Chapter 25: Peptide Drug/Device Combinations

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1 author:

Shahid Uddin
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	Particle	
	Given Name	**Shahid**
	Suffix	
	Division	
	Organization/University	Immunocore
	Address	Oxfordshire, UK
	Email	Shahid.uddin@immunocore.com
Abstract	Peptides play an important role in human physiology, and peptide deficiencies or dysfunction can lead to disease or illness. Therefore, the role that peptides play in disease treatment goes back many years, starting with the use of insulin in patients with type 1 diabetes almost a century ago. The range of targets available for peptide therapeutics and the limitations associated with peptide delivery and therapeutic effect require consideration. This chapter will review the history of the use of peptides as therapeutics, address some challenges in clinical use, and touch on major advances in the use of peptides as drugs. It will also address issues related with formulation and formulation development; peptide bond formation methods; the use of excipients; the synthesis of peptides, as well as aggregation, separation, and purification; and characterisation and stability testing. It further explores the pharmacokinetics of peptides and various routes of drug delivery and challenges related to peptide delivery.	
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Chapter 25
Peptide Drug/Device Combinations

Shahid Uddin

1 Overview of Peptide Therapeutics

The role of peptides in normal human physiology has led to a large amount of interest in the development of peptide therapeutics. But what are peptide therapeutic agents and how are they poised within the context of pharmaceutical patient management strategies? The aim of this section is to provide an overview of the history of peptides as drugs and the uses of peptides in clinical practice. This section provides an insight into how peptide therapy has developed over time, focusing on the advances underlying contemporary uses of peptides as drugs.

1.1 History of Peptides as Drugs

Peptides are defined as amino acids joined through amide bonds and range in length from three amino acids (e.g. thyrotropin-releasing hormone) to 100 amino acids [57]. Long length chains of amino acids are typically not considered peptides and will not be discussed in the present chapter. There are over 7000 naturally occurring peptides, many of which play a role in human physiology [31]. Peptides are essential in the regulation of homeostasis within the human body, performing a range of functions. One of the clearest examples of peptide homeostasis is the role of the peptide insulin in regulating blood glucose levels [80]. Insulin secretion from the pancreas acts on designated receptors to promote uptake of blood glucose into cells while also reducing the synthesis of new glucose and moderating metabolism of

S. Uddin (✉)
Immunocore, Oxfordshire, UK
e-mail: Shahid.uddin@immunocore.com

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glucose. In patients with type 1 diabetes, insulin is not produced by the pancreas, leading to unregulated blood glucose levels; in this context replacement of insulin can provide therapeutic benefits to patients. This example is a classic instance of peptide therapeutics, and replacement of insulin has been used clinically since the 1920s [80]. However, many other peptides may be used therapeutically to manage endocrine and central nervous system disorders, infectious disease, and cancer.

Recent advances and approvals of drugs have led to an emergence of peptides as an innovative and growing therapeutic area. It is estimated that over 140 peptide therapeutics are undergoing clinical trials, while new peptide designs and approaches are being developed routinely [31]. Peptides may be formulated as small molecules (akin to many drugs in the marketplace), larger molecules, or as biologic agents. Biologics was a term that used to include blood or blood components, but this has progressed to include monoclonal antibodies, cytokines, tissue growth factors, vaccines against non-infectious disease targets, and gene transfer products [8]. These agents may have pronounced immunomodulatory effects and illustrate the diversity of peptide therapeutic approaches as a means to prevent immune-mediated disease or to enhance tissue growth, recovery, and protection against disease [8].

Compared with pharmaceutical agents, peptides are generally considered to have a predictable safety profile and tolerance in patients. Furthermore, peptide therapy is selective and potentially efficacious, particularly where naturally occurring peptides are replaced for therapeutic effect [31]. Therefore, interest in peptide therapy is growing with time and changing the pharmaceutical marketplace. Table 1 lists the peptide therapeutics marketed in the last few decades.

Table 1 Marketed therapeutic peptides

Trade name	Generic name	Target	Indication
Forteo	Teriparatide	P1TH1R agonist	Osteoarthritis
Fuzeon	Enfuvirtide	Protein-protein inhibitor	HIV
Prialt	Ziconotide	Calcium channel inhibitor	Pain
Byetta	Exenatide	GLP-1R agonist	Type 2 diabetes
Symlin	Pramlintide	Calcitonin agonist	Type 1 or 2 diabetes
Somatuline	Lanreotide	SST agonist	Acromegaly
Nplate	Romiplostim	Thrombopoietin agonist	Haematology
Egrifta	Tesamorelin	GHRF agonist	Lipodystrophy
Victoza	Liraglutide	GLP-1R agonist	Type 2 diabetes
Bydureon	Exenatide LAR	GLP-1R agonist	Type 2 diabetes
Surfaxin	Lucinactant	Uncertain	IRDS
Omontys	Peginesatide	Erythropoietin analogue	Anaemia
Signifor	Pasireotide	Somatostatin analogue	Cushing's disease
Kyprolis	Carfilzomib	Proteasome inhibitor	Multiple myeloma
Linzess	Linaclotide	Guanidyl cyclase 2C agonist	Irritable bowel syndrome (constipation)
Gattex	Teduglutide	Glucagon-like peptide analogue	Short bowel syndrome

Table taken from Dunn [25, 222]

Peptides offer an enormous potential for growth within the pharmaceutical industry; although many peptides have been developed, traditional peptide design has been modified to allow for a range of putative peptide products [70]. In particular, peptide biologics represent a growing field, including monoclonal antibodies, cytokines, and growth factors [8]. Furthermore, advances in the delivery and efficacy of peptide therapeutics hold great promise for expanding their use in practice. The limitations of peptides in current practice and the potential to overcome these limitations will be considered in the remainder of this section.

1.2 Limiting Factors When Using Peptides in the Clinic

Although peptide therapeutics has grown as a subdivision of the pharmaceutical industry, peptides have a relatively small market share at present [31]. The reasons underlying this observation are numerous, including the limitations of traditional delivery techniques of peptide therapeutics, a limited range of clinical targets, the relative cost of developing peptide therapy, and the practical use of peptides.

One of the characteristics of peptides as therapeutic agents is their molecular size: positioned between small molecules and proteins. Furthermore, the molecular characteristics of peptides differ significantly from either small molecules (most drugs developed) or proteins [31]. The size of peptides and their vulnerability to natural processes of enzymatic degradation and metabolism reduce the potential routes of administration and putative efficacy within the body. Most peptide drugs are administered through injection (intravenous, intramuscular, or subcutaneous) to ensure efficacy within the body and to avoid degradation via the oral route [88]. This may limit the convenience of the use of peptide therapy in the clinic.

