
30. Hernández-Ledesma, B.; Recio, I.; Amigo, L. β-Lactoglobulin as source of bioactive peptides. *Amino Acids* **2008**, *35*, 257–265. [CrossRef]
31. Zhu, K.; Zhou, H.; Qian, H. Antioxidant and free radical-scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with alcalase. *Process Biochem.* **2006**, *41*, 1296–1302. [CrossRef]
32. Je, J.-Y.; Park, P.-J.; Kim, S.-K. Antioxidant activity of a peptide isolated from Alaska pollack (*Theragra chalcogramma*) frame protein hydrolysate. *Food Res. Int.* **2005**, *38*, 45–50. [CrossRef]
33. Ngo, D.-H.; Kim, S.-K. Marine bioactive peptides as potential antioxidants. *Curr. Protein Pept. Sci.* **2013**, *14*, 189–198. [CrossRef]
34. Liu, R.; Zheng, W.; Li, J.; Wang, L.; Wu, H.; Wang, X.; Shi, L. Rapid identification of bioactive peptides with antioxidant activity from the enzymatic hydrolysate of Mactra veneriformis by UHPLC–Q-TOF mass spectrometry. *Food Chem.* **2015**, *167*, 484–489. [CrossRef]

35. Ahmed, A.S.; El-Bassiony, T.; Elmalt, L.M.; Ibrahim, H.R. Identification of potent antioxidant bioactive peptides from goat milk proteins. *Food Res. Int.* **2015**, *74*, 80–88. [CrossRef]
36. Möller, N.P.; Scholz-Ahrens, K.E.; Roos, N.; Schrezenmeir, J. Bioactive peptides and proteins from foods: Indication for health effects. *Eur. J. Nutr.* **2008**, *47*, 171–182. [CrossRef]
37. Silva-Sánchez, C.; De La Rosa, A.P.B.; León-Galván, M.F.; De Lumen, B.O.; de León-Rodríguez, A.; De Mejía, E.G. Bioactive peptides in amaranth (*Amaranthus hypochondriacus*) seed. *J. Agric. Food Chem.* **2008**, *56*, 1233–1240. [CrossRef]
38. Mine, Y.; Li-Chan, E.; Jiang, B. *Bioactive Proteins and Peptides as Functional Foods and Nutraceuticals*; John Wiley & Sons: Hoboken, NJ, USA, 2010; Volume 29.
39. Selamassakul, O.; Laohakunjit, N.; Kerdchoechuen, O.; Ratanakhanokchai, K. A novel multi-biofunctional protein from brown rice hydrolysed by endo/endo-exoproteases. *Food Funct.* **2016**, *7*, 2635–2644. [CrossRef] [PubMed]
40. Singh, B.P.; Vij, S.; Hati, S. Functional significance of bioactive peptides derived from soybean. *Peptides* **2014**, *54*, 171–179. [CrossRef]
41. Gibbs, B.F.; Zougman, A.; Masse, R.; Mulligan, C. Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. *Food Res. Int.* **2004**, *37*, 123–131. [CrossRef]
42. Malaguti, M.; Dinelli, G.; Leoncini, E.; Bregola, V.; Bosi, S.; Cicero, A.F.G.; Hrelia, S. Bioactive peptides in cereals and legumes: Agronomical, biochemical and clinical aspects. *Int. J. Mol. Sci.* **2014**, *15*, 21120–21135. [CrossRef] [PubMed]
43. Wang, L.L.; Xiong, Y.L. Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. *J. Agric. Food Chem.* **2005**, *53*, 9186–9192. [CrossRef] [PubMed]
44. Kim, S.-K.; Wijesekara, I. Development and biological activities of marine-derived bioactive peptides: A review. *J. Funct. Foods* **2010**, *2*, 1–9. [CrossRef]
45. Wu, J.; Majumder, K.; Gibbons, K. Bioactive proteins and peptides from egg proteins. *Bioact. Proteins Pept. Funct. Foods Nutraceuticals* **2010**, *29*, 247.
