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Cell-penetrating peptides for transmucosal delivery of proteins

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ABSTRACT

Enabling non-invasive delivery of proteins across the mucosal barriers promises improved patient compliance and therapeutic efficacies. Cell-penetrating peptides (CPPs) are emerging as a promising and versatile tool to enhance protein and peptide permeation across various mucosal barriers. This review examines the structural and physicochemical attributes of the nasal, buccal, sublingual, and oral mucosa that hamper macromolecular delivery. Recent development of CPPs for overcoming those mucosal barriers for protein delivery is summarized and analyzed. Perspectives regarding current challenges and future research directions towards improving non-invasive transmucosal delivery of macromolecules for ultimate clinical translation are discussed.

1. Introduction

The market of protein (comprising 50 or more amino acids) and peptide (consisting of 2–50 amino acids) drugs has rapidly grown in the recent years [1], with an average annual expansion rate of 20%. This growth has surpassed the annual growth rate of 9% for the overall pharmaceutical market and is projected to reach a value of $388 billion by 2024 [2]. Currently, there are over 500 antibodies and 150 peptides undergoing clinical trials, and >70 antibodies and 110 peptides have received clinical approvals in the United States [1,3–5]. This growth is largely dependent on the increased potency, selectivity and half-life of proteins and peptides relative to small molecules, thus bestowing inherent benefits to protein drugs such as enhanced efficacy, reduced side effects, and decreased dosing frequency [6–8]. However, limited by their poor membrane permeability and susceptibility to enzymatic degradation in the gastrointestinal tract (GIT), parenteral administration through the intravenous (IV), intraperitoneal (IP), intramuscular (IM), and subcutaneous (SC) routes, remains the prevailing delivery approach for protein and peptide drugs [9–13]. The physical discomfort, risk of infection, and high cost associated with needle-based parenteral administrations also diminishes patient compliance, especially among individuals with chronic diseases like diabetes that require frequent dosing [14–17].

Transmucosal delivery offers a great alternative for delivering proteins [18,19]. By permeating the mucosal epithelium barriers, protein and peptide drugs can be absorbed through the underneath microvessels [19]. Due to its non-invasive nature, transmucosal delivery offers the option for self-administration, significantly improving the medication adherence [5]. Exubera® and Afrezza® are inhalable insulin approved by the FDA [20]. Intranasal delivery of proteins has been widely studied with several clinically approved products and many ongoing trials. For example, FluMist Quadrivalent (AstraZeneca) [5], an FDA-approved flu vaccine, is a live attenuated viral vaccine vector administered via a nasal spray [21]. Desmopressin, a synthetic peptide analog of an antidiuretic hormone, is now available in multiple nasal spray formulations (Minirin®, Octostim®, and Stimate®) for patients with diabetes insipidus [22–24]. Oral-lyn (Generex), an insulin product currently in Phase III clinical trial (NCT00668850), is a buccal spray formulation administered orally [5]. These transmucosal formulations obviates the need for needle-based injections, thereby improving the ease and comfort for frequent, repeated dosing. However, both inhalable insulin products were withdrawn from the market in 2007 and 2016, respectively, due to the poor sales and high costs. Pulmonary delivery of insulin also poses risks of chronic airway irritation and lung cancer induction [25–27]. The withdrawals of these two inhalation products have impacted research in the field to switch to other sites of transmucosal delivery. Therefore, this review will focus on intranasal, buccal, sublingual, and oral routes.

Despite these aforementioned successful examples, challenges associated with transmucosal delivery remain. For intranasal delivery, proteins must survive degradation by enzymes in the nasal cavity and rapid cilia clearance before they can penetrate the mucus and epithelium into

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the lamina propria for systemic absorption [28]. Additionally, the limited volume for nasal administration (25–250 Ll) restricts the dose that can be delivered through the nasal route [29]. The buccal and sublingual routes are limited by the small absorption surface areas and the salivary washout that shortens the residence time for the mucosal absorption [30,31]. For oral delivery, the low pH in the gastric environment (pH ~1–2), enzymatic digestion, and layered mucosal barrier hinder the absorption of protein drugs, thus resulting in low bioavailability (typically <1%) [12,32]. Efforts to enhance the mucoadhesion or penetration of proteins have prompted the development of various nanoparticles, microneedles, electroporation, viral-based vectors, and smart devices [33–39]. However, these methods have been associated with either low specificity, high toxicity, low efficiency, the possibility to trigger immune response, or discomfort [40–42]. Alternatively, cell-penetrating peptides (CPPs) have been shown to transport cargos into cells through direct translocation or various endocytosis pathways with minimal cytotoxicity [43]. More recently, CPPs have been widely investigated towards facilitating transmucosal drug delivery [44,45]. In this review, we first analyze major challenges associated with the delivery of protein and peptide drugs through intranasal, buccal, sublingual, and oral routes. We then discuss advantages of using CPPs to address various barriers for transmucosal delivery of proteins. Knowledge and research gaps in CPP-based drug delivery are identified and future research directions that will advance this field are discussed.

2. Mucosae barriers

The mucosal interface is comprised of epithelial cell layers overlaid by a mucus stratum, and interspersed across the mucosal interface are degradative enzymes that serve to minimize macromolecule permeation [46–48]. These components collectively constitute the primary physical and biochemical barricades of mucosal environments. The mucus layer is mostly produced by goblet cells, functioning akin to a hydrogel, primarily consists of negatively charged glycoproteins and water. It acts as a filter that impedes the vertical passive diffusion of macromolecules towards the epithelial surface. The mucus can also interact with drug payloads through non-covalent binding, resulting in trapping of the drugs in the mucus layer. The role of the mucus matrix for perpetually clearance of the epithelial surface through enzymatic digestion and mucus shedding constitute the fundamental barrier for drug delivery [49]. Beneath the mucus layer is the epithelial tissue, which is comprised of a continuous sheet of tightly packed epithelial cells that can adopt shapes of varying complexity (from polyhedral to scutoidal to punakoidal) [50,51]. Epithelia are usually separated from the underlying tissues by an extracellular fibrous basement membrane [52]. Epithelia of different mucosal barriers adopt different tissue structures. For instance, epithelium at the upper respiratory tract is described as pseudostratified columnar epithelium, which is comprised of a single layer of cells but nuclei appearing at different heights, suggesting a stratified structure [53,54]. Mouth mucosae, on the other hand, is stratified epithelium that is made of multiple layers of cells [55]. Regardless of the epithelium structure, they typically have little intercellular space. The apical layer of epithelium can also be parakeratinized [56,57]. Additionally, cellular junctions, particularly tight junctions, serve as barriers to the paracellular route, making the epithelium substantially impermeable [58].

The epithelium absorbs molecules through passive diffusion or receptor-mediated uptake. Solely lipophilic compounds exhibit the ability to passively diffuse across the epithelial layers through direct translocation across cells, and paracellular permeation is largely limited to water and minute solutes [59–61]. On the other hand., larger molecules such as proteins and peptides encounter formidable hindrance [62]. This challenge is exacerbated by their hydrophilic nature and substantial molecular weight (>1000 Da), both of which restrict permeation through the tight and highly impermeable epithelium [63–65].

3. CPPs as a minimally disruptive strategy to cross the mucosal barriers

Strategies for enhancing transmucosal delivery of proteins and peptides can be categorized into two primary approaches: (a) Increasing the mucosal retention and permeability and (b) Reducing drug degradation. Utilization of bio-adhesive materials, such as chitosan (CS), Zein, CS-N-arginine/alginate, and microneedles extends the drug residence on the mucosal surface for improved absorption [66–69]. However, these materials could induce mucosal irritation and mechanical damage in epithelium layers [70–73]. Incorporation of penetration enhancers, such as surfactants, in drug formulations is another common approach to enhance drug permeation through mucosae. Nonetheless, safety concerns arise as such surfactants may cause irreversible disruption of tight junctions [74,75]. Covalent modification strategies such as functionalization with polyethylene glycol (PEG) and fusion-protein designs have been explored to increase the stability and solubility of therapeutic proteins. Yet, these modifications carry the risk of altering the payload's activity [76–79]. Smart devices, such as luminal unfolding microneedle injector (LUMI) [80], have also been developed for protein drugs delivery, but such devices are constrained to the oral delivery route as the nasal, buccal and sublingual space may be too small to host these devices. In addition, orally administered smart devices designed to penetrate the gastric mucosa could induce physical defects in the gastrointestinal track (GIT), risking infections and GI ulceration [81]. Nanocarriers present an intriguing option by encapsulating cargos and shielding them from enzymatic digestion. Various nanocarrier formulations offer means to bolster the retention and penetration of drugs [82,83]. However, efficient drug release from these nanocarriers poses a complex challenge that necessitates further optimization [84–86]. Notably, while a substantial body of literature addresses the potential of nanocarriers for protein and peptide delivery, translation of these findings into clinical practice has not been proportionally aligned with the volume of research.

Cell-penetrating peptides (CPPs), encompassing up to 35 amino acids, offer distinct advantages in terms of biocompatibility, membrane permeability, minimal toxicity and reduced immunogenicity compared to other cationic polymers [87,88]. >1850 different CPPs are disclosed in the CPPsite 2.0 database [89] and can be categorized as cationic, amphipathic and hydrophobic peptides [90]. CPPs have become an attractive delivery system due to their high tissue penetration activity for gene and drug delivery via a non-disruptive mechanism. One of the most extensively used CPPs is Polyarginines (Rx), which have been shown to deliver protein drugs, such as R8 for insulin delivery and R7 for cyclosporine A delivery [87,91,92].

4. Mechanisms of CPPs translocation across epithelia

The negatively charged character of the mucus establishes an opportunity for electrostatic engagement by positively charged CPPs, thereby extending the retention period of CPP-functionalized cargoes. Due to their high degree of positive charges from the guanidinium groups, polyarginines effectively bind with the negatively charged oligosaccharide chain of mucins or proteoglycan constituents (e.g. glycosaminoglycans (GAGs) and sialic acids) of the plasma membrane [93,94]. Cellular uptake pathways for CPPs or CPP/cargo complexes are generally divided into paracellular delivery and transcellular delivery [95,96]. For paracellular delivery, CPPs have been shown to open cellular junctions, tight junctions, and adhesion junctions by regulating key proteins such as claudins, cadherins, and desmosomes, allowing for direct diffusion of cargos [10,97–99]. Transcellular delivery can be categorized into direct translocation and endocytosis (Fig. 1). CPPs can directly translocate into cells upon contact through electrostatic interaction or hydrogen bonding between CPPs or CPP/cargoes and the cell membrane. These processes can mediate pore formation or membrane destabilization, permitting for subsequent cargo ingress [100,101].