As a general rule, naturally occurring peptides have a short plasma half-life, which can limit their therapeutic potential [31]. Half-life control forms an essential aspect of the homeostatic regulation of peptides as part of an endocrinological system, so strategies have to be devised to overcome this limitation [52]. Enzymatic cleavage is a common means for degradation of peptides, and prevention of enzymatic activity, potentially through alteration of cleavage site amino acid sequences, is one strategy to extend the life of peptides in the body. However, stability of naturally occurring peptides has been a major obstacle to the development of many peptides as viable therapeutic agents [31].

Additional imitations to peptide use in clinical practice include the limited range of targets available or peptide therapy and the cost of peptide therapy. Targets are typically limited to replacement therapy in many clinical contexts, thereby only covering a small range of conditions. There is the potential to develop peptides for many more therapeutic purposes – the supply of available agents does not match this potential, however. The relative cost of peptide therapy may also be higher than other forms of therapy, particularly as manufacturing techniques become more

advanced [52]. Therefore, limitations to peptide use in practice are numerous but represent challenges that can be potentially overcome using alternations in peptide formulation and advances in peptide modification and development.

1.3 Advances in the Use of Peptides as Drugs

Although there are limitations to the widespread use of peptides in clinical practice, advances in drug development and refinement of the peptide therapeutic approach have opened up multiple avenues for expansion of this therapeutic area. Initially, peptides were endogenously sourced, derived from animals and acting as replacement therapies for human diseases. This is the case with insulin, as well as adrenocorticotrophic hormone (ACTH), which was isolated from bovine or porcine pituitary glands in the 1950s [49]. Once sequencing of peptides became possible, synthetic peptides were manufactured during the 1960s and 1970s, leading to a rapid expansion in available agents, including oxytocin, vasopressin, calcitonin, and octreotide [49]. The genomic era has seen massive leaps in peptide therapeutic technology, with identification of receptors and novel agents activating receptors for potential therapeutic effects [7]. As manufacturing approaches of peptide therapeutics have advanced over time, so too have the potential applications of this type of therapy.

Increasing the pharmacological potency of peptides has also been a key research focus, and peptide modifications to promote cell entry and increase stability have been developed accordingly [7]. Although native peptides do not typically cross cell membranes, cell-penetrating peptides (e.g. penetratin) have been devised to overcome this limitation and expand molecular targets to include intracellular targets [61]. Balancing the potential to expand the range of targets of peptides with the increased volume of distribution and potential for lower potency of the peptide is an important factor for refinement [31].

Oral bioavailability of peptides has generally been poor, requiring routes of administration through injection [82]. Improving oral bioavailability is considered an important therapeutic hurdle, which would make peptide therapy simpler and more attractive to patients. Chemical strategies to overcome acidic and enzymatic digestion in the gastrointestinal tract have emerged, including features of peptide stabilisation, such as hydrophobic face construction, cyclisation, methylation of amino acid N-terminals, and introduction of intramolecular hydrogen bonds [31]. However, advances in injectable peptide delivery have also been pursued in order to improve the convenience of delivery and patient experience [82]. Therefore, changes to peptide stabilisation can have an impact on the attractiveness of these therapeutic options.

Peptide sequencing techniques have developed dramatically over time, allowing for an expansion of the available targets of peptides as well as the techniques used to synthesise peptide therapies [87]. Sequencing of peptides allows for accurate characterisation of the likely chemical properties of the peptide, including involvement in degradation pathways and likely shelf-life of the drug, as well as efficacy in targeting specific clinical conditions [7]. Sequencing techniques have

become more rapid and dynamic, allowing for high-throughput approaches to refining drug candidates and use strategic design approaches to drug synthesis [7]. Similarly, techniques used to synthesise peptides are advancing, reducing the cost and length of time required to take a peptide from the laboratory to the clinic [87].

The generation of peptide libraries has also facilitated coordinated research efforts on a global scale [51]. These libraries catalogue identified peptides and allow researchers to identify and optimise peptides for a range of clinical uses, including antimicrobials [5]. Libraries allow for rapid screening of peptides for use as drugs and can facilitate early stage drug development, making this strategy a powerful tool for expanding the repertoire of peptides available for clinical use [51]. These libraries may also include information on modifications and formulations of peptides and their corresponding pharmacokinetic and pharmacodynamic profiles, advancing the potential to formulate peptides for specific uses [26].

One of the most promising avenues of research is the potential to target peptides to cells or tissue, allowing for highly specific therapeutic effects [30]. Peptides interact with cell surface receptors in a highly specific manner, affording the opportunity to modify peptide sequences to target specific cellular or tissue receptors [30]. These peptides may be used alone for therapeutic purposes or may be associated with other drugs and delivery systems, facilitating tissue-specific drug delivery [42]. The combinations of advances in library catalogues of peptides and synthesis approaches have generated a massive interest in the potential for targeted activity with these drugs, opening up many avenues to future pharmacological development.

2 Formulation of Peptides

The formulation of peptides refers to the process of managing bulk raw materials and producing therapeutic peptides through a series of manufacturing and processing stages. The strategies employed in refining peptides and ensuring a viable clinical product are diverse and remain integral to the potential utility of peptide therapeutics in practice. Different formulation strategies may also have implications for the efficacy and pharmacokinetics of peptides produced, highlighting the need to balance the outcomes of different formulation strategies. This section will consider how peptide formulation is facilitated in practice, with a focus on newer pharmaceutical methods, as well as essential quality control processes used to ensure the viability of peptides for clinical use.

2.1 Pre-formulation Studies

The use of pre-formulation studies as an initial stage in evaluating bulk material is essential prior to formulation of peptides. These studies provide the basis for development of optimal dosage forms of the peptides and the design of a suitable delivery

system, with the overarching goal of achieving maximal stability and bioavailability [59]. Pre-formulation studies are often used in small molecule drug development, including the use of crystallography, nuclear magnetic resonance, and mass spectrometry to characterise bulk materials and determine the atomic-level structures of molecules [59]. The complexity of proteins and peptides, including the formation of higher-order forms of these molecules, complicates this process, but lower-resolution methodologies may be applied [85]. Gel electrophoresis and high-performance liquid chromatography can be used to analyse bulk materials, peptides, and the presence of any impurities allowing for refinement of the peptide product for subsequent formulation into a pharmaceutical agent [37].