46. Korhonen, H.; Pihlanto, A. Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. *Curr. Pharm. Des.* **2007**, *13*, 829–843. [CrossRef] [PubMed]
47. Kitts, D.D.; Weiler, K. Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. *Curr. Pharm. Des.* **2003**, *9*, 1309–1323. [CrossRef]
48. Chelliah, R.; Wei, S.; Daliri, E.B.-M.; Elahi, F.; Yeon, S.-J.; Tyagi, A.; Liu, S.; Madar, I.H.; Sultan, G.; Oh, D.-H. The role of bioactive peptides in diabetes and obesity. *Foods* **2021**, *10*, 2220. [CrossRef]
49. Kristinsson, H.G.; Rasco, B.A. Fish protein hydrolysates: Production, biochemical, and functional properties. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 43–81. [CrossRef]
50. Wang, X.; Yu, H.; Xing, R.; Li, P. Characterization, preparation, and purification of marine bioactive peptides. *BioMed Res. Int.* **2017**, *2017*, 9746720. [CrossRef]
51. Sila, A.; Sayari, N.; Balti, R.; Martinez-Alvarez, O.; Nedjar-Arroume, N.; Moncef, N.; Bougatef, A. Biochemical and antioxidant properties of peptidic fraction of carotenoproteins generated from shrimp by-products by enzymatic hydrolysis. *Food Chem.* **2014**, *148*, 445–452. [CrossRef]
52. Je, J.-Y.; Lee, K.-H.; Lee, M.H.; Ahn, C.-B. Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis. *Food Res. Int.* **2009**, *42*, 1266–1272. [CrossRef]
53. Ko, S.-C.; Kim, D.; Jeon, Y.-J. Protective effect of a novel antioxidative peptide purified from a marine Chlorella ellipsoidea protein against free radical-induced oxidative stress. *Food Chem. Toxicol.* **2012**, *50*, 2294–2302. [CrossRef]
54. Zarei, M.; Ebrahimpour, A.; Abdul-Hamid, A.; Anwar, F.; Saari, N. Production of defatted palm kernel cake protein hydrolysate as a valuable source of natural antioxidants. *Int. J. Mol. Sci.* **2012**, *13*, 8097–8111. [CrossRef] [PubMed]
55. Chang, O.K.; Roux, É.; Awussi, A.A.; Miclo, L.; Jardin, J.; Jameh, N.; Dary, A.; Humbert, G.; Perrin, C. Use of a free form of the Streptococcus thermophilus cell envelope protease PrtS as a tool to produce bioactive peptides. *Int. Dairy J.* **2014**, *38*, 104–115. [CrossRef]
56. Lassoued, I.; Mora, L.; Barkia, A.; Aristoy, M.C.; Nasri, M.; Toldrá, F. Bioactive peptides identified in thornback ray skin's gelatin hydrolysates by proteases from Bacillus subtilis and Bacillus amyloliquefaciens. *J. Proteom.* **2015**, *128*, 8–17. [CrossRef]
57. Ryder, K.; Bekhit, A.E.-D.; McConnell, M.; Carne, A. Towards generation of bioactive peptides from meat industry waste proteins: Generation of peptides using commercial microbial proteases. *Food Chem.* **2016**, *208*, 42–50. [CrossRef] [PubMed]
58. Wongputtisin, P.; Khanongnuch, C.; Pongpiachan, P.; Lumyong, S. Antioxidant activity improvement of soybean meal by microbial fermentation. *Res. J. Microbiol.* **2007**, *2*, 577–583.
59. Hayes, M.; Ross, R.P.; Fitzgerald, G.F.; Stanton, C. Putting microbes to work: Dairy fermentation, cell factories and bioactive peptides. Part I: Overview. *Biotechnol. J. Healthc. Nutr. Technol.* **2007**, *2*, 426–434. [CrossRef] [PubMed]
60. Rui, X.; Wen, D.; Li, W.; Chen, X.; Jiang, M.; Dong, M. Enrichment of ACE inhibitory peptides in navy bean (Phaseolus vulgaris) using lactic acid bacteria. *Food Funct.* **2015**, *6*, 622–629. [CrossRef]
61. Gonzalez-Gonzalez, C.; Gibson, T.; Jauregi, P. Novel probiotic-fermented milk with angiotensin I-converting enzyme inhibitory peptides produced by Bifidobacterium bifidum MF 20/5. *Int. J. Food Microbiol.* **2013**, *167*, 131–137. [CrossRef] [PubMed]
62. Vijaykrishnaraj, M.; Prabhasankar, P. Marine protein hydrolysates: Their present and future perspectives in food chemistry—A review. *RSC Adv.* **2015**, *5*, 34864–34877. [CrossRef]

63. Loow, Y.-L.; Wu, T.Y.; Jahim, J.M.; Mohammad, A.W.; Teoh, W.H. Typical conversion of lignocellulosic biomass into reducing sugars using dilute acid hydrolysis and alkaline pretreatment. *Cellulose* **2016**, *23*, 1491–1520. [CrossRef]
64. Navab, M.; Anantharamaiah, G.M.; Reddy, S.T.; Van Lenten, B.J.; Datta, G.; Garber, D.; Fogelman, A.M. Human apolipoprotein AI and AI mimetic peptides: Potential for atherosclerosis reversal. *Curr. Opin. Lipidol.* **2004**, *15*, 645–649. [CrossRef]