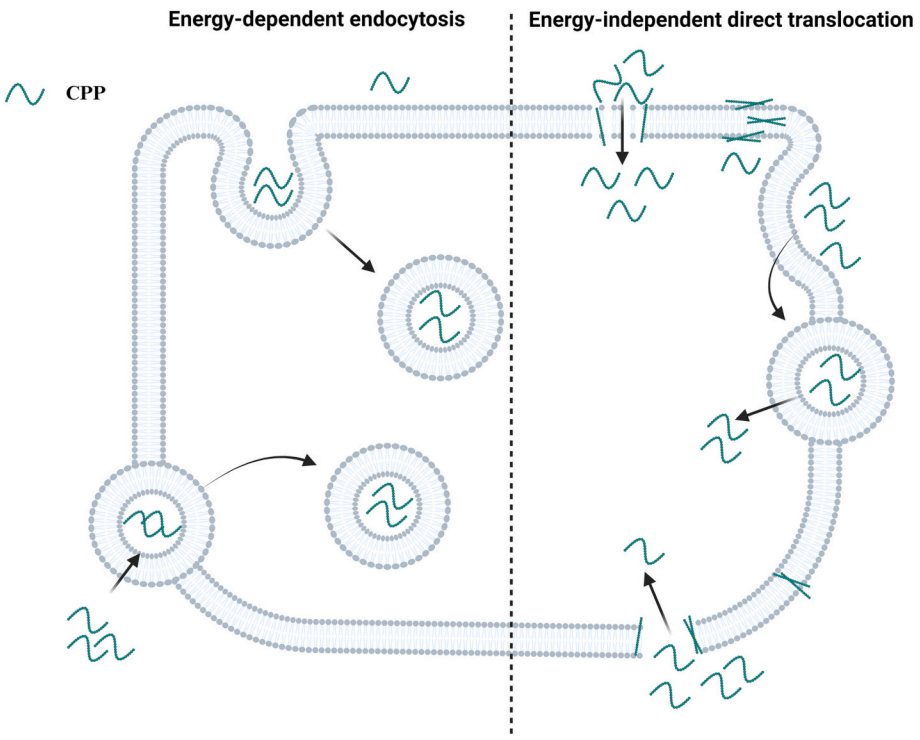

Fig. 1. Schematic representation of proposed mechanisms of CPP internalization.

Notably, the mechanisms behind the direct translocation by CPP-mediated transcellular delivery has been delineated in three distinctive models: (a) CPP-mediated membrane translocation. (b) CPP-mediated membrane pore formation. (c) CPP-mediated endocytosis. Some CPPs are associated with amphiphilic properties that allow them to insert into the lipophilic cell membranes, thereby allowing for direct translocation across the membrane barrier [102,103]. In addition, transient disruption of the lipid layer allows CPPs and associated cargoes to translocate through the compromised cell membrane barrier through passive diffusion [102,104]. Furthermore, CPPs or CPP/cargoes have also been shown to access cellular interior through endocytosis. Notably, energy-dependent endocytosis pathways, such as micropinocytosis, caveolin-mediated endocytosis, and clathrin-mediated endocytosis, predominantly govern the cellular uptake of bulkier CPPs or CPP/cargo assemblies. Among these, macropinocytosis, rooted in receptor-independent and lipid raft-dependent mechanisms, emerges as a favored endocytic avenue for CPPs in tandem with substantial cargoes [102,105].

5. CPP-mediated delivery of protein drugs

CPPs offer a versatile approach for enhancing delivery of peptides and proteins across biologically impermeable barriers. CPPs can be linked to various drugs through covalent or non-covalent associations, enabling them to facilitate the crossing of cellular membranes via mechanisms described above [106,107]. CPPs, when conjugated to macromolecules, have been shown to enhance the membrane permeability of the associated cargo. For instance, an 11-amino acid, positively charged peptide sequence derived from the transactivator of transcription (TAT) protein of HIV-1, has been engineered with various peptides and proteins to enhance their cellular penetration [108–110]. A notable example lies in D-penetratin mixed with insulin, which has been demonstrated to cross the GI mucosa and elevate the bioavailability of orally administered insulin [111]. Similarly, L-penetratin-insulin, administered intranasally, has been shown to overcome the nasal epithelium barrier to exert insulin's activity in blood glucose (BG) control [112]. Some CPP sequences have been identified to target specific sites, such as RGD for targeting αvβ3 integrin widely expressed on tumor cells [113]. As covalent linking of CPPs and cargoes could potentially disrupt the drugs' inherent functions, payload incorporation through physical mixture with CPPs has been explored for transmucosal delivery. Cargo association with CPPs during physical mixing is dependent on intermolecular interactions, such as hydrophobic and electrostatic interactions and hydrogen bonding [114,115]. Numerous studies have demonstrated in vivo transmucosal delivery of proteins admixed with CPPs. The efficacy of absorption is influenced not only by the binding efficiency between CPP and cargo but also by other factors such as the internalization efficiency of CPPs [90,116]. Fusion of CPPs with therapeutic proteins has also shown promise. Researchers have fused paraoxonase1 (PON1) proteins with a cell permeable peptide (PEP-1), which facilitates the cellular transduction to counteract oxidized-LDL-associated inflammation, a hallmark of Parkinson's disease [117]. However, the fusion method usually requires sophisticated biochemistry process [118].

The realm of nanocarriers has also been harnessed for peptide and protein delivery, capitalizing on their ability to shield encapsulated therapeutics from enzymatic and chemical degradation for sustained release. Incorporating CPPs onto the surface of nanocarriers enhances their interactions with mucus or epithelial layers, thus extending their retention time. For instance, Keum et al. [119] have employed CPP-decorated liposomes to bolster drug absorption across the buccal mucosa. CPPs can be physically combined with nanocarriers and protein complexes via layer-by-layer complexation. Typically, cationic CPPs would be deposited at the outermost layer to facilitate nanoparticulate penetration into cells [120,121]. Recent advances with specific examples are discussed in the following section.

6. CPPs for intranasal delivery

6.1. Nasal structure

The nasal cavity encompasses three distinct regions: the vestibular

region, respiratory region, and olfactory region. These segments are responsible for filtration, humidification, temperature regulation, and olfaction [122]. In humans, the vestibular region spans approximately 0.6 cm^2, the respiratory region occupies an extensive area of 150 cm^2, and the olfactory region covers around 10 cm^2 [123]. The substantial surface area, coupled with the presence of vascularized subepithelial tissues, facilitates absorption of molecules into the systemic circulation [124]. The nasal route features an intranasal pathway characterized by a pH range of 4 to 6.5 and avoids the first-pass metabolism, thus amplifying the bioavailability of drugs [125,126]. An additional advantage of the nasal route is its high accessibility, enabling patients to engage self-medication [127].

6.2. Advantages of nasal delivery for protein and peptide drugs

Utilizing the nasal route for the delivery of protein and peptide drugs holds the advantage for achieving a more rapid onset of action, typically within minutes, compared to other non-parenteral administration methods [129]. The respiratory mucosa is highly vascularized with a large surface area and permeable thickness (0.3-5 mm) [130], which are conducive to drug absorption. Lower enzymatic degradation in the nasal cavity compared to GIT also makes nasal route attractive for biologics delivery [131]. In addition, intranasal delivery enhances patients' satisfaction to increase medical adherence and serves as a viable for drug delivery to the brain (Fig. 2) [132]. Studies have shown that CPP-mediated intranasal delivery results in higher bioavailability of protein drugs and can be achieved with no toxic effects on biological membranes [133]. For instance, L-penetratin is a widely adopted CPP for intranasal delivery, which has been demonstrated to improve the bioavailability of insulin, glucagon-like-peptide-1 (GLP-1), and exendin-4 [112,134,135].

6.3. Intranasal barriers for drug delivery

Transmucosal delivery of protein and peptide drugs via the nasal route is faced with several formidable barriers inherent to the nasal epithelium. Firstly, the mucus layer (10–15 μm thick) presents a substantial obstacle by filtrating protein and peptide drugs, impeding their journey towards the intended site [136]. Although degradative enzymatic activity within the mucus is less than that in the GIT, it remains a non-negligible factor that compromises drug integrity [137]. In addition, the epithelial cells on the nasal surface bear hair-like cilia that undergo rhythmic movements, a process termed mucociliary clearance with a half-life of approximately 7–15 min. This dynamic clearance mechanism results in the significant loss of administered drugs [138,139]. The limited physical space in the nasal cavity also imposes a constraint on the applicable drug dosing [140,141]. Beyond these initial challenges, a multilayered epithelium awaits [141,142]. Proteins and peptides must traverse through either the transcellular or paracellular pathways in order to reach the ultimate destination, lamina propria, for systemic absorption.

6.4. CPP-mediated intranasal delivery of protein and peptide drugs

Intranasal delivery of peptide and protein drugs via nasal spraying has emerged as a viable and effective therapeutic approach for various medical conditions, such as diabetes insipidus, osteoporosis, and central precocious puberty [143–145]. To amplify the efficiency of intranasal delivery, CPPs have been integrated to serve as facilitators for enhanced penetration of peptide and protein drugs across mucosal layers. A variety of CPP-based delivery technologies have been developed for intranasal delivery of proteins. For instance, Akita et al. [146] demonstrated that CPP conjugation effectively safeguards GLP-2 from degradation, expediting its transit from the nasal route to the brain. Driven by the versatility of nanoparticles for payload incorporation and surface functionalization, researchers have employed nanoparticles to enhance the protection, solubility, stability, and bioavailability of peptide and protein drugs. CPPs have been linked to the surface of a broad variety of nanoparticles, including liposomes, polymeric nanoparticles, and micelles, to augment the mucus-penetrating capability. Eliete et al. [147] demonstrated that the CPP-modified liposome-encapsulated peptide drugs exhibited a prolonged release profile, and this coupling notably bolstered nanoparticle absorption. Despite the advantages offered by nanocarrier designs, physically mixing CPP with cargo remains the preferred approach because of formulation simplicity and minimal payload alteration. By mixing CPP with insulin, Bae et al. [148] showcased effective intranasal absorption of insulin, resulting in significant reduction in BG levels in diabetic rats. Khafagy et al. [149] demonstrated that the co-administration of CPP with leptin through the nasal route notably amplified the leptin concentrations both in the systemic circulation and within the anterior brain. Other peptides, such as exendin-4 and GLP-1 have been delivered through a physical mixture with CPPs. Examples of intranasally administered CPPs include TCTP-PTD, penetratin, and their variants [112,150,151]. A summary of intranasal formulations involving CPPs is shown in Table 1. It is noted that additional procedures to animals were required in these studies