2.2 Formulation Development

Following completion of pre-formulation studies, formulation development aims to characterise impurities in the product, including the presence of any degradation products [37]. Furthermore, the packaging and environmental conditions under which the peptide can remain stable should be investigated and optimised [3]. A combination of literature review and analytical methods can determine the likelihood of the presence of leachable elements from protein/peptide storage vessels. Specific challenges regarding the formulation of lyophilised or high-concentration formulations are also noted [3].

Buffer systems need to be selected carefully in order to prevent small pH changes from adversely affecting the stability or function of the peptide. Phosphate buffers are commonly used but are limited when applied to zinc insulin (zinc phosphate precipitations arise) or in peptides that require a low pH to maintain stability (e.g. gamma-interferon) [31]. For lower pH solutions, organic acid buffers, such as lactate, may be useful [76]. However, generally inorganic buffers are used in practice in order to achieve the desirable characteristics of being zwitterionic, excluded from the peptide domain, acting as a scavenger of free radicals and preventing mechanical stress in the peptide [31]. For instance, histidine buffer has a pH of 7.4 and is commonly used for monoclonal antibody preparations [72, 75]. Buffers also need to be considered in terms of how the solubility of the protein or peptide is affected [31].

In addition to the buffer system used, the pH of the formulation can affect stability and bioavailability; often a compromise is needed to prevent deamidation reactions but minimising oxidation reactions [31]. The solution pH and use of excipients may also affect the solubility of the peptide formulation [54]. Ideally, solubility should be achieved where the maximum amount of peptide is dissolved without precipitation in a medium. This may be predicted, in part, from the structure of the peptide, although other methods are needed in practice to optimise solubility. This includes extrapolation of peptide solubility based on polyethylene glycol precipitation values, a time-consuming process, or light scatter solubility assessment [54].

Similarly, the selection of solvents, preservation agents, and container are all important during peptide formulation, as these affect stability, solubility, and the

bioavailability of the peptide solution [31]. Polyhydric alcohols, including glycerol, can stabilise peptides solutions. Preservatives may be added to stabilise molecules for a longer shelf-life, although these should be considered cautiously and in-line with regulatory requirements. Containers, including glass or composite materials, should be selected to increase stability and minimise the potential for alterations of peptides, as well as for practical use in the clinical setting.

2.3 Pharmaceutical Excipients

Another important part of the peptide formulation process is the use of pharmaceutical excipients, non-medicinal substances added to facilitate stability and desirable characteristics to facilitate drug delivery in the body [54]. Common excipients for peptide therapeutics include albumin, amino acids, carbohydrates, chelating and reducing agents, cyclodextrins, surfactants, salts, alcohols, and glycol. These excipients have varying biochemical roles but all act to alter the chemical environment, reducing the rate of peptide degradation or enhancing the stability of peptides in specific tissues [33].

Excipients may also play a role as solid supports and linkers, which assist in peptide synthesis and in stabilising the peptides once formed [33]. Solid supports include resins, which are stable and inert, often comprising polystyrene beads cross-linked with divinylbenzene, although many other solid supports are used in contemporary peptide therapeutic formulation [74]. Linkers may be used to attach amino acids of the peptide to the resin or solid support, and the characteristics of these linkers may influence their functionality. Cleavage of linkers often occurs under acidic conditions, allowing for pH-based control over formulation of peptides once stabilised [74].

Finally, the use of excipients as protecting groups has been observed as essential to ensuring amino acids are protected (as well as side chains) from degradation or alteration during peptide synthesis and storage [54]. Two commonly used protecting groups are fluorenylmethyloxycarbonyl (Fmoc) and tert-butyloxy-carbonyl (tBoc), of which many molecules can be used to protect different amino acid residues [84]. Introduction of protecting groups is a complex process and requires careful consideration of the effects of the protecting groups on subsequent synthesis reactions and formulation of the peptide.

2.4 Aggregation in Protein Formulations

Numerous processes and unintended reactions within peptide and protein therapeutic solutions can affect the synthesis and formulation of an effective drug. Aggregation of proteins occurs under numerous environmental conditions and is governed by the intrinsic structural or chemical features of the protein, as well as the

external environment [31]. The consequences of protein aggregation may be a reduction in biological activity or the potential development of immunogenicity, which can limit the therapeutic use of proteins, as well as peptide agents [86]. Aggregation of proteins and peptides may occur in an orderly fashion, often with linear aggregate formation (as seen with amyloid proteins in Alzheimer's disease) [20], or in a disorder manner, termed amorphous aggregation [58]. In both instances, aggregates can serve as seed nuclei for the generation of larger aggregates and visible particles, which can have damaging effects on the cellular environment [86].

Aggregation is dependent on the environmental conditions where the protein or peptide is located. These conditions include temperature, pH, the presence of solvent compounds, and the presence of additional environmental stressors [72]. These conditions affect the intrinsic molecular bonds within peptides and proteins, affecting secondary, tertiary, and quaternary structures, potentially resulting in unfolding, dimerisation, and then formation of oligomers of peptides [81]. The susceptibility of different peptides to aggregation depends on the molecular characteristics of the peptide. Different peptides or proteins may have more desirable environmental resilience, depending on the intended use of the agent and the association of additional drugs or adjuvants [81].

Control of aggregation is essential in preventing loss of biological efficacy of the peptide, as well as preventing immunogenicity, characterised by immunological reactions to the aggregates [14]. The use of protecting groups and microwave heating are techniques associated with the prevention of aggregation in synthesis techniques. Scavenger agents within the working solution can also be used to prevent aggregation, by removing substances that promote aggregation of modify the binding characteristics of peptides [14, 31]. However, these techniques are diverse and individualised for specific peptides, adding complexity to this discussion and suggesting the need for transparent synthesis strategies where aggregation is managed appropriately.

2.5 Peptide Bond Formation (Coupling Methods)

Peptide bonds (amide bonds) form the basis for joining amino acid residues together in order to form peptides [11]. These bonds are formed between a C-terminal (carboxyl group) and N-terminal (amino group) of different amino acids. Although peptide bonds form naturally, facilitating these bonds during peptide synthesis is essential to produce the desired end product. Essentially, the strategy for bond formation involves the presence of amino acid residues, a peptide bond forming reagent, and a target activating group on the amino acid to be joined [11].

Peptide bond forming reagents are numerous, but the most common agents are carbodiimides, symmetric anhydrides, and acid halides [72]. Carbodiimides are water-soluble molecules with the general formula RN = C = NR [9]. These molecules are advantageous in that they hydrolyse to form urea, which does not interfere with peptide synthesis reactions. Carbodiimides activate the carboxyl group of an

amino acid allowing for formation of peptide bonds under certain conditions [9]. Symmetrical anhydrides are carboxyl acid anhydrides that are transient but persist in solution long enough to complete peptide bond formation [72]. Mixed and N-carboxy anhydrides may also be used in peptide synthesis, facilitating the formation of peptides and protected amino acids in solution or solid phase [72].