65. Nguyen, S.D.; Jeong, T.-S.; Kim, M.R.; Sok, D.-E. Broad-spectrum antioxidant peptides derived from His residue-containing sequences present in human paraoxonase 1. *Free. Radic. Res.* **2006**, *40*, 349–358. [CrossRef] [PubMed]
66. Kadam, S.U.; Tiwari, B.K.; Álvarez, C.; O'Donnell, C.P. Ultrasound applications for the extraction, identification and delivery of food proteins and bioactive peptides. *Trends Food Sci. Technol.* **2015**, *46*, 60–67. [CrossRef]
67. Yin, X.B.; Wu, P.; Li, Y.; Yan, X.P. 3.22-Mercury Speciation and Binding to Biomacromolecules. In *Comprehensive Sampling and Sample Preparation*; Pawliszyn, J., Ed.; Academic Press: Oxford, UK, 2012; pp. 435–460. [CrossRef]
68. Cruz-Casas, D.E.; Aguilar, C.N.; Ascacio-Valdés, J.A.; Rodríguez-Herrera, R.; Chávez-González, M.L.; Flores-Gallegos, A.C. Enzymatic hydrolysis and microbial fermentation: The most favorable biotechnological methods for the release of bioactive peptides. *Food Chem. Mol. Sci.* **2021**, *3*, 100047. [CrossRef]
69. Akbarian, M.; Khani, A.; Eghbalpour, S.; Uversky, V.N. Bioactive Peptides: Synthesis, Sources, Applications, and Proposed Mechanisms of Action. *Int. J. Mol. Sci.* **2022**, *23*, 1445. [CrossRef]
70. Nimalaratne, C.; Bandara, N.; Wu, J. Purification and characterization of antioxidant peptides from enzymatically hydrolyzed chicken egg white. *Food Chem.* **2015**, *188*, 467–472. [CrossRef]
71. Jang, H.L.; Liceaga, A.M.; Yoon, K.Y. Purification, characterisation and stability of an antioxidant peptide derived from sandfish (*Arctoscopus japonicus*) protein hydrolysates. *J. Funct. Foods* **2016**, *20*, 433–442. [CrossRef]
72. Vandanjon, L.; Johannsson, R.; Derouiniot, M.; Bourseau, P.; Jaouen, P. Concentration and purification of blue whiting peptide hydrolysates by membrane processes. *J. Food Eng.* **2007**, *83*, 581–589. [CrossRef]
73. Pouliot, Y.; Gauthier, S.F.; l'Heureux, J. Effect of peptide distribution on the fractionation of whey protein hydrolysates by nanofiltration membranes. *Le Lait* **2000**, *80*, 113–120. [CrossRef]
74. Mant, C.T.; Kondejewski, L.H.; Cachia, P.J.; Monera, O.D.; Hodges, R.S. [19] Analysis of synthetic peptides by high-performance liquid chromatography. *Methods Enzymol.* **1997**, *289*, 426–469. [PubMed]
75. Puchalska, P.; Marina Alegre, M.L.; Garcia Lopez, M.C. Isolation and characterization of peptides with antihypertensive activity in foodstuffs. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 521–551. [CrossRef] [PubMed]
76. Levison, P.R. Large-scale ion-exchange column chromatography of proteins: Comparison of different formats. *J. Chromatogr. B* **2003**, *790*, 17–33. [CrossRef]
77. Andrew, B.E. Determination of perchlorate accumulation in flame atomic absorption systems by ion-pairing chromatography. *J. Anal. At. Spectrom.* **1988**, *3*, 401–405. [CrossRef]
78. Di Palma, S.; Hennrich, M.L.; Heck, A.J.R.; Mohammed, S. Recent advances in peptide separation by multidimensional liquid chromatography for proteome analysis. *J. Proteom.* **2012**, *75*, 3791–3813. [CrossRef]
79. Ebere, E.C.; Obinna, I.B.; Wirnkor, V.A. Applications of column, paper, thin layer and ion exchange chromatography in purifying samples: Mini review. *SF J. Pharm. Anal. Chem.* **2019**, *2*, 1018.
80. Lee, J.K.; Hong, S.; Jeon, J.-K.; Kim, S.-K.; Byun, H.-G. Purification and characterization of angiotensin I converting enzyme inhibitory peptides from the rotifer, Brachionus rotundiformis. *Bioresour. Technol.* **2009**, *100*, 5255–5259. [CrossRef]
81. Yang, J.; Huang, J.; Dong, X.; Zhang, Y.; Zhou, X.; Huang, M.; Zhou, G. Purification and identification of antioxidant peptides from duck plasma proteins. *Food Chem.* **2020**, *319*, 126534. [CrossRef]
82. Xia, J.; Song, H.; Huang, K.; Li, S.; Guan, X. Purification and characterization of antioxidant peptides from enzymatic hydrolysate of mungbean protein. *J. Food Sci.* **2020**, *85*, 1735–1741. [CrossRef]
83. Morgan, A.A.; Rubenstein, E. Proline: The distribution, frequency, positioning, and common functional roles of proline and polyproline sequences in the human proteome. *PLoS ONE* **2013**, *8*, e53785. [CrossRef]
84. Léonil, J.; Gagnaire, V.; Mollé, D.; Pezennec, S.; Bouhallab, S.d. Application of chromatography and mass spectrometry to the characterization of food proteins and derived peptides. *J. Chromatogr. A* **2000**, *881*, 1–21. [CrossRef] [PubMed]