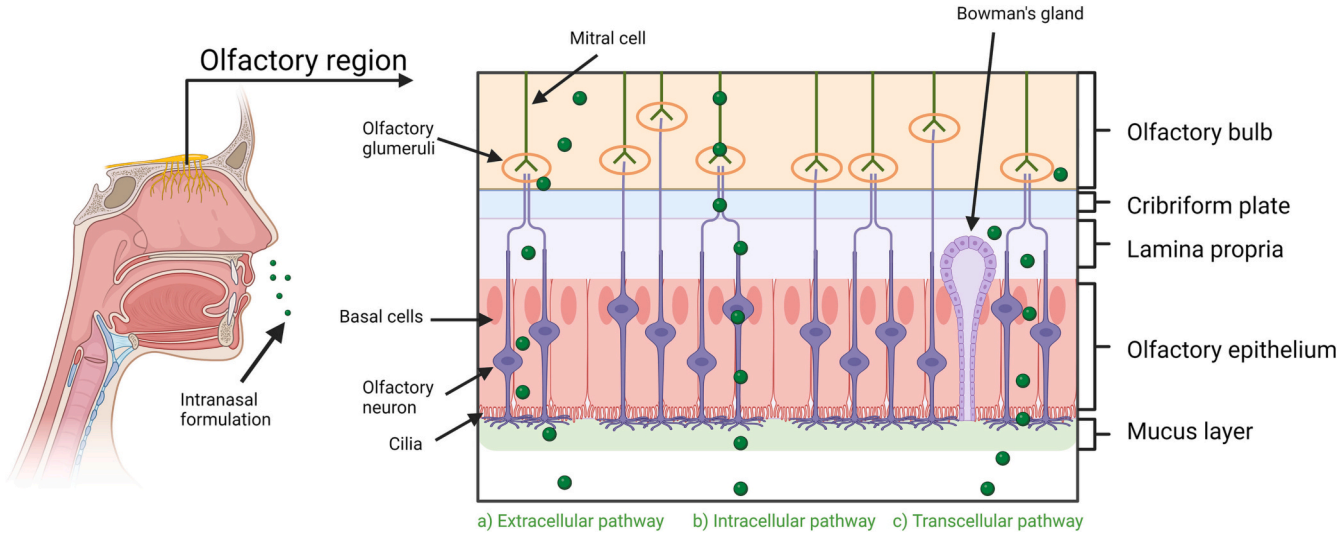

Fig. 2. Schematic representation of various possible mechanisms involved in intranasal delivery and direct nose-to-brain drug transport from the olfactory region [128].

Table 1
Intranasal formulations of CPP-containing protein drugs in preclinical research.

Cargo	CPPs	Methodology	Extra procedure for intranasal delivery	Results	Reference
dnRas	TAT	fusion protein	nasal catheter insertion to increase pulmonary delivery and reduce dosage clearance through nasal exhalation	TAT-dnRas effectively reduces eosinophil migration, airway inflammation, and hyperresponsiveness in immune-sensitive mice.	[153]
insulin	TCTP-PTD 13	simple mixing	anesthesia	Enhances nasal insulin absorption with a relative pharmacological bioavailability (BA) of 21.3% (compared to s.c.) without causing mucosal damage. BG control for ~2 h	[150]
insulin	TCTP-PTD 13 M2	simple mixing	anesthesia	Enhances intranasal insulin delivery with a relative BA of 37.1% (compared to s.c.). BG control for ~4 h.	[154]
insulin	L-penetratin	simple mixing	anesthesia and cannulation of trachea and esophagus	Improves nasal insulin absorption with up to 76.7% pharmacological availability (PA) and 50.7% BA (compared to s.c.). BG control for ~2 h.	[112]
insulin	shuffle (R, K fix) 2	simple mixing	anesthesia and cannulation of trachea and esophagus	Enhances nasal insulin absorption by 1.85-fold compared to the original penetratin. BG control for ~2 h	[155]
insulin	penetraMax	simple mixing	anesthesia and cannulation of trachea and esophagus	Achieves almost 100% relative BA (compared to s.c.). No BG results reported.	[151]
insulin	protamine	simple mixing	no additional procedure required	Promotes intracellular uptake, directs the cargo to nuclei instead of lysosomes. The BA is 28.6% compared to s.c. BG control for ~6 h	[156]
exendin-4	TCTP-PTD 13 M2, TCTP-PTD 13 M3, penetratin, shuffle (R, K fix) 2	simple mixing	anesthesia and cannulation of trachea and esophagus micropipette insertion	Penetratin increases the nasal absorption of exendin-4 (7.7% BA) more than the intestinal (1.8% BA) absorption. Shuffle (R,K fix) 2 significantly enhances nasal absorption of exendin-4 compared to L-penetratin. TCTP-PTD 13 M2 shows the highest exendin-4 uptake in normal rats, and decreases BG levels by 43.3% (compared to exendin-4 alone) and by 18.6% (compared to exendin-4 plus TCTP-PTD 13) in diabetic mice.	[134,135,157]
GLP-1	penetratin, shuffle (R, K fix) 2	simple mixing	anesthesia and cannulation of trachea and esophagus micropipette insertion	Penetratin significantly increases the nasal absorption of GLP-1 (15.9% BA) more than the intestinal (5% BA) absorption. Shuffle (R,K fix) 2 significantly enhances nasal absorption of GLP-1 compared to L-penetratin.	[134,135]
GLP-2	R8	fusion protein	anesthesia	PAS-CPP-GLP-2 prevents degradation of GLP-2, enhances cellular uptake and efficiently delivers to the CNS, demonstrating rapid antidepressant-like effects comparable to i.c.v. administration in mice.	[158]
recombinant influenza hemagglutinin (HA)	CPE	covalent	anesthesia	Conjugating CPE to recombinant HA induces increased mucosal IgA responses in mice but does not affect the systemic IgG responses.	[159]
leptin	penetratin	simple mixing	anesthesia and cannulation of trachea and esophagus micropipette insertion	Intranasal coadministration of leptin with L-penetratin efficiently delivers leptin to the brain, reducing appetite, body weight gain, and plasma triglyceride levels in rats.	[160]
IFN-beta	penetratin	simple mixing	anesthesia and cannulation of trachea and esophagus micropipette insertion	Penetratin increases the nasal absorption of IFN-beta (11.1% BA) more than the intestinal (0.17% BA) absorption.	[134]

involved with intranasal delivery to enhance the absorption. Animals were anesthetized during the drug administration to transiently block swallowing and removal of drug formulation through nasal exhalation, allowing prolonged nasal retention and increased delivery to the lower respiratory tract, such as lungs [152]. To further increase the nasal retention of the drug formulations, the trachea and esophagus were cannulated during the nasal administration. These procedures cannot be translated clinically, and the need of them reflects the suboptimal efficiency of these CPP formulations. Very recently, Wu et al. [10] demonstrated that protamine, a clinically used arginine-rich peptide as an antidote for heparin overdose, facilitated systemic absorption of bovine serum albumin (BSA) and insulin by nasal administration using the physical mixing formulation in conscious animals, without any additional procedures. This formulation effectively reduced the BG in diabetic mice 0.5 h after administration, and the effect lasted for ~6 h, comparable to s.c. injected insulin at the same dose. This is a significant breakthrough in this field, and the promising results may be due to that protamine could bypass the cellular lysosomal degradation [10], while payloads delivered by other CPPs such as R8 were mostly trapped in the lysosomes.

In summary, the integration of CPPs into intranasal drug delivery strategies stands as a promising avenue to amplify the permeation of peptide and protein drugs across the mucosal layers. These techniques, as exemplified by the aforementioned studies, hold potential to optimize therapeutic outcomes and broaden the spectrum of treatable conditions.

6.5. *Challenges and future prospect of CPP-mediated intranasal delivery*

Despite the promise presented in multiple preclinical studies, it should be noted that the nasal cavity is a highly sensitive area that can rapidly accelerate mucus build-up and shedding upon irritation. Such alteration can vastly impact the pharmacokinetic of intranasally administered drugs, and the interplay between drug dosing and nasal irritation should be carefully examined. In addition, the aforementioned barrier factors can be complicated by the influence of nasal pathologies such as rhinitis and the common cold. Potential efficacy reduction among subjects with respiratory illness should be carefully assessed for CPP-formulated intranasal proteins. More efforts should be made to

simplify the intranasal delivery procedure to improve patient compliance and reduce efficacy variation.

7. CPP for buccal and sublingual delivery

7.1. Buccal and sublingual mucosa structure

The epithelial composition of the oral mucosa varies by regions of the mouth. Approximately 40% of the surface area of the oral cavity consists of keratinized squamous epithelium, which covers the hard palate and gingiva and is tightly bound to the bone underlying these tissues [161–164]. In these regions, squamous cells in the apical stratum corneum layer possess a proteinaceous coating reinforced by the keratin filaments they are linked to, which creates a more rigid layer designed to protect the underlying tissue. Beneath the basement layer of this keratinized epithelium sits the loose areolar connective tissue of the lamina propria, containing capillary loops that drain into the jugular vein [165,166].

The stratified, non-keratinized epithelium of the buccal and sublingual mucosa makes up the remaining 60% of the cavity's surface area. In these regions, permeation is much more feasible due to the lack of keratin barrier. These areas are therefore highly suited for drug absorption and present an intriguing delivery route of choice [167,168].

Although their epithelium is quite similar, the buccal and sublingual mucosa differ in size and thickness: the buccal epithelium is generally 400–700 μm thick spanning 40–50 cell layers, totaling roughly 50 cm^2 of surface area [164]. Meanwhile, the sublingual mucosa is thinner, averaging only 100–200 μm thick across 8–12 cell layers, comprising roughly 27 cm^2 of surface area [166] (Fig. 3 and Table 2).

7.2. Advantages of buccal and sublingual delivery of protein and peptide drugs

There are several advantages that buccal and sublingual delivery methods offer over other routes of administration due to the physiology of the oral cavity. The pH of this environment generally ranges from 6.5 to 7.5, which protects proteins from acid-catalyzed denaturation. Combined with the decreased presence of enzymes that may degrade the cargo being delivered, the buccal and sublingual routes offer a desirable environment for drugs than typical oral administration that subjects biologic payloads to the gastric cavity [164].