2.6 Synthesis Approaches

Numerous synthesis approaches have been employed in peptide therapeutics, and a brief discussion of these approaches and their key differences should be considered. The first synthesis of oxytocin occurred in the 1950s using a classical approach, termed solution-phase synthesis (SPS), or synthesis in solution, which remains the main synthesis techniques used in contemporary peptide therapeutics [9]. The principle of this method is to add amino acids to a central amino acid or group or amino acids in sequence, with all reagents in a solution (i.e. homogeneous phase) [9]. The SPS approach is considered beneficial for large peptide synthesis, as the control over soluble elements of the solution can be greatly enhanced by refining the technique [9].

Solid-phase synthesis is an alternative to SPS and is commonly based on the Fmoc/tBu strategy in association with activated carboxyl groups and the use of modified polystyrene resins [21]. Essentially, the C-terminal amino acid is anchored to the solid supporting resin, often using a linking agent, allowing for the removal of the N-terminal protecting group [67]. When performed in sequence, this process protects the side chains from alteration and provides a sequential approach to peptide synthesis as each amino acid is introduced in turn [67]. This process is illustrated in Fig. 1.

Hybrid synthesis involves the use of a solid-/solution-phase approach, whereby peptides are synthesised on solid supports within a solution [9]. For instance, small peptides (e.g. up to ten amino acids) can be produced in solid phase on resins, and then segment condensations are completed in solution to construct the entire sequence of the peptide [2]. This combined approach can overcome the disadvantages of solid-phase approaches, including extensive cost and limits to the size of peptides produced, while taking advantage of the ability to utilise peptides that are not amenable to bacterial expression, required for synthesis in solution [2].

The synthesis of cyclic peptides is an area of specific interest, as cyclic peptides are generally more stable, have greater resistance to degradation, and have longer-lasting (depot) effects in the body [11]. The synthesis of cyclic peptides requires the formation of disulphide bridges of amide bonds between sulfhydryl groups or other groups [90]. These cyclic peptides may be formed using chain-to-side chain, head-to-side chain, side chain-to-tail, or head-to-tail strategies; the choice of technique depends on the peptide structure and the cyclisation position [17]. Most commonly, disulphide bridges are formed between two amino acid elements through a variation of solid- or solution-phase techniques.

Finally, the depsipeptide method for peptide synthesis is designed to overcome the challenges of folding and aggregation with other techniques [21]. Despipeptides

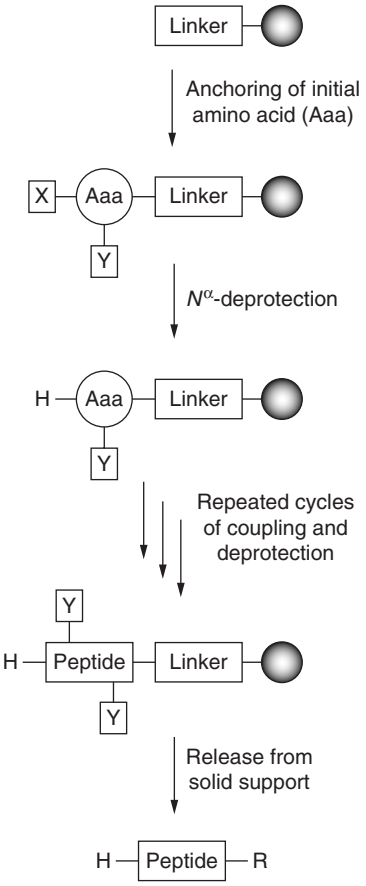

Fig. 1 The principle of solid-phase peptide synthesis. X, temporary protecting group; Y, semi-permanent side-chain amino acid (Aaa) protecting groups; R, C-terminal functionality [67]

are *O*-acyl isopeptides and are ester isomers of the intended peptide sequence, which are more advantageous during synthesis as they are (1) easier to assemble, (2) easier to purify, and (3) can be easily converted to parent amides [21]. This method is generally employed for challenging peptide sequences prone to folding and aggregation using other methods, with a solid-phase basis [21].

2.7 Separation and Purification (Chromatography)

Once peptides have undergone synthesis, it is essential that the resulting solution is separated and purified to remove contaminants and substances that may affect the degradation potential of the peptide product [9]. Chromatography techniques are typically used for this purpose, allowing for separation of molecules based on numerous characteristics, including charge or molecular size, depending on the type of chromatography employed.

Gas, ion-exchange, and affinity chromatography techniques may be employed allowing for separation of peptides and proteins based on molecular size, charge, polarity, solubility, and/or covalent interactions [72]. Selection of appropriate chromatography techniques is dependent on laboratory resources and technician experiences as well as the presence of specialised classes of proteins and the need for amino acid residue distribution and modifications.

2.8 Characterisation with Mass Spectrometry

Following separation and purification of the peptide agent, mass spectrometry can be used as an accurate means of characterising the final product [9]. Ion mobility mass spectrometry (IMMS) has emerged as a powerful analytical tool and is increasingly used to characterise peptides and proteins in complex samples [27]. The principle of ion mobility is the separation of ions based on their size and charge ratios, as well as considering interactions of ions with a buffer gas [41]. This technique allows for an accurate and sensitive way of separating proteins and peptides within a complex mixture, as well as allowing for careful characterisation of all present elements [19]. Five stages are used during IMMS: sample introduction, compound ionisation, ion mobility separation, mass separation, and ion detection [41].

The potential use of IMMS for peptide therapeutics includes the ability not only to separate complex mixtures but also to characterise peptides or proteins within complex mixtures. The complementary approaches of ion mobility spectrometry and mass spectrometry allow for combination into IMMS, which serves as an adjunct to traditional structural techniques. For instance, IMMS can identify rotationally averaged cross-sectional area, which may not be achievable using other techniques, as well as the conformational dynamics of the peptide solution, as well as appreciating folding mechanisms and aggregation profiles of proteins and peptides [48]. High separation selectivity during bioanalysis has been observed [41], emphasising the role of IMMS in peptide therapeutics characterisation.

The reliability of structural interpretation and identification of ions relies on careful calibration of the IMMS equipment and consideration of variables within the analytical process, including gas pressure, gas compositions, temperature, and modes of separation [13]. Therefore, as IMMS technology continues to advance, calibration and regulation of this analytical procedure is needed to ensure consistency in results and utility in drug manufacturing.