85. Singh, G.K.S.; Turner, L.; Desai, R.; Jimenez, M.; Handelsman, D.J. Pharmacokinetic-pharmacodynamic study of subcutaneous injection of depot nandrolone decanoate using dried blood spots sampling coupled with ultrapressure liquid chromatography tandem mass spectrometry assays. *J. Clin. Endocrinol. Metab.* **2014**, *99*, 2592–2598. [CrossRef] [PubMed]
86. Xia, Y.; Yu, J.; Miao, W.; Shuang, Q. A UPLC-Q-TOF-MS-based metabolomics approach for the evaluation of fermented mare's milk to koumiss. *Food Chem.* **2020**, *320*, 126619. [CrossRef] [PubMed]
87. Sarmadi, B.H.; Ismail, A. Antioxidative peptides from food proteins: A review. *Peptides* **2010**, *31*, 1949–1956. [CrossRef] [PubMed]
88. Baindara, P.; Mandal, S.M.; Chawla, N.; Singh, P.K.; Pinnaka, A.K.; Korpole, S. Characterization of two antimicrobial peptides produced by a halotolerant Bacillus subtilis strain SK. DU. 4 isolated from a rhizosphere soil sample. *AMB Express* **2013**, *3*, 2. [CrossRef] [PubMed]
89. Collmer, A.; Keen, N.T. The role of pectic enzymes in plant pathogenesis. *Annu. Rev. Phytopathol.* **1986**, *24*, 383–409. [CrossRef]
90. Dowling, P.; Hayes, C.; Ting, K.R.; Hameed, A.; Meiller, J.; Mitsiades, C.; Anderson, K.C.; Clynes, M.; Clarke, C.; Richardson, P. Identification of proteins found to be significantly altered when comparing the serum proteome from Multiple Myeloma patients with varying degrees of bone disease. *BMC Genom.* **2014**, *15*, 904. [CrossRef]

91. Matougui, N.; Boge, L.; Groo, A.-C.; Umerska, A.; Ringstad, L.; Bysell, H.; Saulnier, P. Lipid-based nanoformulations for peptide delivery. *Int. J. Pharm.* **2016**, *502*, 80–97. [CrossRef]
92. Shah, L.; Kulkarni, P.; Ferris, C.; Amiji, M.M. Analgesic efficacy and safety of DALDA peptide analog delivery to the brain using oil-in-water nanoemulsion formulation. *Pharm. Res.* **2014**, *31*, 2724–2734. [CrossRef]
93. Pattani, A.S.; Mandawgade, S.D.; Patravale, V.B. Development and comparative anti-microbial evaluation of lipid nanoparticles and nanoemulsion of polymyxin B. *J. Nanosci. Nanotechnol.* **2006**, *6*, 2986–2990. [CrossRef]
94. Niu, Z.; Zhao, W.; Zhang, Z.; Xiao, F.; Tang, X.; Yang, J. The Molecular Structure of Alzheimer β-Amyloid Fibrils Formed in the Presence of Phospholipid Vesicles. *Angew. Chem.* **2014**, *126*, 9448–9451. [CrossRef]
95. Omri, A.; Suntres, Z.E.; Shek, P.N. Enhanced activity of liposomal polymyxin B against Pseudomonas aeruginosa in a rat model of lung infection. *Biochem. Pharmacol.* **2002**, *64*, 1407–1413. [CrossRef] [PubMed]
96. Almeida, A.J.; Souto, E. Solid lipid nanoparticles as a drug delivery system for peptides and proteins. *Adv. Drug Deliv. Rev.* **2007**, *59*, 478–490. [CrossRef]
97. Müller, R.; Maaben, S.; Weyhers, H.; Mehnert, W. Phagocytic uptake and cytotoxicity of solid lipid nanoparticles (SLN) sterically stabilized with poloxamine 908 and poloxamer 407. *J. Drug Target.* **1996**, *4*, 161–170. [CrossRef] [PubMed]
98. Yuan, H.; Miao, J.; Du, Y.-Z.; You, J.; Hu, F.-Q.; Zeng, S. Cellular uptake of solid lipid nanoparticles and cytotoxicity of encapsulated paclitaxel in A549 cancer cells. *Int. J. Pharm.* **2008**, *348*, 137–145. [CrossRef]
99. Garcia-Fuentes, M.; Torres, D.; Alonso, M.J. New surface-modified lipid nanoparticles as delivery vehicles for salmon calcitonin. *Int. J. Pharm.* **2005**, *296*, 122–132. [CrossRef]
100. Martin, N.I.; Breukink, E. The expanding role of lipid II as a target for lantibiotics. *Future Med.* **2007**, *2*, 513–525. [CrossRef]
101. Matougui, N.; Groo, A.-C.; Umerska, A.; Cassisa, V.; Saulnier, P. A comparison of different strategies for antimicrobial peptides incorporation onto/into lipid nanocapsules. *Nanomedicine* **2019**, *14*, 1647–1662. [CrossRef]
102. Zhao, Z.; Li, Y.; Xie, M.-B. Silk fibroin-based nanoparticles for drug delivery. *Int. J. Mol. Sci.* **2015**, *16*, 4880–4903. [CrossRef]
103. Li, B.; Yang, Y.; Wang, F.; Wang, R.; Fei, H.; Duan, S.; Huang, L.; Liao, N.; Zhao, S.; Ma, X. Biodegradable silk fibroin nanocarriers to modulate hypoxia tumor microenvironment favoring enhanced chemotherapy. *Front. Bioeng. Biotechnol.* **2022**, *10*, 1246.