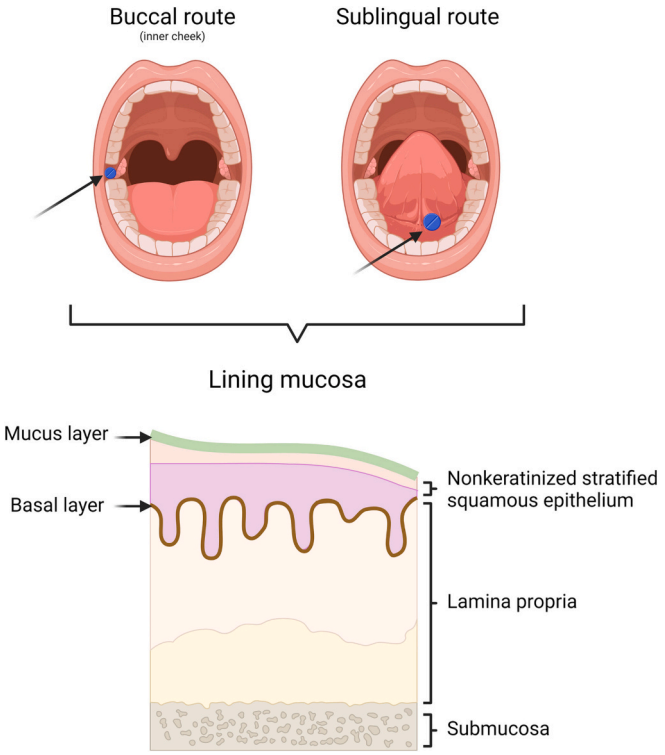

Fig. 3. Sketch map of the oral mucosa.

Absorption of drugs across the buccal and sublingual mucosa is also aided by the high level of vascularity in these tissues. The vast capillary beds of this region that drain into the jugular vein offer quick access to systemic circulation. This direct entry to the bloodstream allows drugs to bypass first-pass metabolism to maximize bioavailability [166,169].

The quick absorption and low degradation presented by these routes is particularly useful for a wide array of applications, including administration of drugs required in emergent situations. Furthermore, due to the non-invasive nature of the buccal and sublingual routes, administration of protein-based vaccines via non-invasive buccal and sublingual routes could lead to higher rates of acceptance and coverage [164]. Mucosal vaccination also induces mucosal immunity, which offers improved protection against pathogens invading through the mucosal route [167,170].

7.3. Buccal and sublingual barriers to drug delivery

Although the non-keratinized epithelium of the sublingual and buccal mucosa is more conducive to drug absorption, several layers of barriers remain for proteins to move from the oral cavity into the systemic circulation. In order to reach the capillary beds in the lamina propria, drugs must be able to traverse the mucosal layer through either transcellular or paracellular mechanisms [171]. In particular, paracellular routes require drugs to overcome anchoring junctions, such as adherens junctions and desmosomes, that tightly bind cells in the superficial layers of the epithelium. Since these junctions are highly present in tissues that undergo frequent and significant mechanical stress, this issue is particularly relevant to the mobile epithelium of the buccal and sublingual regions [172,173]. Additionally, washout from salivary secretion can significantly limit the residence time of drugs in the oral cavity. In general, saliva is secreted at a rate of 0.5–2.0 L per day, with roughly 1 mL being maintained in the oral cavity at all times [174,175]. This frequent refreshment of saliva poses challenges to adhesion and retention for molecules that are larger and less lipophilic, limiting their ability to be absorbed into the mucosal layer before being diluted in new saliva and swallowed. In addition to this, although the buccal surface area is larger than that of the sublingual region, the decreased drug retention in this area due to salivary dilution and swallowing poses an additional challenge to drug delivery [176].

7.4. CPP-mediated buccal and sublingual delivery of protein and peptide drugs

Since salivary washout significantly limits the time available for drug absorption across the sublingual and buccal mucosa, enhancing quick absorption is key to shuttling proteins and peptides across these oral mucosal barriers. CPPs have been found to dramatically elevate the bioavailability of drugs delivered through this route, as they allow for significantly increased penetration of cargo through mucosal barriers to the underlying blood vessels. For instance, Xu et al. [177] explored the ability of insulin-conjugated low molecular weight protamine for buccal uptake in rabbits, finding that the hypoglycemic effect was significantly increased with the conjugate as compared to the group treated with buccal insulin alone. In another example, Keum et al. [119] examined CPP-mediated buccal absorption with penetratin, conjugating the CPP to phospholipids and forming liposomes to deliver salmon calcitonin (sCT). It was found that their liposomal formulation increased sCT permeation across the porcine buccal tissue by 92-fold, highlighting CPP-functionalized carriers as a valuable strategy for increasing buccal uptake.

Table 2
Characteristics of buccal and sublingual mucosa [166].

Tissues	Keratinization degree	Thickness/ μm	Surface area/cm^2	Permeability	Residence time	Blood flow velocity/ml*min-1*100 g^{-1}
Buccal mucosa	Non-keratinization	500–600	50.2 ± 2.9	Medium	Medium	20.3
Sublingual mucosa	Non-keratinization	100–200	26.5 ± 4.2	High	Low	12.2

7.5. Challenges and future prospect of CPP-mediated buccal and sublingual delivery

Facilitating quick absorption to overcome the frequent refreshment of saliva is the primary challenge facing transmucosal delivery in the oral cavity, and CPPs offer a promising avenue to increase absorption of drugs. Covalent linkage of CPPs to payloads as well as to biologic-loaded nanocarriers have been shown to increase buccal uptake [178,179]. The field of CPP-mediated transmucosal delivery in the oral cavity remain relatively underexplored, and significant potential remains. Because of the quick absorption associated with the buccal and sublingual routes, this mode of delivery may be tailored for emergency syndrome such as acute allergy reaction.

8. CPP for oral delivery

8.1. Oral route structure

The stomach, small intestine, and colon are the main locations in GIT that absorb orally ingested protein. The length of human GIT can be up to 9 m, containing segments with large variations in object transit time, pH, degradative enzymes, and mucosal layer composition (Fig. 4) [180–182]. These variations and complexities present design challenges for orally ingested drug formulations. In a healthy adult, the transit time in the stomach is around 1 h, and the environment is highly acidic (pH 1–2), rich in gastric enzymes, and surrounded by thick mucus layer with relatively low surface area (0.05m^2) [183–185]. The stomach is characterized by fast epithelial regeneration and it is a rapidly accessible site [38,186]. The small intestine has the highest surface area in the GIT (32 m^2), averaging 6 m in length, and is rich in microvilli structures [187,188]. Its near-neutral pH of 6.5–7 and long turnover time (4–6 h) establish it as the primary organ for drug and nutrient absorption [189]. Furthermore, the thickness of mucus in the small intestine varies from 15 to 450 μm [28], which provides a lot of flexibility for oral formulation designs. Lastly, the colon is the final absorption segment in the GIT. It has a pH of 5–7 and a surface area of 2 m^2. Due to its long transit time (~20 h), lower fluid volume, and minimal digestive enzymes, the colon is another major target for oral drug absorption [189].

8.2. Advantages of oral delivery for protein and peptide drugs

The convenience of oral drug delivery makes this route particularly

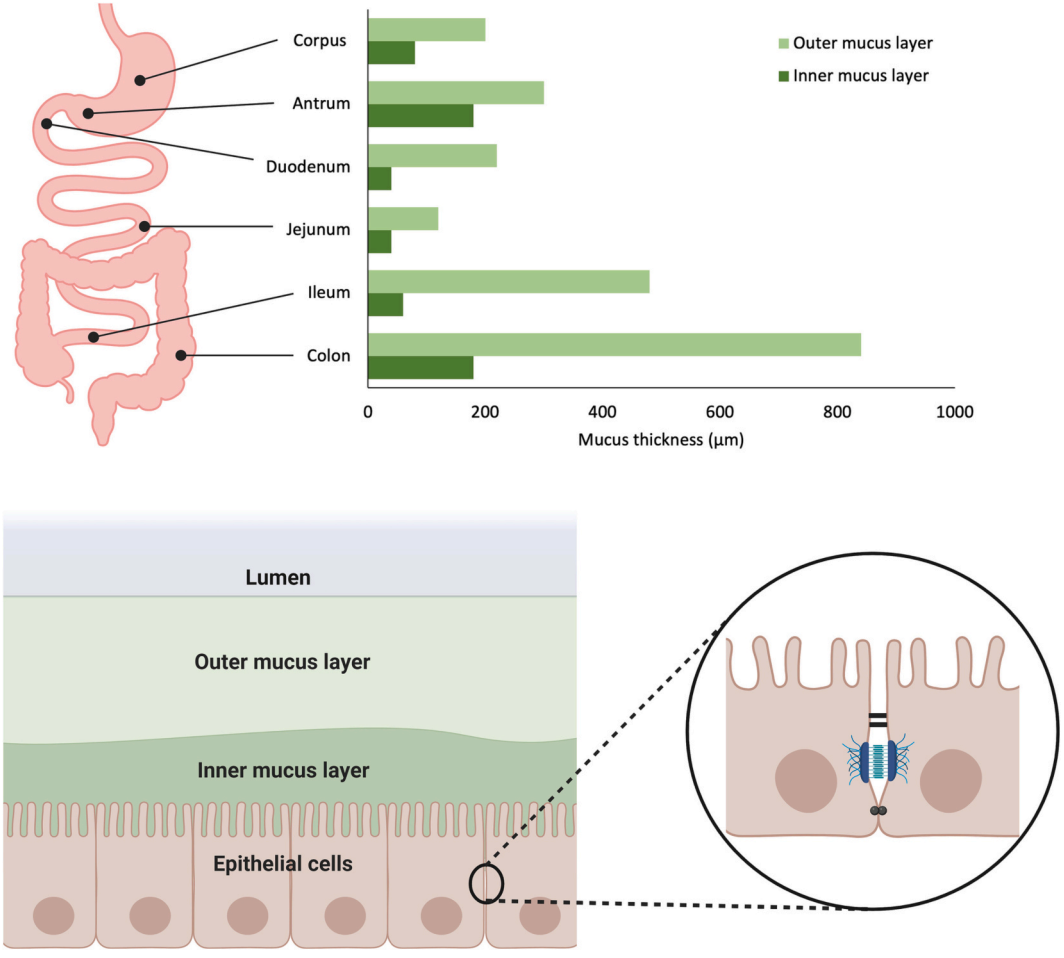

Fig. 4. Schematic illustration of the mucus layers covering GIT. The mucus depth varies with GIT sites. The mucus layer is composed of two layers, including the outer mucus layer, which is loosely adherent and the inner mucus layer, which is firmly adherent on epithelia [81].

attractive for patients as compared to parenteral injections. Ouyang et al. [28] reported that over 62% of pharmaceutical products in 2018 are administered via oral route. The recent FDA approval of an oral formulation of the glucagon-like peptide 1 (GLP1) receptor agonist semaglutide for the treatment of type 2 diabetes (T2D) is a major milestone and landmark in orally delivered protein therapeutics. The oral protein and peptide therapeutics market is anticipated to grow to 8233 million USD by 2028 [190].