2.9 Stability Testing

Stability of peptides is a principle concern for drug manufacturers, as stability can be indicative of the lifespan of the drug in storage and during clinical use [10]. It is essential to determine stability characteristics of any peptide agent to understand the lifespan of the drug.

Many aspects of the environment can affect stability, and stability testing involves monitoring the effects of pH, temperature, humidity, and light exposure on peptide structure, function, and efficacy [9]. Characterisation and stability testing of oral peptide agents' procedures highlight the need for rigor when investigating drugs at this stage of development, but criteria for stability will likely evolve as drugs move from preclinical to clinical development [10]. The complexity of formulations and the use of excipients to facilitate oral delivery of peptides raise an intriguing challenge to stability testing in the future, and standards and testing regimens will need to follow the example of small molecule development to ensure drug longevity and patient safety [6].

3 Delivery System Considerations for Peptide Therapeutics

This section illustrates the role of delivery systems in the development of peptide therapeutics. The route of administration and the delivery method of peptides are heavily dependent on the pharmacokinetic properties of the drug, and the implications of delivery system design are numerous. This section highlights the main delivery techniques used in current practice while highlighting novel strategies and developments for the future of this therapeutic field.

3.1 Pharmacokinetics of Peptides

Pharmacokinetics covers a range of characteristics of a putative drug or molecule when introduced into the body [66]. The term encompasses a range of features of the drug, including bioavailability, volume of distribution, clearance characteristics, half-life, stability, and concentration characteristics (i.e. peak concentration and trough concentration). As noted in previous sections, peptides are prone to degradation and have a short half-life, which impacts on their overall pharmacokinetic profile, making them less suited for pharmaceutical purposes than small molecules [72]. For instance, peptides with a large molecular weight, susceptibility to digestive enzymes, low permeability through the intestine, and hydrophilicity (features common to most peptides) can yield a low potential for distribution throughout the body and the achievement of biological concentration to elicit a therapeutic effect [66].

The pharmacokinetics of peptide agents has implications for administration and device design. Most importantly, the route of delivery is largely determined by the extent to which the peptide drug can survive in the body – degradation due to proteolytic enzymes and acidic conditions can limit oral delivery of many agents [31]. Furthermore, short half-lives of peptides in the body suggest the need for rapid delivery, close to the target organ, often favouring parenteral (i.e. injected) delivery [9].

3.2 Delivery Approaches

Delivery of peptides has seen a massive increase in diversity and design over the past few decades, underlining advances made in the formulation of effective products [80]. The main routes of administration include parenteral, transdermal, oral, inhalation, intranasal, and ocular. Each administration route is associated with unique device characteristics designed to optimise effective dosage and reduce or minimise patient side effects [6]. Each of these routes of administration is associated with distinct advantages and disadvantages in practice and has implications for the design of device used to administer medications [7].

Drug delivery approaches must be carefully considered and should ensure that pharmacokinetic factors are reflected in the delivery route of the drug [86]. Table 2 illustrates the range of delivery technologies used for intra- or transdermal peptide delivery and oral peptide delivery. The delivery approaches of peptides may be related to their specific drug type, i.e. the differentiation between small molecules and biologics. Biologics are often regulated with greater scrutiny than small molecules, and their use as intravenous or injectable agents (e.g. vaccines, monoclonal antibodies, and cytokines) may be preferred to oral routes to enhance delivery and minimise instability [8].

Structural modifications of peptides are diverse and may be used to modify degradation and half-life characteristics in complex ways but potentially compromise the efficacy of the drug [72]. This principle applies to peptide delivery, which may be facilitated by polymers of peptides that are biodegradable or non-biodegradable, the use of enzyme inhibitors, the use of permeation enhancers, and consideration of strategies used to target individual tissues or organs (e.g. transport across the blood-brain barrier).

Delivery systems must be designed with the specific qualities of peptides for which they are intended to deliver. A range of characteristics influence the design of a delivery system, including pharmacokinetics of peptides, available delivery approaches, site of action of the drug, and the clinical use of the drug [7]. Only where peptide stability can be ensured can a specific route of administration be considered for widespread use in practice [87]. Modifications to peptide formulations may yield impressive benefits in stabilising and improving the pharmacokinetic profile of the drug once delivered, but the device characteristics may still provide limitations to the dosage needed and the therapeutic effect [85]. For instance, inhaled peptides used in the management or respiratory conditions may be prone to deposition in the oropharynx, particularly where inhaler technique is suboptimal, limiting the therapeutic efficacy of the delivered dose and increasing the risk of local side effects [83]. Hence, delivery system design and use by the patient can significantly influence how a well-formulated peptide drug impacts the clinical status of the patient. All these factors therefore need to be considered in the context of peptide delivery.

Protection of the peptide against enzymatic or environmental degradation can be achieved using delivery of peptides combined with polymers, designed to either

Table 2 Peptide delivery strategies undergoing or receiving approval, p. 44 [50]

Company	Details	Technology	Reports/claims
Intra- and transdermal delivery of peptides			
3 M	Solid and hollow microneedle patches	sMTS, hMTS	hPTH, PTHrP
Corium	Dissolvable peptide microneedle patch	MicroCor	hPTH
Isis biopolymer	Iontophoresis	IsisIQ	Collagen-stimulating peptides
NanoPass	Intradermal microneedle injection system	MicronJet	Proteins, vaccines
Pantec Biosolutions	Laser-assisted ablation	PLEASE	Triptorelin
Phosphagenics	Topical	Targeted Penetration Matrix	Insulin
TheraJect	Dissolvable peptide microneedle patch	TheraJectMAT	hPTH
Vaxxas	Microprojection patch	Nanopatch	Vaccines
Vysteris	Iontophoresis	SmartPatch	Peptides
Zosano	Solid coated microneedle patch	ZP Patch	hPTH
Oral delivery of peptides			
Access	Oral, receptor-mediated uptake	CobOral	Insulin, hGH
Aegis	Buccal, oral	Intravail	AFREP, octreotide
ArisGen	Buccal, oral	ArisCrown	Exendin, hPTH, insulin
Biodel	Sublingual film tablet	VIAtab	Insulin
Proxima Concepts	Oral, enteric-coated capsule	Axcess	Calcitonin, hPTH
Chiasma	Oral, oily suspension of enhances	TPE Technology	Octreotide
Emisphere	Oral, passive transcellular uptake	Eligen	Calcitonin, insulin, GLP-1, PYY
Merrion	Oral, enteric-coated tablet	GIPET	Insulin, GLP-1, GnRH analogue
Midatech/ MonoSol	Buccal film, nanoparticles	PharmFilm	Insulin
NanoMega Medical	Oral, nanoparticles	–	Insulin
NOD Pharmaceuticals	Oral, nanoparticles	NOD	Insulin
Oramed	Oral, enteric-coated tablet	–	Insulin, exenatide
Unigene	Oral, enteric-coated tablet	Peptelligence	Calcitonin, hPTH, CR845