104. Yeguas, V.; Altarsha, M.; Monard, G.; López, R.; Ruiz-López, M.F. Peptide binding to β-cyclodextrins: Structure, dynamics, energetics, and electronic effects. *J. Phys. Chem. A* **2011**, *115*, 11810–11817. [CrossRef] [PubMed]
105. Jóhannsdóttir, S.; Kristinsson, J.K.; Fülöp, Z.; Ásgrímsdóttir, G.; Stefánsson, E.; Loftsson, T. Formulations and toxicologic in vivo studies of aqueous cyclosporin A eye drops with cyclodextrin nanoparticles. *Int. J. Pharm.* **2017**, *529*, 486–490. [CrossRef] [PubMed]
106. Kantner, I.; Erben, R.G. Long-term parenteral administration of 2-hydroxypropyl-β-cyclodextrin causes bone loss. *Toxicol. Pathol.* **2012**, *40*, 742–750. [CrossRef] [PubMed]
107. Li, Z.; Zheng, Z.; Su, S.; Yu, L.; Wang, X. Hydroxypropyl-β-CD vs. its α-homologue for a 3D modified polyrotaxane network formation and properties: The relationship between modified CD and polymer revealed through comparison. *Soft Matter* **2016**, *12*, 7089–7101. [CrossRef]
108. Knauer, N.; Pashkina, E.; Apartsin, E. Topological aspects of the design of nanocarriers for therapeutic peptides and proteins. *Pharmaceutics* **2019**, *11*, 91. [CrossRef]
109. Yu, J.; Patel, S.A.; Dickson, R.M. In vitro and intracellular production of peptide-encapsulated fluorescent silver nanoclusters. *Angew. Chem. Int. Ed.* **2007**, *46*, 2028–2030. [CrossRef]
110. Jagani, H.; Rao, J.; Palanimuthu, V.; Hariharapura, R.; Gang, S. A nanoformulation of siRNA and its role in cancer therapy: In vitro and in vivo evaluation. *Cell. Mol. Biol. Lett.* **2013**, *18*, 120–136. [CrossRef]
111. Bawa, R.; Fung, S.-Y.; Shiozaki, A.; Yang, H.; Zheng, G.; Keshavjee, S.; Liu, M. Self-assembling peptide-based nanoparticles enhance cellular delivery of the hydrophobic anticancer drug ellipticine through caveolae-dependent endocytosis. *Nanomed. Nanotechnol. Biol. Med.* **2012**, *8*, 647–654. [CrossRef]
112. Gomes, M.R.F.; Schuh, R.S.; Jacques, A.L.B.; Augustin, O.A.; Bordignon, S.A.L.; Dias, D.O.; Kelmann, R.G.; Koester, L.S.; Gehring, M.P.; Morrone, F.B. Citotoxic activity evaluation of essential oils and nanoemulsions of Drimys angustifolia and D. brasiliensis on human glioblastoma (U-138 MG) and human bladder carcinoma (T24) cell lines in vitro. *Rev. Bras. Farmacogn.* **2013**, *23*, 259–267. [CrossRef]
113. Kulsharova, G.K.; Lee, M.B.; Cheng, F.; Haque, M.; Choi, H.; Kim, K.; O'Brien, W.D.; Liu, G.L. In vitro and in vivo imaging of peptide-encapsulated polymer nanoparticles for cancer biomarker activated drug delivery. *IEEE Trans. Nanobiosci.* **2013**, *12*, 304–310. [CrossRef]
114. Nishikawa, T.; Nakagami, H.; Maeda, A.; Morishita, R.; Miyazaki, N.; Ogawa, T.; Tabata, Y.; Kikuchi, Y.; Hayashi, H.; Tatsu, Y. Development of a novel antimicrobial peptide, AG-30, with angiogenic properties. *J. Cell. Mol. Med.* **2009**, *13*, 535–546. [CrossRef] [PubMed]
115. Imanparast, F.; Faramarzi, M.A.; Vatannejad, A.; Paknejad, M.; Deiham, B.; Kobarfard, F.; Amani, A.; Doosti, M. mZD7349 peptide-conjugated PLGA nanoparticles directed against VCAM-1 for targeted delivery of simvastatin to restore dysfunctional HUVECs. *Microvasc. Res.* **2017**, *112*, 14–19. [CrossRef] [PubMed]
116. De Campos, A.M.; Diebold, Y.; Carvalho, E.L.S.; Sánchez, A.; Alonso, M.J. Chitosan nanoparticles as new ocular drug delivery systems: In vitro stability, in vivo fate, and cellular toxicity. *Pharm. Res.* **2004**, *21*, 803–810. [CrossRef] [PubMed]

117. Narayanan, D.; Anitha, A.; Jayakumar, R.; Nair, S.V.; Chennazhi, K.P. Synthesis, characterization and preliminary in vitro evaluation of PTH 1-34 loaded chitosan nanoparticles for osteoporosis. *J. Biomed. Nanotechnol.* **2012**, *8*, 98–106. [CrossRef]
118. Kim, R.M.; Feng, T.; Zhang, Q.; Chan, Y.H.; Chau, Y. Co-Encapsulation and Co-Delivery of Peptide Drugs via Polymeric Nanoparticles. *Polymers* **2019**, *11*, 288. [CrossRef]
119. Silva, A.L.; Rosalia, R.A.; Sazak, A.; Carstens, M.G.; Ossendorp, F.; Oostendorp, J.; Jiskoot, W. Optimization of encapsulation of a synthetic long peptide in PLGA nanoparticles: Low-burst release is crucial for efficient CD8+ T cell activation. *Eur. J. Pharm. Biopharm.* **2013**, *83*, 338–345. [CrossRef]