8.3. Oral barriers to drug delivery

The lining of the GIT has evolved a sophisticated set of cellular and mucus barriers to restrict the access of protein drugs. The GIT is characterized by highly denaturing environments, a plethora of digestive enzymes, and enteral microbiota and pathogens, which collectively make transmucosal biologics delivery challenging [191–194]. In particular, unanticipated disruption of the GIT mucosal barrier or microbiota can have profound influence on the subject's health and well-being [195,196].

8.4. CPP-mediated oral delivery of protein and peptide drugs

Oral delivery strategies for protein and peptide drugs can be divided into 3 distinctive categories, including improving the oral route retention time, enhancing permeation, and combating enzymatic degradation. Among these strategies, CPPs function primarily by enhancing intestinal permeation of protein and peptide payloads. CPPs may interact with membrane glycosaminoglycans located on the enterocyte surfaces of microvilli, traversing into the cells through endocytic pathways, and ultimately delivering their protein cargos to the systemic circulation upon exocytosis. Degradation of CPPs and payloads in the GIT is the major hurdle when using CPPs to facilitate oral drug absorption. For example, the stability half-life of penetratin in the rat intestinal fluid was only 0.7 min [197]. Therefore, early studies focused on direct injection of a mixture of CPPs and payloads into the intestinal lumen to enable effective absorption (supplementary table 1). Alternatively, CPPs and their payloads can be encapsulated in nanoparticles or specific devices to reduce the degradation and denaturation in the low pH gastric fluid before the release in the intestine or colon for absorption. PEG-modified mesostructured silica nanoparticles have been used to protect CPPs and protein payloads while enhancing their retention and penetration in the intestinal mucosae [197]. CPPs released in the gut lumen can facilitate drug penetration through the mucosae for systemic absorption, or nanoparticles may penetrate into the mucosae before releasing CPPs and payloads. In another case, Guo et al. [198] encapsulated insulin and a CPP in PLGA nanoparticles that were grafted with modified chitosan. This formulation survived the environments in the stomach and intestine, but was digested by colon flora to release the CPP and insulin for systemic absorption, leading to a 40% reduction in BG levels. Alternatively, CPPs can be attached to nanoparticle surface, and Tan et, al. [197] showed that this approach enhanced the mucus-penetrating ability and achieved a 5-fold increase in bioavailability following oral delivery. However, it is to be noted that CPPs attached on particle surface could be susceptible to degradation by enzymes in the GIT [12].

Nielsen et al. [111] was the first group showing that a CPP enabled oral absorption of a peptide through a physical mixing formulation without nanoparticles or any device. They first demonstrated that D-penetratin was more stable in the diluted gastrointestinal fluid compared to L-penetratin, with a stability half-life of 67 min. D-penetratin bound with insulin via non-covalent intermolecular interactions and increased its stability half-life from 25 min to 91 min. However, the efficacy was only moderate with a PA of 18.2% compared to s.c. insulin. A summary of oral formulations involving CPPs is shown in Table 3.

Table 3
Oral formulations of CPP-containing protein drugs in preclinical research.

Cargo	CPP	Methodology	Results	Reference
insulin	polyarginine $[R]_6$	P(MAA-g-EG) microparticles	These hybrid hydrogels efficiently load insulin and R6, show rapid release properties at pH 7.4, and cause a substantial hypoglycemic response when administered in rat ileal segments.	[199]
insulin	SAR6EW	chitosan (CS)-based nanocarriers	Orally administered SAR6EW/CS/insulin-NPs exhibit superior hypoglycemic effects compared to CS/insulin-NPs in diabetic rats.	[200]
insulin	LMWP	mucoadhesive NPs (MNPs)	MNPs loaded with Insulin-LMWP conjugates demonstrate a long-lasting hypoglycemic effect with a faster onset and a PA of 17.98% (compared to s.c. insulin injection) in diabetic rats.	[201]
insulin	D-penetratin	simple mixing	A PA of 18.2% is achieved in mice dosed with insulin and D-penetratin.	[111]

8.5. Challenges and future prospect of CPP-mediated oral delivery

A major challenge in CPP-mediated oral proteins delivery lies in the acidic and degradative environment in GIT, to which CPPs are highly susceptible. Carrier technologies are often required to shuttle CPP-functionalized proteins and peptides past the gastric cavity, and sustained release in the small intestine or colon allows CPPs to mediate enhanced cargo transcytosis. Although significant efficacy has been demonstrated with the above approach, optimization of drug loading and release kinetics remains to be a major task. The success of LNP in COVID-19 mRNA vaccine delivery is speeding up the developmental efforts in nanocarrier engineering. Combining carrier technologies with CPPs for oral biologics delivery presents a promising prospect for future translation. CPP formulations based on physical mixing is preferable; however, this approach tends to only facilitate absorption of payloads that have strong interactions with CPPs. It is possible that payloads could dissociate from the complexes with CPPs in the presence of food and electrolytes [111], and this is yet to be examined. CPP stability in the GIT can be improved through sequence optimization, which is crucial to enable efficient oral absorption.

8.6. Immunological issues of CPPs

Most of the research on CPPs conducted in laboratory settings indicated their general nonimmunogenicity and low toxicity [202]. However, a more thorough investigation, especially involving in vivo, is necessary due to the positively charged nature and origin of most CPPs, which may impact cell membrane integrity and potentially trigger immune responses. The immunogenicity of CPPs can be influenced by various physicochemical properties, including molecular weight, charge, amino acid sequence, hydrophilicity, morphology, and the type of conjugated cargo [202].

In rare occasions, the immune response to CPPs may occur when they

are recognized as antigens and taken up by immune cells, such as dendritic cells (DC) [203]. Most well-characterized CPPs consist of positively charged amino acid sequences, and under normal conditions, their electrostatic interaction with GAGs facilitates the internalization of cargo into the cytoplasm [204]. This process, along with the toxicity of CPPs, is dependent on the dosage administered [205]. CPPs do not facilitate steps like proteolytic processing, major histocompatibility complex (MHC) presentation, and T-cell receptor (TCR) binding for a successful T cell response [206]. Therefore, they normally do not cause immunotoxicity when administered alone within an optimized dosage.

In vivo experiments have shown that certain CPPs, like D-form arginine oligomer (R9) and Penetratin, can cause severe systemic toxicity at high doses (5 μmol/kg for R9 and 10 μg for Penetratin) [207]. Penetratin, in particular, influences the innate immune system through interaction with the Toll-like receptor pathway [207]. Amphipathic sequences exhibit greater toxicity on cell metabolism compared to cationic ones, but modifications, such as reducing the hydrophobicity or the overall cationic charge, have been successful in reducing cytotoxicity and immunotoxicity in vitro and in vivo [205,208,209].

In another aspect, CPPs have been used as immune-enhancers, and low concentrations of CPPs (e.g. 2 mM for Penetratin) have been used to deliver nasal vaccines [210]. Studies have shown that CPPs promote DC uptake of antigens, enhance vaccine effectiveness in various animal models including nonhuman primates, and improve the antitumor efficacy of cancer vaccines [206]. Recent studies showed that CPPs increased intracellular delivery of antigens into antigen-presenting cells (APCs), leading to enhanced T cell priming in vivo, predominantly through cross-presentation [206].

Coralie and colleagues [206] noted a significant enhancement in antigen accumulation in draining lymph nodes when delivered by CPP formulations. This outcome was linked to the CPP's ability to bind to lymph-trafficking lipoproteins and safeguard the CPP-antigen from degradation. These dual effects led to an extended presentation of the CPP-peptide in draining lymph nodes, resulting in robust priming and expansion of T cells. The combined impact of improved antigen stability, augmented lymph node accumulation, and effective uptake by APCs contributes to the prolonged presentation of antigens following CPP-peptide immunization.

Overall, CPPs alone without an antigen are unlikely to cause immunotoxicity. However, dosage optimization of CPPs is needed.

8.7. Clinical trials for CPP-based drug delivery

Although clinical translation of transmucosal delivery of proteins using CPPs remains in the infancy, a few local and systemic delivery systems derived from CPPs have entered clinical trials (Table 4). While most of the CPP clinical trials focus on systemic delivery, localized applications of CPPs appear to be more clinically successful with one product (DAXI) approved. The first CPP clinical trial was topically delivered cyclosporine linked with polyarginines by CellGate for the treatment of psoriasis [204]. This topical treatment appeared to be well tolerated without systemic adverse effects. In 2016, Revance Therapeutics released its Phase 3 trial results of Daxibotulinumtoxin Topical Gel (RT001) based on a TATp technology (TransMTS™) that enables topical delivery of botulinum toxin to treat patients with moderate to severe lateral canthal lines or cow feet. DaxibotulinumtoxinA-lanm for injection (DAXI) is the first FDA-approved CPP-containing product, in which the CPP, RTP004, binds noncovalently with the cargo, botulinum toxin type A. RTP004 is a 35 amino acid peptide rich in lysine and arginine, which improves the formulation stability by reducing self-aggregation of the cargo and facilitates the drug penetration [211]. DAXI is used to treat frown lines and cervical dystonia in adults by local injection [211]. Although CPP technologies have mostly been applied for protein delivery via systemic administration, with the first trial conducted in 2003, no product in this category has received regulatory approvals [114], to our best knowledge. Additionally, safety concerns about systemic exposure of CPPs have been raised, as cationic CPPs non-specifically bind with blood components and cell membranes via charge-charge interactions, which could induce immune responses and cytotoxicity. Local administration of CPP formulations, on the other hand, minimizes the systemic exposure.