resist or undergo biodegradation, including conjugation with carriers or polymers, adsorption to carriers, or encapsulation in carrier systems [16]. The principle is that these polymers will facilitate delivery of peptides to target tissues within the body, either by persisting or degrading in a controlled manner, allowing release of the peptide into the bloodstream or target tissue [65]. Polymeric nanoparticles have been used widely in pharmaceuticals for this very purpose and may be designed to release peptides or proteins gradually over time, up to weeks or months [65]. However, this is a complex process, and application to peptide therapeutics is promising, but not complete (Fig. 2).

Enzyme inhibitors may be introduced with the peptide as a means of avoiding degradation upon oral delivery or delivery through other routes [22]. Soybean trypsin inhibitor, FT-448, is a leading inhibitor against chymotrypsin degradation and can enhance peptide absorption as well as prevent degradation when co-administered with peptides in animal models [12]. Other enzyme inhibitors have been trialled for use with insulin and other peptides, with mixed results. Enzyme inhibitors may also disrupt the absorption of normal dietary peptides and may have toxic effects over time [72].

However, although degradation by enzymes remains one of the major challenges to peptide use in therapeutics, peptides are also limited by their poor permeability across membranes and structures [55]. Permeation enhancers have been proposed and include modifications to the peptide structure, to facilitate entry into cells and

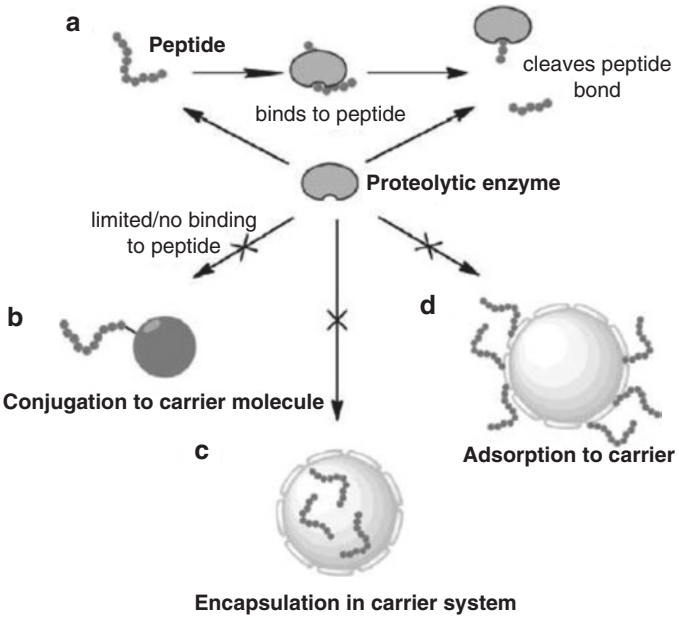

Fig. 2 Conjugation, adsorption, and encapsulation of peptide therapeutics to reduce proteolysis and degradation. (a) Free peptides are rapidly degraded, but the use of carriers (b–d) can block degradation [33]

across membranes [65]. Chitosans are polymer derivatives of chitin, which are known to enhance absorption of macromolecules in the gut, while not being absorbed themselves, potentially limiting side effects of their use [55]. Chitosans have been used in conjunction with insulin, atenolol, and vasopressin and have shown increased permeability and absorption of these peptides, with a good safety profile [62]. Furthermore, lectins and certain types of toxin, which have natural roles in facilitating cross-membrane transportation of macromolecules, have shown promise in enhancing permeation of peptides [62].

Delivery across the blood-brain barrier is a specific challenge for peptide delivery and applies to agents that would be considered to have a primary mode of action on cerebral structures [12]. Liposomes may be used to enhance transport across the blood-brain barrier in animal models, although subsequent liver accumulation of these carriers is a concern [60]. More research is needed to ensure safe and effective transport into the brain prior to human studies.

3.3 Parenteral Peptide Drugs

One of the key delivery strategies for peptides and proteins used for therapeutic purposes is the ability to control the release of the agents, allowing for long-term use without repeat administrations. Furthermore, optimisation of the parenteral use of the peptide, including enhancing stability and targeting specific tissues, is an important feature of modern delivery methods [72]. Microspheres represent one strategy to encapsulate peptides and control their release over time while avoiding degradation [73]. The type of microsphere used in practice is dependent on the polymer used and the sphere-forming method, including the use of phase separation, emulsions, spray-drying, and cryogenic techniques [47]. Typically, the microsphere product is a dry powder that is suspended in the delivery device (e.g. syringe) prior to injection [47]. A similar approach to peptide delivery is the use of injectable implants, essentially polymers inserted subcutaneously and permitting controlled release of the drug over time [1]. Implants can protect peptides from degradation and may be combined with gelling agents to improve their efficacy and length of drug delivery [1]. Concerns over the toxicity and limited lifespan of implants have impeded this area of research and development, although phospholipid-based phase separation gel technology appears to be a low toxicity approach with great promise, as demonstrated in octreotide delivery [89].

Liposomes and nanoparticles have generated a great deal of interest as nanosized drug delivery mechanisms, affording optimal pharmacokinetic and drug release control in parenteral peptide systems [56]. Liposomes are phospholipid-enclosed bilayer spheres, which can be used to transport drugs and peptides and have been shown to improve delivery of anticancer drugs in animal and human studies [4]. Similarly, nanoparticles are colloidal carriers of peptides, fabricated from lipids or polymers, with uniform drug distribution within a matrix [12]. Nanoparticles contain a cargo peptide within a lipid (solid) core, surrounded by