120. Shenoy, D.; Little, S.; Langer, R.; Amiji, M. Poly (ethylene oxide)-modified poly (β-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs. 1. In vitro evaluations. *Mol. Pharm.* **2005**, *2*, 357–366. [CrossRef]
121. Fu, X.; Yang, Y.; Li, X.; Lai, H.; Huang, Y.; He, L.; Zheng, W.; Chen, T. RGD peptide-conjugated selenium nanoparticles: Antiangiogenesis by suppressing VEGF-VEGFR2-ERK/AKT pathway. *Nanomed. Nanotechnol. Biol. Med.* **2016**, *12*, 1627–1639. [CrossRef]
122. Kaliaperumal, J.; Padarthi, P.; Elangovan, N.; Hari, N. Anti-tumorigenic effect of nano formulated peptide pACC1 by diminishing de novo lipogenisis in DMBA induced mammary carcinoma rat model. *Biomed. Pharmacother.* **2014**, *68*, 763–773. [CrossRef]
123. Wei, Y.; Gao, L.; Wang, L.; Shi, L.; Wei, E.; Zhou, B.; Zhou, L.; Ge, B. Polydopamine and peptide decorated doxorubicin-loaded mesoporous silica nanoparticles as a targeted drug delivery system for bladder cancer therapy. *Drug Deliv.* **2017**, *24*, 681–691. [CrossRef]
124. Sarangthem, V.; Seo, B.-Y.; Yi, A.; Lee, Y.-J.; Cheon, S.-H.; Kim, S.K.; Singh, T.D.; Lee, B.-H.; Park, R.-W. Effects of molecular weight and structural conformation of multivalent-based elastin-like polypeptides on tumor accumulation and tissue biodistribution. *Nanotheranostics* **2020**, *4*, 57. [CrossRef] [PubMed]
125. Zhao, Y.; Black, A.S.; Bonnet, D.J.; Maryanoff, B.E.; Curtiss, L.K.; Leman, L.J.; Ghadiri, M.R. In vivo efficacy of HDL-like nanolipid particles containing multivalent peptide mimetics of apolipoprotein AI. *J. Lipid Res.* **2014**, *55*, 2053–2063. [CrossRef] [PubMed]
126. Wang, J.; Wang, H.; Li, J.; Liu, Z.; Xie, H.; Wei, X.; Lu, D.; Zhuang, R.; Xu, X.; Zheng, S. iRGD-decorated polymeric nanoparticles for the efficient delivery of vandetanib to hepatocellular carcinoma: Preparation and in vitro and in vivo evaluation. *ACS Appl. Mater. Interfaces* **2016**, *8*, 19228–19237. [CrossRef]
127. Wang, K.; Zhang, X.; Liu, Y.; Liu, C.; Jiang, B.; Jiang, Y. Tumor penetrability and anti-angiogenesis using iRGD-mediated delivery of doxorubicin-polymer conjugates. *Biomaterials* **2014**, *35*, 8735–8747. [CrossRef] [PubMed]
128. Kang, T.; Gao, X.; Hu, Q.; Jiang, D.; Feng, X.; Zhang, X.; Song, Q.; Yao, L.; Huang, M.; Jiang, X. iNGR-modified PEG-PLGA nanoparticles that recognize tumor vasculature and penetrate gliomas. *Biomaterials* **2014**, *35*, 4319–4332. [CrossRef] [PubMed]
129. Feng, X.; Jiang, D.; Kang, T.; Yao, J.; Jing, Y.; Jiang, T.; Feng, J.; Zhu, Q.; Song, Q.; Dong, N. Tumor-homing and penetrating peptide-functionalized photosensitizer-conjugated PEG-PLA nanoparticles for chemo-photodynamic combination therapy of drug-resistant cancer. *ACS Appl. Mater. Interfaces* **2016**, *8*, 17817–17832. [CrossRef]
130. Liang, D.-S.; Su, H.-T.; Liu, Y.-J.; Wang, A.-T.; Qi, X.-R. Tumor-specific penetrating peptides-functionalized hyaluronic acid-d-α-tocopheryl succinate based nanoparticles for multi-task delivery to invasive cancers. *Biomaterials* **2015**, *71*, 11–23. [CrossRef]
131. Xiao, K.; Li, Y.; Lee, J.S.; Gonik, A.M.; Dong, T.; Fung, G.; Sanchez, E.; Xing, L.; Cheng, H.R.; Luo, J. "OA02" peptide facilitates the precise targeting of paclitaxel-loaded micellar nanoparticles to ovarian cancer in vivo. *Cancer Res.* **2012**, *72*, 2100–2110. [CrossRef]
132. Miyano, K.; Cabral, H.; Miura, Y.; Matsumoto, Y.; Mochida, Y.; Kinoh, H.; Iwata, C.; Nagano, O.; Saya, H.; Nishiyama, N. cRGD peptide installation on cisplatin-loaded nanomedicines enhances efficacy against locally advanced head and neck squamous cell carcinoma bearing cancer stem-like cells. *J. Control. Release* **2017**, *261*, 275–286. [CrossRef]
133. Fang, Y.; Jiang, Y.; Zou, Y.; Meng, F.; Zhang, J.; Deng, C.; Sun, H.; Zhong, Z. Targeted glioma chemotherapy by cyclic RGD peptide-functionalized reversibly core-crosslinked multifunctional poly (ethylene glycol)-b-poly (ε-caprolactone) micelles. *Acta Biomater.* **2017**, *50*, 396–406. [CrossRef]