8.8. Future prospect

Needle-free delivery of biological drugs provides a non-invasive alternative to patients and is particularly attractive for chronic health conditions that require frequent and long-term medications. This mode of delivery increases medication adherence and reduces the cost. The approval of nasal Foralumab [212], oral semaglutide [213], and inhalable insulin attests this need. CPP delivery has enabled approvals of a protein drug [211], and its use for transmucosal delivery has advanced significantly over the past few years. This relatively local mode of delivery is anticipated to minimize systemic toxicity of CPPs, which has been the major concern for their clinical translation. We anticipate that products enabled by CPP delivery through transmucosal routes will become ready for clinical trials in the near future. To facilitate the clinical translation, thorough toxicology studies in larger animals such as dogs and non-human primates must be conducted with CPP formulations. Immune responses triggered by CPPs, if any, need to be studied. Under this consideration, CPPs that have been used in human with an established safety profile will be highly favorable. For example, protamine has been used clinically for many years as an antidote for heparin overdose as well as an excipient to prepare NPH insulin for sustained BG-lowering effect via s.c. delivery.

Efforts on developing CPP formulations for transmucosal delivery have been mainly devoted to small proteins (Mw $<$ 6 kDa) such as insulin and GLP-1 agonist. Improving the delivery of large protein drugs remains a topic of interest in the field, as more and more large protein drugs are approved such as mAb (150 kDa) and antibody-drug conjugate (ADC). Transmucosal delivery efficiency of CPPs must be improved to mediate transport of these large molecules. Sequence mutation and optimization through introducing cationic or hydrophobic amino acids and cyclization appear to be viable approaches. It has been found that mutating specific amino acids in L-penetratin improved the stability in diluted gastrointestinal fluids [214]. Tools of artificial intelligence, machine learning, and molecular modeling are anticipated to facilitate the progress of CPP optimization through rational design and virtual screening. Additionally, D-form CPPs have been demonstrated to exhibit improved stability, providing increased protection over the payloads [111]. Another viable strategy could be the development of multivalent CPPs [215]. It is noted that the density of CPPs per particle affects the membrane transduction of CPP-decorated nanoparticles. Generally, 10 to 20 copies of CPP in one nanoparticle achieve effective transportation [102]. Therefore, cross-linking multiple CPP molecules may increase the membrane permeating activity. For examples, compared with a TAT monomer, TAT dimer displayed 10-fold increased delivery of proteins into the cultured cells [102,216]. PAMAM has been used to develop multivalent CPPs (penetratin-PAMAM) that showed enhanced cell permeation [217].

It appears that buccal and sublingual delivery of protein remains under-studied compared to oral and intranasal delivery. The oral cavity provides several advantages for protein delivery, including better tolerability compared to the nasal cavity and a less harsh environment relative to the GIT. The most significant challenge for buccal and sublingual delivery is the short residence time, which requires rapid absorption to offset. More efficient CPPs are needed to achieve significant delivery through buccal and sublingual administration. Another understudied topic is using CPPs to improve local delivery of proteins for localized diseases. Through local administration, CPPs will enhance penetration of drugs while minimizing their systemic exposure and side effects compared to systemic delivery. For example, PsorBan (CGC1072) is topically delivered, displaying increased drug concentrations in the

Table 4
CPP-containing protein drugs being investigated in clinical trials.

	CPP	Cargo	Administration route	Methodology	Indication	Phase	Company/ Organization	ClinicalTrials.gov identifier
localized injection	polyarginine (R7)	cyclosporine A (PsorBan, CGC1072)	topical	covalent	psoriasis	II	Cellgate Inc.	N/A
	RTP004	botulinumtoxin A (RT001)	topical	simple mixing	lateral canthal lines, Crow's feet, facial wrinkles	II	Revance Therapeutics, Inc.	NCT01064518 completed 2010
	RTP004	botulinumtoxin A (RT002)	intramuscular injection	simple mixing	glabellar lines	approved	Revance Therapeutics, Inc.	NCT02303002 completed 2015
	TAT	brimapitide (XG-102)	sub-conjunctival injection	fusion protein	post-cataract surgery intraocular inflammation and pain	III	Xigen SA	NCT02508337 completed 2016
	TAT	brimapitide (AM-111)	intratympanic injection	fusion protein	hearing loss	III	Auris Medical AG	NCT02561091 completed 2017
	TAT	delcasertib (KAI-9803)	intracoronary injection	covalent	myocardial infarction	II	KAI Pharmaceuticals	NCT00093197 completed 2006
	AZX100 (cell-permeable anti-fibrotic peptide bearing an "enhanced" PTD (PTD4))	a phosphorylatedregion from heat shock-related protein HSP20	intradermal injection	fusion protein	scar prevention/ reduction	II	Capstone Therapeutics	NCT00451256 NCT00892723 completed 2010 NCT00811577 completed 2010
systemic injection	TAT	active inhibitor of εPKC (KAI-1678)	subcutaneous infusion	covalent	postoperative pain	II	KAI Pharmaceuticals	NCT01015235 completed 2010
	TAT	εPKC activator (KAI-1455)	intravenous infusion	covalent	ischemic organ injury	I	KAI Pharmaceuticals, Inc.	N/A
	TAT	PSD-95 inhibitor (Nerinetide, NA-1)	intravenous infusion	fusion protein	major acute ischemic stroke (AIS)	III	NoNO Inc.	NCT02930018 completed 2019
	TAT	PSD-95 inhibitor (AVLX-144)	intravenous infusion	covalent	acute ischemic stroke, chronic inflammatory pain, neuropathic pain and subarachnoid hemorrhage	I	Avilex Pharma	NCT04689035 completed 2023
	TAT	MAGE-A3- and HPV16-specific peptide immunomodulatory vaccines (GL-0810, GL-0817)	subcutaneous injection	fusion protein	head and neck carcinoma	I	Gliknik Inc.	N/A
	P28	glutathione-S-transferase (P28GST)	subcutaneous injections	fusion protein	Crohn's ileocolitis	II	University Hospital, Lille	NCT02281916 completed 2018
	P28	P28	intravenous infusion	N/A	pediatric central nervous system tumors	I	Pediatric Brain Tumor Consortium	NCT01975116 completed 2015
	P28	P28	intravenous infusion	N/A	refractory solid tumors	I	CDG Therapeutics, Inc.	NCT00914914 completed 2011
	PEP-010 (DPT-C9h) (DPT as a penetrating peptide and PEP1 as the interfering active peptide)	PEP-010 (DPT-C9h), and in combination with paclitaxel	intravenous infusion	fusion protein	metastatic solid tumor cancer	I	PEP-Therapy	NCT04733027
	ATP128	TLR agonist-derived peptide and a multi-antigenic domain (KISIMA™)	subcutaneous injection/ intravenous infusion	fusion protein	stage IV colorectal cancer	I	Amal Therapeutics	NCT04046445
	ATX-101 (APIM (AlkB homolog 2 protein PCNA (proliferating cell nuclear antigen) interacting motif)-containing peptide)	ATX-101 plus carboplatin and pegylated liposomal doxorubicin (ACD)	intravenous infusion	fusion protein	platinum-sensitive ovarian cancer	I/II	THERAPIM PTY LTD	NCT04814875
	ATX-101	ATX-101	intravenous infusion	fusion protein	liposarcoma and leiomyosarcoma	II	Columbia University	NCT05116683

skin without systemic absorption. PsorBan is well-tolerated in patients [218,219].

9. Conclusion

Intranasal, buccal, sublingual, and oral delivery of proteins using CPPs has showed encouraging progress, but mostly focuses on small proteins <6 kDa. Continuous optimization of CPPs through tools such as artificial intelligence and high throughput screening is warranted to achieve increased and consistent efficacy and safety as well as to expand its application for delivering large proteins including mAb and ADC. More efforts should be put on buccal and sublingual delivery and exploration of CPP use in localized applications. We maintain a positive outlook for this field and expect transmucosal CPP products entering clinical trials soon. These new clinical data will further propel research and development of CPPs.

CRediT authorship contribution statement

Jiamin Wu: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sophie Roesger:** Visualization, Validation, Investigation. **Natalie Jones:** Investigation. **Che-Ming J. Hu:** Writing – review & editing. **Shyh-Dar Li:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