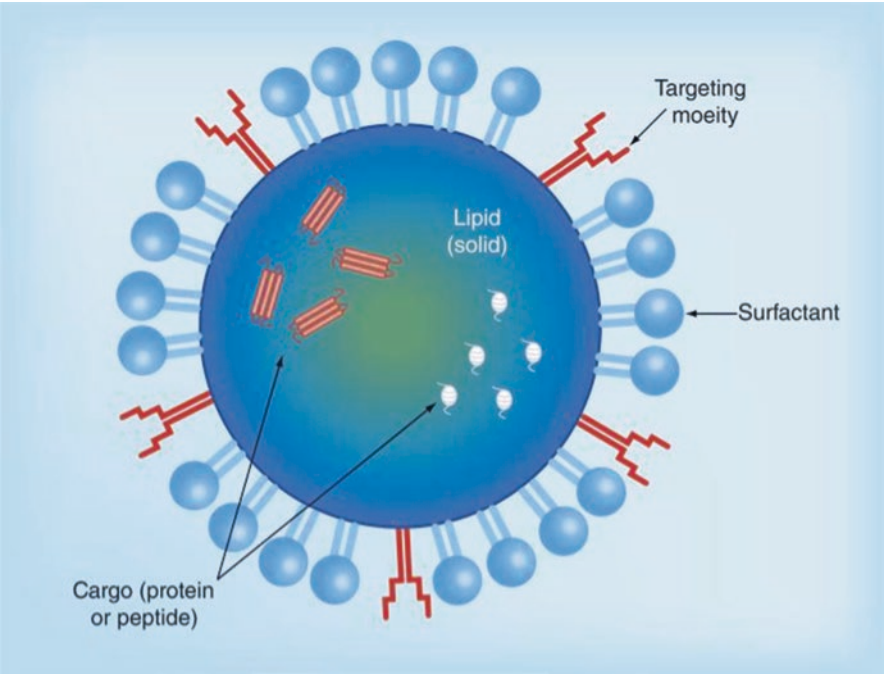

Fig. 3 Solid lipid nanoparticle. The solid lipid core contains the cargo peptide and is surrounded by surfactant, with or without targeting moieties to guide delivery to specific tissues [12]

targeting receptors/ligands and surfactants, allowing evasion of degradation and targeting to specific tissues (Fig. 3). Although both approaches offer the opportunity to overcome degradation and target specific tissues, through promotion of membrane entry, few clinical studies have verified the use of these technologies with peptide delivery [65].

Other approaches to peptide delivery across the skin include the use of microneedles, iontophoresis (electrical charge mediated drug transfer), and patches of drugs applied to the skin (Table 2). The microneedle system (Fig. 4) can involve the use of hollow or soluble microneedles and may rely on skin porations and then drug patch application, needle dissolution in the skin, or infusion of drug formulations through hollow needles. These approaches are actively being explored as patient-friendly approaches to deliver drugs (e.g. human parathyroid hormone (Table 2)). Although a potentially promising route of delivery, there are a couple of key limitations to this approach: (a) immunogenicity (an immune response to the peptide in the transdermal space) and (b) a limitation in the volume of drug product which can be delivered and absorbed in the subcutaneous space.

Vaccines are another interesting area of peptide therapeutics, as peptides may be a preferred vaccination strategy than traditional strategies [53]. The use of proteins and whole or partial microorganisms in vaccines leads to a high antigenic load and the delivery of many substances that may provoke unintended immunological

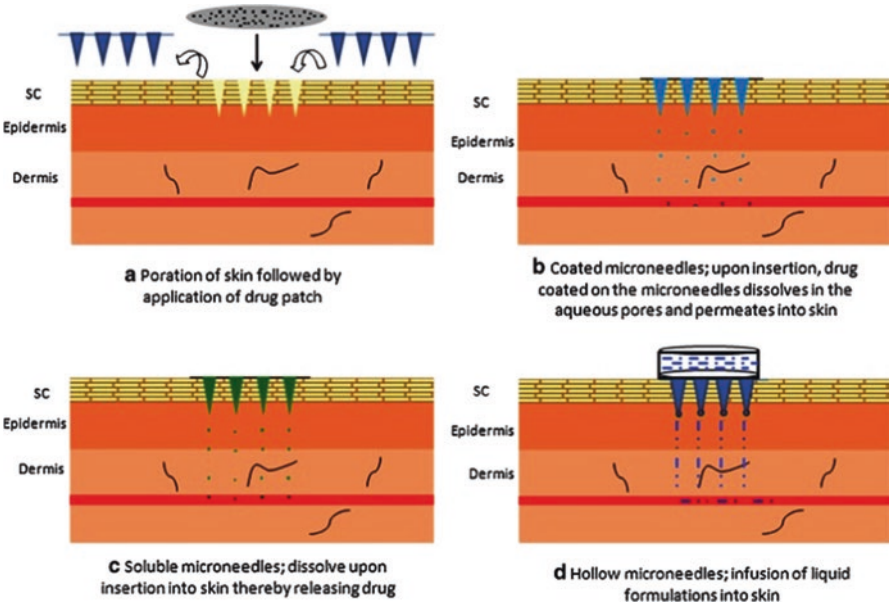

Fig. 4 Microneedle drug delivery systems. (**a**) Solid microneedles cause skin poration, and a drug-loaded patch is placed onto the skin; (**b**) drug-coated solid microneedles are inserted into the skin; (**c**) drug-encapsulated soluble microneedles are inserted into the skin; and (**d**) hollow microneedles allow for liquid formulations to be infused into the skin [40]

reactions [53]. In this application, the potential immunological response is exactly the intended action. Peptide vaccines can avoid this antigenic load, potentially increasing efficacy and reducing the potential for adverse reactions. Emulsions, liposomes, and polymer-based systems have been applied to peptide vaccine development, but efficacy remains weak compared to traditional standards. Adjuvant development is essential to maximise the potential of peptide vaccination for a range of conditions [53].

3.4 Intranasal and Enteral Delivery

The intranasal delivery of peptides has been considered an attractive option for drug delivery due to the potential to bypass first-pass hepatic metabolism and enter the bloodstream rapidly [24]. The use of intranasal drug delivery is considered particularly relevant to central nervous system therapeutics, as the olfactory neurons provide a direct route to this system [64]. Microspheres and liposomes have been used to facilitate intranasal delivery of peptides, but results remain limited in many regards [9]. This may be due to the challenges of accurate dosing with intranasal

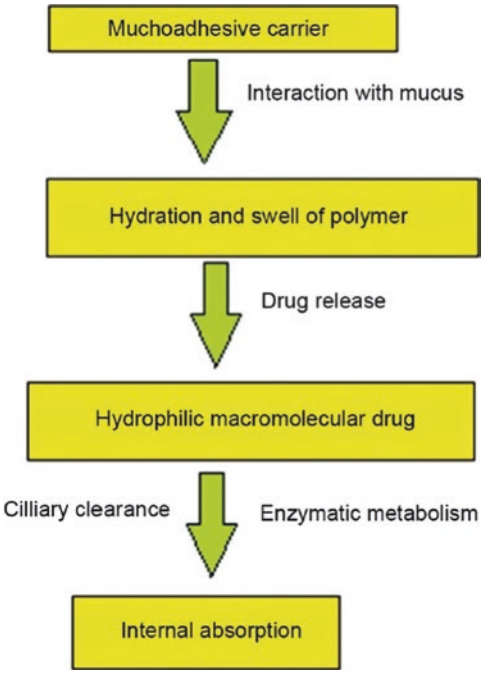

Fig. 5 Schematic mucoadhesive drug delivery for intranasal delivery [18]

methods, including the need for larger doses than delivered via the parenteral route, as well as the presence of degrading enzymes within the nasal cavity [24]. The principle of passing drugs through the nasal mucosa includes consideration of how drugs interact with mucus, can effectively avoid mucociliary clearance, can be effectively released, and can be absorbed (Fig. 5).