134. Bi, Y.; Liu, L.; Lu, Y.; Sun, T.; Shen, C.; Chen, X.; Chen, Q.; An, S.; He, X.; Ruan, C. T7 peptide-functionalized PEG-PLGA micelles loaded with carmustine for targeting therapy of glioma. *ACS Appl. Mater. Interfaces* **2016**, *8*, 27465–27473. [CrossRef] [PubMed]
135. Ji, S.; Czerwinski, A.; Zhou, Y.; Shao, G.; Valenzuela, F.; Sowiński, P.; Chauhan, S.; Pennington, M.; Liu, S. 99mTc-Galacto-RGD2: A novel 99mTc-labeled cyclic RGD peptide dimer useful for tumor imaging. *Mol. Pharm.* **2013**, *10*, 3304–3314. [CrossRef] [PubMed]
136. Liu, X.; Wu, F.; Ji, Y.; Yin, L. Recent advances in anti-cancer protein/peptide delivery. *Bioconjug. Chem.* **2018**, *30*, 305–324. [CrossRef] [PubMed]
137. Yu, G.; Baeder, D.Y.; Regoes, R.R.; Rolff, J. Combination effects of antimicrobial peptides. *Antimicrob. Agents Chemother.* **2016**, *60*, 1717–1724. [CrossRef]
138. Sun, H.; Dong, Y.; Feijen, J.; Zhong, Z. Peptide-decorated polymeric nanomedicines for precision cancer therapy. *J. Control. Release* **2018**, *290*, 11–27. [CrossRef]
139. Kim, J.E.; Kim, S.H.; Jung, Y. In situ chondrogenic differentiation of bone marrow stromal cells in bioactive self-assembled peptide gels. *J. Biosci. Bioeng.* **2015**, *120*, 91–98. [CrossRef]
140. Zhang, P.; Sun, F.; Liu, S.; Jiang, S. Anti-PEG antibodies in the clinic: Current issues and beyond PEGylation. *J. Control. Release* **2016**, *244*, 184–193. [CrossRef]
141. Rodriguez, P.L.; Harada, T.; Christian, D.A.; Pantano, D.A.; Tsai, R.K.; Discher, D.E. Minimal" Self" peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. *Science* **2013**, *339*, 971–975. [CrossRef]

142. Li, M.; Ning, Y.; Chen, J.; Duan, X.; Song, N.; Ding, D.; Su, X.; Yu, Z. Proline isomerization-regulated tumor microenvironment-adaptable self-assembly of peptides for enhanced therapeutic efficacy. *Nano Lett.* **2019**, *19*, 7965–7976. [CrossRef]
143. Zhao, Y.; Ashcroft, B.; Zhang, P.; Liu, H.; Sen, S.; Song, W.; Im, J.; Gyarfas, B.; Manna, S.; Biswas, S. Single-molecule spectroscopy of amino acids and peptides by recognition tunnelling. *Nat. Nanotechnol.* **2014**, *9*, 466–473. [CrossRef]
144. Mehrotra, N.; Kharbanda, S.; Singh, H. Peptide-based combination nanoformulations for cancer therapy. *Nanomedicine* **2020**, *15*, 2201–2217. [CrossRef]
145. Wang, T.-T.; Xia, Y.-Y.; Gao, J.-Q.; Xu, D.-H.; Han, M. Recent progress in the design and medical application of in situ self-assembled polypeptide materials. *Pharmaceutics* **2021**, *13*, 753. [CrossRef]
146. Liu, Y.; Naumenko, E.; Akhatova, F.; Zou, Q.; Fakhrullin, R.; Yan, X. Self-assembled peptide nanoparticles for enhanced dark-field hyperspectral imaging at the cellular and invertebrate level. *Chem. Eng. J.* **2021**, *424*, 130348. [CrossRef]
147. Samec, T.; Boulos, J.; Gilmore, S.; Hazelton, A.; Alexander-Bryant, A. Peptide-based delivery of therapeutics in cancer treatment. *Mater. Today Bio* **2022**, *14*, 100248. [CrossRef]
148. Mousavizadeh, A.; Jabbari, A.; Akrami, M.; Bardania, H. Cell targeting peptides as smart ligands for targeting of therapeutic or diagnostic agents: A systematic review. *Colloids Surf. B Biointerfaces* **2017**, *158*, 507–517. [CrossRef]
149. Yu, M.-Z.; Pang, W.-H.; Yang, T.; Wang, J.-C.; Wei, L.; Qiu, C.; Wu, Y.-F.; Liu, W.-Z.; Wei, W.; Guo, X.-Y. Systemic delivery of siRNA by T7 peptide modified core-shell nanoparticles for targeted therapy of breast cancer. *Eur. J. Pharm. Sci.* **2016**, *92*, 39–48. [CrossRef]
150. Sorolla, A.; Wang, E.; Clemons, T.D.; Evans, C.W.; Plani-Lam, J.H.C.; Golden, E.; Dessauvagie, B.; Redfern, A.D.; Swaminathan-Iyer, K.; Blancafort, P. Triple-hit therapeutic approach for triple negative breast cancers using docetaxel nanoparticles, EN1-iPeps and RGD peptides. *Nanomed. Nanotechnol. Biol. Med.* **2019**, *20*, 102003. [CrossRef] [PubMed]
151. Lin, W.; Ma, G.; Yuan, Z.; Qian, H.; Xu, L.; Sidransky, E.; Chen, S. Development of zwitterionic polypeptide nanoformulation with high doxorubicin loading content for targeted drug delivery. *Langmuir* **2018**, *35*, 1273–1283. [CrossRef] [PubMed]
152. Li, C.; Qi, Y.; Wang, Y.; Nie, G.; Zhao, Y. Application of peptide-based nanoformulations for targeting and regulating tumor microenvironment. *J. Funct. Polym.* **2019**, *32*, 567–581.