[1] A.M. Vargason, A.C. Anselmo, S. Mitragotri, The evolution of commercial drug delivery technologies, Nat. Biomed. Eng. 5 (2021) 951–967, https://doi.org/10.1038/s41551-021-00698-w.
[2] L. Qin, Z. Cui, Y. Wu, H. Wang, X. Zhang, J. Guan, S. Mao, Challenges and strategies to enhance the systemic absorption of inhaled peptides and proteins, Pharm. Res. 40 (2023) 1037–1055, https://doi.org/10.1007/s11095-022-03435-3.
[3] K. Sharma, K.K. Sharma, A. Sharma, R. Jain, Peptide-based drug discovery: current status and recent advances, Drug Discov. Today 28 (2023) 103464, https://doi.org/10.1016/j.drudis.2022.103464.
[4] R.M. Lu, Y.C. Hwang, I.J. Liu, C.C. Lee, H.Z. Tsai, H.J. Li, H.C. Wu, Development of therapeutic antibodies for the treatment of diseases, J. Biomed. Sci. 27 (2020) 1, https://doi.org/10.1186/s12929-019-0592-z.
[5] A.C. Anselmo, Y. Gokarn, S. Mitragotri, Non-invasive delivery strategies for biologics, Nat. Rev. Drug Discov. 18 (2019) 19–40, https://doi.org/10.1038/nrd.2018.183.
[6] L. Wang, N. Wang, W. Zhang, X. Cheng, Z. Yan, G. Shao, X. Wang, R. Wang, C. Fu, Therapeutic peptides: current applications and future directions, Signal Transduct. Target. Ther. 7 (2022) 48, https://doi.org/10.1038/s41392-022-00904-4.
[7] S.B. Ebrahimi, D. Samanta, Engineering protein-based therapeutics through structural and chemical design, Nat. Commun. 14 (2023) 2411, https://doi.org/10.1038/s41467-023-38039-x.
[8] V.Y. Berdan, P.C. Klauser, L. Wang, Covalent peptides and proteins for therapeutics, Bioorg. Med. Chem. 29 (2021) 115896, https://doi.org/10.1016/j.bmc.2020.115896.
[9] M. Shi, K.J. McHugh, Strategies for overcoming protein and peptide instability in biodegradable drug delivery systems, Adv. Drug Deliv. Rev. 199 (2023) 114904, https://doi.org/10.1016/j.addr.2023.114904.
[10] J. Wu, N. Jones, N.A.L. Fayez, P.H. Chao, A. Wu, D.R. de Araujo, E. Rouhollahi, A. Jia, S.D. Li, Protamine-mediated efficient transcellular and transmucosal delivery of proteins, J. Control. Release 356 (2023) 373–385, https://doi.org/10.1016/j.jconrel.2023.03.002.
[11] A. Yamamoto, H. Ukai, M. Morishita, H. Katsumi, Approaches to improve intestinal and transmucosal absorption of peptide and protein drugs, Pharmacol. Ther. 211 (2020) 107537, https://doi.org/10.1016/j.pharmthera.2020.107537.
[12] S. Verma, U.K. Goand, A. Husain, R.A. Katekar, R. Garg, J.R. Gayen, Challenges of peptide and protein drug delivery by oral route: current strategies to improve the bioavailability, Drug Dev. Res. 82 (2021) 927–944, https://doi.org/10.1002/ddr.21832.
[13] S. Pechenov, J. Revell, S. Will, J. Naylor, P. Tyagi, C. Patel, L. Liang, L. Tseng, Y. Huang, A.I. Rosenbaum, K. Balic, A. Konkar, J. Grimsby, J.A. Subramony, Development of an orally delivered GLP-1 receptor agonist through peptide engineering and drug delivery to treat chronic disease, Sci. Rep. 11 (2021) 22521, https://doi.org/10.1038/s41598-021-01750-0.
[14] D.A. Provenzano, M.C. Hanes, T.R. Deer, Interventional pain procedures and the risk of infection, in: Essentials of Pain Medicine, 2018, pp. 813–824.e813.
[15] W. Suksatan, S.A. Jasim, G. Widjaja, A.T. Jalil, S. Chupradit, M.J. Ansari, Y. F. Mustafa, H.A. Hammoodi, M.J. Mohammadi, Assessment effects and risk of nosocomial infection and needle sticks injuries among patents and health care worker, Toxicol. Rep. 9 (2022) 284–292, https://doi.org/10.1016/j.toxrep.2022.02.013.
[16] B. Mangla, S. Javed, M.H. Sultan, W. Ahsan, G. Aggarwal, K. Kohli, Nanocarriers-assisted needle-free vaccine delivery through Oral and intranasal Transmucosal routes: a novel therapeutic conduit, Front. Pharmacol. 12 (2021) 757761, https://doi.org/10.3389/fphar.2021.757761.
[17] A.Z. Alkilani, J. Nasereddin, R. Hamed, S. Nimrawi, G. Hussein, H. Abo-Zour, R. F. Donnelly, Beneath the skin: a review of current trends and future prospects of transdermal drug delivery systems, Pharmaceutics 14 (2022), https://doi.org/10.3390/pharmaceutics14061152.
[18] M. Durán-Lobato, A.M. López-Estévez, A.S. Cordeiro, T.G. Dacoba, J. Crecente-Campo, D. Torres, M.J. Alonso, Nanotechnologies for the delivery of biologicals: historical perspective and current landscape, Adv. Drug Deliv. Rev. 176 (2021) 113899, https://doi.org/10.1016/j.addr.2021.113899.
[19] J.K.W. Lam, C.C.K. Cheung, M.Y.T. Chow, E. Harrop, S. Lapwood, S.I.G. Barclay, I.C.K. Wong, Transmucosal drug administration as an alternative route in palliative and end-of-life care during the COVID-19 pandemic, Adv. Drug Deliv. Rev. 160 (2020) 234–243, https://doi.org/10.1016/j.addr.2020.10.018.
[20] M. Karimi, H. Kamali, M. Mohammadi, M. Tafaghodi, Evaluation of various techniques for production of inhalable dry powders for pulmonary delivery of peptide and protein, J. Drug Deliv. Sci. Technol. 69 (2022), https://doi.org/10.1016/j.jddst.2022.103186.
[21] Influenza Vaccine for 2019-2020, JAMA 323 (2020) 84–85, https://doi.org/10.1001/jama.2019.18016.
[22] J.A. Cohn, C.G. Kowalik, W.S. Reynolds, M.R. Kaufman, D.F. Milam, R. R. Dmochowski, A.J. Wein, Desmopressin acetate nasal spray for adults with nocturia, Expert. Rev. Clin. Pharmacol. 10 (2017) 1281–1293, https://doi.org/10.1080/17512433.2017.1394185.
[23] MedBroadcast, Drug and Medications, Octostim. https://medbroadcast.com/drug/getdrug/octostim, 2024.
[24] Mayo Clinic, Drugs and Supplements, Desmopressin (Nasal Route). https://www.mayoclinic.org/drugs-supplements/desmopressin-nasal-route/description/drg-20066827, 2024.
[25] L. Heinemann, The failure of exubera: are we beating a dead horse? J. Diabetes Sci. Technol. 2 (2008) 518–529, https://doi.org/10.1177/193229680800200325.
[26] F.M. Kolitsopoulos, N.M. Gatto, K. Sweetland, M.B. Bracken, N. Jackson, Implications of product withdrawal on a post-approval pragmatic trial: the VOLUME study experience, Contemp. Clin. Trials Commun. 16 (2019) 100477, https://doi.org/10.1016/j.conctc.2019.100477.
[27] J. Oleck, S. Kassam, J.D. Goldman, Commentary: why was inhaled insulin a failure in the market? Diabet. Spectr. 29 (2016) 180–184, https://doi.org/10.2337/diaspect.29.3.180.
[28] Y. Mohammed, A. Holmes, P.C.L. Kwok, T. Kumeria, S. Namjoshi, M. Imran, L. Matteucci, M. Ali, W. Tai, H.A.E. Benson, M.S. Roberts, Advances and future perspectives in epithelial drug delivery, Adv. Drug Deliv. Rev. 186 (2022) 114293, https://doi.org/10.1016/j.addr.2022.114293.
[29] Y. Ozsoy, S. Gungor, E. Cevher, Nasal delivery of high molecular weight drugs, Molecules 14 (2009) 3754–3779, https://doi.org/10.3390/molecules14093754.
[30] F. Chen, H. Liu, B. Wang, L. Yang, W. Cai, Z. Jiao, Z. Yang, Y. Chen, Y. Quan, X. Xiang, H. Wang, Physiologically based pharmacokinetic modeling to understand the absorption of risperidone Orodispersible film, Front. Pharmacol. 10 (2019) 1692, https://doi.org/10.3389/fphar.2019.01692.
[31] M. Rawas-Qalaji, H.E. Thu, Z. Hussain, Oromucosal delivery of macromolecules: challenges and recent developments to improve bioavailability, J. Control. Release 352 (2022) 726–746, https://doi.org/10.1016/j.jconrel.2022.10.059.