Intranasal efficacy for peptides has been achieved at levels comparable to those seen with parenteral peptide administration when using transmucosal delivery agents, including alkylsaccharides [64]. Furthermore, penetration enhancers with or without protease inhibitors offer attractive intranasal delivery methods for peptides [78]. Indeed, chitosan-based delivery methods and alkylsaccharides have both been shown to have applications in the nasal delivery of drugs and peptides in particular [35]. Nanotechnology approaches also hold great promise, despite the lack of clinical breakthroughs in recent years [78]. Chitosan nanoparticle delivery of intranasal peptides appears to maximise the transport of drugs from the nose to brain, compared with simpler chitosan formulations, suggesting that this approach may be worth exploring in the future [15].

Enteral delivery, including delivery through the oral route, has seen similar advances but remains problematic compared with parenteral delivery due to bioavailability issues, the need for higher drug concentrations or dosages, and the issue of degradation. Microemulsions, liposomes, nanoparticles, and microspheres have all been proposed to facilitate delivery of peptides via the oral route [9]. Some of these

strategies have progressed to clinical trial phase and are worthy of greater discussion. For instance, the delivery of insulin using the POD technology approach has been developed by Oramed Pharmaceuticals, Inc. [63], whereby the oral insulin formulation combined with protease inhibitors and absorption enhancers in enteric-coated capsules. This approach is associated with glucose-lowering effects in clinical trials, but safety of the approach needs further validation [83].

3.5 Challenges in Delivery of Peptides

There are multiple challenges to the delivery of peptides for therapeutic effect in the human body. Principally, peptides are easily degraded through enzymatic and chemical processes within the gastrointestinal system, and oral administration of drugs requires significant modification to the formulation to ensure any form of efficacy [34]. The typical route of administration is the use of subcutaneous or intramuscular formulation, which bypasses gastrointestinal enzymes and has a more stable pharmacokinetic profile [31]. However, it is important to note that this route of administration is associated with a range of challenges, some specific to the peptide injected and many ubiquitous across all forms of peptide therapy.

One of the challenges with multidose protein or peptide formulations is the ability to maintain peptide stability and prevent contamination. Most peptide formulations are available in single-dose forms, but multiple-dose forms have the advantage or amenability to dose titration or dose combination. However, preservatives are required within the multidose formulation in order to prevent contamination with microbes and/or microbial growth that may occur during container closure/opening or transient loss of integrity. Bactericidal agents (e.g. 0.1–0.2% phenol or cresol) may be used within the formulation to ensure control of bacterial contamination [23], while specific limitations on the size of the container and the amount of uses permitted can reduce the risk of contamination during use. The amphiphilic nature of peptides encourages adsorption onto materials such as glass, rubber, and plastic, which can reduce the quantity of active materials during processing and storage, and therefore compatibility with primary containers and closures should be evaluated [6, 68]. Specific testing protocols may be applied to determine the effectiveness of stopper mechanisms and/or preservatives, which vary according to geographical region and national standards.

However, it is important to note that the addition of preservatives to the peptide formulation inevitably modifies the stability of the drug. This may lead to product aggregation or precipitation and can affect the shelf-life of the product substantially. Surface binding sites are general finite in nature, and the use of human serum albumin or surfactant agents can effectively prevent active peptide binding during storage, while surfactants may also act to stabilise formulations by preventing denaturation and the tendency of hydrophilic reactions to cause adsorption [39]. Some surfactants can cause reduced stability in peptides, including polysorbate sur-

factants that contain oxidative impurities [6]. Manufacturers should rigorously explore these possibilities and take appropriate remedial actions.

Another consideration which can arise in subcutaneous delivery of peptides is the potential for local toxicity and irritation at the site of application of parenteral peptide therapeutics. Lipohypertrophy is a common complaint among individuals who inject insulin and results from specific effects of insulin on subcutaneous fat (lipodystrophic reactions) that cause a swelling to appear in commonly used injection sites [32]. When these sites are continually used, the absorption of the drug may be erratic, and glycaemic control may be substantially reduced [29, 38]. Although rotation of injection sites and associated patient education is essential in preventing this complication, it is important that device designs are consistent with minimising this risk and optimising drug delivery [32].

4 Conclusion

This chapter has provided an insight into a complex and emerging class of drugs: peptides. Peptide therapeutics is a broad field, and although traditionally dominated by insulin and hormone delivery in states of deficiency, increasingly complex mechanisms are being established through which peptides may exert biological effects. This includes the potential for biologic agents, such as monoclonal antibodies, growth factors, cytokines, and vaccines, all of which can have profound effects on disease courses.

The formulation of peptides remains a complex challenge to maximising the therapeutic potential of these agents. Peptides generally have a poor bioavailability and unstable pharmacokinetics when delivered orally, and they are routinely degraded quickly as part of a natural homeostatic mechanism. Modifications to peptide structure, as well as encapsulation in various devices or delivery methods, can overcome some of these limitations. However, the use of various devices and the development of novel delivery strategies must be cost-effective and should minimise the risk of harm or side effects to the patient.

The delivery of peptides through intranasal, transdermal, intradermal, and oral routes has been achieved in practice, and many delivery methods are being devised to optimise therapeutic effects. In the future it will be vital to optimise delivery strategies to enhance patient adherence and acceptability of therapeutic peptide treatment. Furthermore, the use of nanoparticles and emerging technologies represents a unique opportunity to regulate peptide use in the body and provide a means of achieving modifiable, responsive, and controlled release of peptides over time. This revolutionary approach to drug delivery could minimise the need for repeat administrations while facilitating natural homeostatic mechanisms to release peptides over time. Safety, convenience, and cost all need to be considered in these approaches, as formulation approaches look set to advance with the promise of peptide therapeutics.

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