153. Bae, M.; Rusu, L.; Castellon, M.; Stojanovic-Terpo, A.; Onyuksel, H.; Du, X.; Minshall, R. Novel endothelial cell targeted peptide nanoformulation for inhibiting von Willebrand factor secretion to reduce thrombotic complications in sepsis. *FASEB J.* **2019**, *33*, 680.11. [CrossRef]
154. Dissanayake, S.; Denny, W.A.; Gamage, S.; Sarojini, V. Recent developments in anticancer drug delivery using cell penetrating and tumor targeting peptides. *J. Control. Release* **2017**, *250*, 62–76. [CrossRef] [PubMed]
155. Samec, T.; Alatise, K.L.; Boulos, J.; Gilmore, S.; Hazelton, A.; Coffin, C.; Alexander-Bryant, A. Fusogenic peptide delivery of bioactive siRNAs targeting CSNK2A1 for treatment of ovarian cancer. *Mol. Ther.-Nucleic Acids* **2022**, *30*, 95–111. [CrossRef] [PubMed]
156. Liu, K.; Li, Y.; Yu, B.; Wang, F.; Mi, T.; Zhao, Y. Silencing non-SMC chromosome-associated polypeptide G inhibits proliferation and induces apoptosis in hepatocellular carcinoma cells. *Can. J. Physiol. Pharmacol.* **2018**, *96*, 1246–1254. [CrossRef]
157. Jing, H.; Cheng, W.; Li, S.; Wu, B.; Leng, X.; Xu, S.; Tian, J. Novel cell-penetrating peptide-loaded nanobubbles synergized with ultrasound irradiation enhance EGFR siRNA delivery for triple negative Breast cancer therapy. *Colloids Surf. B Biointerfaces* **2016**, *146*, 387–395. [CrossRef] [PubMed]
158. Xie, J.; Bi, Y.; Zhang, H.; Dong, S.; Teng, L.; Lee, R.J.; Yang, Z. Cell-penetrating peptides in diagnosis and treatment of human diseases: From preclinical research to clinical application. *Front. Pharmacol.* **2020**, *11*, 697. [CrossRef]
159. He, W.; Xing, X.; Wang, X.; Wu, D.; Wu, W.; Guo, J.; Mitragotri, S. Nanocarrier-mediated cytosolic delivery of biopharmaceuticals. *Adv. Funct. Mater.* **2020**, *30*, 1910566. [CrossRef]
160. Fuchs, H.; Bachran, C.; Flavell, D.J. Diving through membranes: Molecular cunning to enforce the endosomal escape of antibody-targeted anti-tumor toxins. *Antibodies* **2013**, *2*, 209–235. [CrossRef]
161. Yao, P.; Zhang, Y.; Meng, H.; Sun, H.; Zhong, Z. Smart polymersomes dually functionalized with cRGD and fusogenic GALA peptides enable specific and high-efficiency cytosolic delivery of apoptotic proteins. *Biomacromolecules* **2018**, *20*, 184–191. [CrossRef]
162. Lv, S.; Sylvestre, M.; Song, K.; Pun, S.H. Development of D-melittin polymeric nanoparticles for anti-cancer treatment. *Biomaterials* **2021**, *277*, 121076. [CrossRef]
163. Ahmad, A.; Khan, J.M. pH-sensitive endosomolytic peptides in gene and drug delivery: Endosomal escape and current challenges. *J. Drug Deliv. Sci. Technol.* **2022**, *76*, 103786. [CrossRef]
164. Yang, G.; Zhang, J.; You, W.; Zhao, X.; Hou, P.; He, W.; Yan, J.; Guo, H. Targeted disruption of the BCL9/β-catenin interaction by endosomal-escapable nanoparticles functionalized with an E-cadherin-derived peptide. *Nanotechnology* **2019**, *31*, 115102. [CrossRef] [PubMed]
165. Ahmad, A.; Khan, J.M.; Haque, S. Strategies in the design of endosomolytic agents for facilitating endosomal escape in nanoparticles. *Biochimie* **2019**, *160*, 61–75. [CrossRef] [PubMed]
166. García-Mora, P.; Martín-Martínez, M.; Bonache, M.A.; González-Múniz, R.; Peñas, E.; Frias, J.; Martinez-Villaluenga, C. Identification, functional gastrointestinal stability and molecular docking studies of lentil peptides with dual antioxidant and angiotensin I converting enzyme inhibitory activities. *Food Chem.* **2017**, *221*, 464–472. [CrossRef] [PubMed]