[32] T.D. Brown, K.A. Whitehead, S. Mitragotri, Materials for oral delivery of proteins and peptides, Nat. Rev. Mater. 5 (2019) 127–148, https://doi.org/10.1038/s41578-019-0156-6.
[33] R. Bajracharya, J.G. Song, S.Y. Back, H.K. Han, Recent advancements in non-invasive formulations for protein drug delivery, Comput Struct, Biotechnol. J. 17 (2019) 1290–1308, https://doi.org/10.1016/j.csbj.2019.09.004.
[34] A.S. Rzhevskiy, T.R.R. Singh, R.F. Donnelly, Y.G. Anissimov, Microneedles as the technique of drug delivery enhancement in diverse organs and tissues, J. Control. Release 270 (2018) 184–202, https://doi.org/10.1016/j.jconrel.2017.11.048.
[35] A. Banerjee, R. Chen, S. Arafin, S. Mitragotri, Intestinal iontophoresis from mucoadhesive patches: a strategy for oral delivery, J. Control. Release 297 (2019) 71–78, https://doi.org/10.1016/j.jconrel.2019.01.037.
[36] Y. Yang, R. Zhou, Y. Wang, Y. Zhang, J. Yu, Z. Gu, Recent advances in Oral and transdermal protein delivery systems, Angew. Chem. Int. Ed. Eng. 62 (2023) e202214795, https://doi.org/10.1002/anie.202214795.
[37] K. Baxi, S. Sawarkar, M. Momin, V. Patel, T. Fernandes, Vaginal siRNA delivery: overview on novel delivery approaches, drug Deliv, Transl. Res. 10 (2020) 962–974, https://doi.org/10.1007/s13346-020-00741-4.
[38] V. Hearnden, V. Sankar, K. Hull, D.V. Juras, M. Greenberg, A.R. Kerr, P. B. Lockhart, L.L. Patton, S. Porter, M.H. Thornhill, New developments and opportunities in oral mucosal drug delivery for local and systemic disease, Adv. Drug Deliv. Rev. 64 (2012) 16–28, https://doi.org/10.1016/j.addr.2011.02.008.
[39] A. Abramson, M.R. Frederiksen, A. Vegge, B. Jensen, M. Poulsen, B. Mouridsen, M.O. Jespersen, R.K. Kirk, J. Windum, F. Hubálek, J.J. Water, J. Fels, S. B. Gunnarsson, A. Bohr, E.M. Straarup, M.W.H. Ley, X. Lu, J. Wainer, J. Collins, S. Tamang, K. Ishida, A. Hayward, P. Herskind, S.T. Buckley, N. Roxhed, R. Langer, U. Rahbek, G. Traverso, Oral delivery of systemic monoclonal antibodies, peptides and small molecules using gastric auto-injectors, Nat. Biotechnol. 40 (2022) 103–109, https://doi.org/10.1038/s41587-021-01024-0.
[40] S. Talkar, S. Dhoble, A. Majumdar, V. Patravale, Transmucosal Nanoparticles: Toxicological Overview, Adv. Exp. Med. Biol. 1048 (2018) 37–57, https://doi.org/10.1007/978-3-319-72041-8_3.
[41] N.P. Lang, M.S. Tonetti, J.E. Suvan, J. Pierre Bernard, D. Botticelli, I. Fourmousis, M. Hallund, R. Jung, L. Laurell, G.E. Salvi, D. Shafer, H.P. Weber, Immediate implant placement with transmucosal healing in areas of aesthetic priority. A multicentre randomized-controlled clinical trial I. Surgical outcomes, Clin. Oral Implants Res. 18 (2007) 188–196, https://doi.org/10.1111/j.1600-0501.2006.01371.x.
[42] Y. Meng, X.J. Li, Y. Li, T.Y. Zhang, D. Liu, Y.Q. Wu, F.F. Hou, L. Ye, C.J. Wu, X. D. Feng, X.J. Ju, L. Jiang, Novel double-layer dissolving microneedles for Transmucosal sequential delivery of multiple drugs in the treatment of Oral mucosa diseases, ACS Appl. Mater. Interfaces 15 (2023) 13892–13906, https://doi.org/10.1021/acsami.2c19913.
[43] J. Xie, Y. Bi, H. Zhang, S. Dong, L. Teng, R.J. Lee, Z. Yang, Cell-penetrating peptides in diagnosis and treatment of human diseases: from preclinical research to clinical application, Front. Pharmacol. 11 (2020) 697, https://doi.org/10.3389/fphar.2020.00697.
[44] T. Miwa, K. Tachii, F.-Y. Wei, T. Kaitsuka, K. Tomizawa, Intranasal drug delivery into mouse nasal mucosa and brain utilizing arginine-rich cell-penetrating peptide-mediated protein transduction, Int. J. Pept. Res. Ther. 26 (2019) 1643–1650, https://doi.org/10.1007/s10989-019-09971-8.
[45] E. de Souza Von, J.O. Zuben, V.H.S. Eloy, M.P.D. Araujo, M. Chorilli Gremião, Insulin-loaded liposomes functionalized with cell-penetrating peptides: influence on drug release and permeation through porcine nasal mucosa, Colloids Surf. A Physicochem. Eng. Asp. 622 (2021), https://doi.org/10.1016/j.colsurfa.2021.126624.
[46] S.K. Linden, P. Sutton, N.G. Karlsson, V. Korolik, M.A. McGuckin, Mucins in the mucosal barrier to infection, Mucosal Immunol. 1 (2008) 183–197, https://doi.org/10.1038/mi.2008.5.
[47] A.P. Corfield, Mucins: a biologically relevant glycan barrier in mucosal protection, Biochim. Biophys. Acta 2015 (1850) 236–252, https://doi.org/10.1016/j.bbagen.2014.05.003.
[48] M. Boegh, H.M. Nielsen, Mucus as a barrier to drug delivery – understanding and mimicking the barrier properties, Basic Clin. Pharmacol. Toxicol. 116 (2015) 179–186, https://doi.org/10.1111/bcpt.12342.
[49] R.A. Cone, Barrier properties of mucus, Adv. Drug Deliv. Rev. 61 (2009) 75–85, https://doi.org/10.1016/j.addr.2008.09.008.
[50] P. Gómez-Gálvez, P. Vicente-Munuera, S. Anbari, J. Buceta, L.M. Escudero, The complex three-dimensional organization of epithelial tissues, Development 148 (2021), https://doi.org/10.1242/dev.195669.
[51] H.F. Gómez, M.S. Dumond, L. Hodel, R. Vetter, D. Iber, 3D cell neighbour dynamics in growing pseudostratified epithelia, Elife 10 (2021), https://doi.org/10.7554/eLife.68135.
[52] A.A. Torricelli, V. Singh, M.R. Santhiago, S.E. Wilson, The corneal epithelial basement membrane: structure, function, and disease, Invest. Ophthalmol. Vis. Sci. 54 (2013) 6390–6400, https://doi.org/10.1167/iovs.13-12547.
[53] N. Kia'i, T. Bajaj, Histology, Respiratory Epithelium, StatPearls Publishing, Treasure Island (FL), 2022.
[54] R.B. Nagle, R. Moll, H. Weidauer, H. Nemetschek, W.W. Franke, Different patterns of cytokeratin expression in the normal epithelia of the upper respiratory tract, Differentiation 30 (1985) 130–140, https://doi.org/10.1111/j.1432-0436.1985.tb00524.x.
[55] P. Asikainen, T.J. Ruotsalainen, J.J. Mikkonen, A. Koistinen, C. Ten Bruggenkate, A.M. Kullaa, The defence architecture of the superficial cells of the oral mucosa, Med. Hypotheses 78 (2012) 790–792, https://doi.org/10.1016/j.mehy.2012.03.009.
[56] Wikipedia, Epithelium. https://en.wikipedia.org/wiki/Epithelium, 2024.
[57] S. Groeger, J. Meyle, Oral mucosal epithelial cells, Front. Immunol. 10 (2019) 208, https://doi.org/10.3389/fimmu.2019.00208.
[58] G.H. Liang, C.R. Weber, Molecular aspects of tight junction barrier function, Curr. Opin. Pharmacol. 19 (2014) 84–89, https://doi.org/10.1016/j.coph.2014.07.017.
[59] A.L. Daugherty, R.J. Mrsny, Transcellular uptake mechanisms of the intestinal epithelial barrier part one, Pharm. Sci. Technol. Today 4 (1999) 144–151, https://doi.org/10.1016/s1461-5347(99)00142-x.
[60] M. Takano, M. Kawami, A. Aoki, R. Yumoto, Receptor-mediated endocytosis of macromolecules and strategy to enhance their transport in alveolar epithelial cells, Expert Opin. Drug Deliv. 12 (2015) 813–825, https://doi.org/10.1517/17425247.2015.992778.
[61] J.H. Hamman, P.H. Demana, E.I. Olivier, Targeting receptors, transporters and site of absorption to improve oral drug delivery, Drug Target Insights 2 (2007) 71–81.
[62] D.J. Drucker, Advances in oral peptide therapeutics, Nat. Rev. Drug Discov. 19 (2020) 277–289, https://doi.org/10.1038/s41573-019-0053-0.
[63] R. Suri, S. Beg, K. Kohli, Target strategies for drug delivery bypassing ocular barriers, J. Drug Deliv. Sci. Technol. 55 (2020), https://doi.org/10.1016/j.jddst.2019.101389.
[64] C. Liu, Y. Kou, X. Zhang, H. Cheng, X. Chen, S. Mao, Strategies and industrial perspectives to improve oral absorption of biological macromolecules, Expert Opin. Drug Deliv. 15 (2018) 223–233, https://doi.org/10.1080/17425247.2017.1395853.
[65] V. Gote, M. Ansong, D. Pal, Prodrugs and nanomicelles to overcome ocular barriers for drug penetration, Expert Opin. Drug Metab. Toxicol. 16 (2020) 885–906, https://doi.org/10.1080/17425255.2020.1803278.
[66] D. Esposito, C. Conte, I. d'Angelo, A. Miro, F. Ungaro, F. Quaglia, Mucoadhesive zein/beta-cyclodextrin nanoparticles for the buccal delivery of curcumin, Int. J. Pharm. 586 (2020) 119587, https://doi.org/10.1016/j.ijpharm.2020.119587.
[67] Z. Sang, J. Qian, J. Han, X. Deng, J. Shen, G. Li, Y. Xie, Comparison of three water-soluble polyphosphate tripolyphosphate, phytic acid, and sodium hexametaphosphate as crosslinking agents in chitosan nanoparticle formulation, Carbohydr. Polym. 230 (2020) 115577, https://doi.org/10.1016/j.carbpol.2019.115577.
[68] M.C. Gómez-Guillén, M.P. Montero, Enhancement of oral bioavailability of natural compounds and probiotics by mucoadhesive tailored biopolymer-based nanoparticles: a review, Food Hydrocoll. 118 (2021), https://doi.org/10.1016/j.foodhyd.2021.106772.
[69] R.L. Creighton, K.A. Woodrow, Microneedle-mediated vaccine delivery to the Oral mucosa, Adv. Healthc. Mater. 8 (2019) e1801180, https://doi.org/10.1002/adhm.201801180.
[70] G. Muniraj, R.H.S. Tan, Y. Dai, R. Wu, M. Alberti, G. Sriram, Microphysiological modeling of gingival tissues and host-material interactions using gingiva-on-Chip, Adv. Healthc. Mater. e2301472 (2023), https://doi.org/10.1002/adhm.202301472.
[71] M. Bertolini, T. Sobue, A. Thompson, A. Dongari-Bagtzoglou, Chemotherapy induces Oral mucositis in mice without additional noxious stimuli, Transl. Oncol. 10 (2017) 612–620, https://doi.org/10.1016/j.tranon.2017.05.001.
[72] P.H. Hoet, I. Brüske-Hohlfeld, O.V. Salata, Nanoparticles - known and unknown health risks, J. Nanobiotechnol. 2 (2004) 12, https://doi.org/10.1186/1477-3155-2-12.
[73] C.C. Davis, C.A. Squier, G.E. Lilly, Irritant contact stomatitis: a review of the condition, J. Periodontol. 69 (1998) 620–631, https://doi.org/10.1902/jop.1998.69.6.620.
[74] Z. Ujhelyi, F. Fenyvesi, J. Váradi, P. Fehér, T. Kiss, S. Veszelka, M. Deli, M. Vecsernyés, I. Bácskay, Evaluation of cytotoxicity of surfactants used in self-micro emulsifying drug delivery systems and their effects on paracellular transport in Caco-2 cell monolayer, Eur. J. Pharm. Sci. 47 (2012) 564–573, https://doi.org/10.1016/j.ejps.2012.07.005.
[75] M.M. Alvi, P. Chatterjee, A prospective analysis of co-processed non-ionic surfactants in enhancing permeability of a model hydrophilic drug, AAPS PharmSciTech 15 (2014) 339–353, https://doi.org/10.1208/s12249-013-0065-8.
[76] F. Khodabakhsh, M. Salimian, M.H. Hedayati, R. Ahangari Cohan, D. Norouzian, Challenges and advancements in the pharmacokinetic enhancement of therapeutic proteins, Prep. Biochem. Biotechnol. 51 (2021) 519–529, https://doi.org/10.1080/10826068.2020.1839907.
[77] P.J. Carter, Introduction to current and future protein therapeutics: a protein engineering perspective, Exp. Cell Res. 317 (2011) 1261–1269, https://doi.org/10.1016/j.yexcr.2011.02.013.
[78] I.-U.-R. Tak, F. Ali, J.S. Dar, A.R. Magray, B.A. Ganai, M.Z. Chishti, Posttranslational modifications of proteins and their role in biological processes and associated diseases, in: Protein Modificomics, 2019, pp. 1–35.
[79] C. Le Sueur, H.M. Hammarén, S. Sridharan, M.M. Savitski, Thermal proteome profiling: insights into protein modifications, associations, and functions, Curr. Opin. Chem. Biol. 71 (2022) 102225, https://doi.org/10.1016/j.cbpa.2022.102225.
[80] Z. Zhang, L. Shang, Smart ingestible devices: orally delivering macromolecules and beyond, Matter 4 (2021) 3379–3381, https://doi.org/10.1016/j.matt.2021.10.003.
[81] Q. Zhu, Z. Chen, P.K. Paul, Y. Lu, W. Wu, J. Qi, Oral delivery of proteins and peptides: challenges, status quo and future perspectives, Acta Pharm. Sin. B 11 (2021) 2416–2448, https://doi.org/10.1016/j.apsb.2021.04.001.

[82] T. Tong, L. Wang, X. You, J. Wu, Nano and microscale delivery platforms for enhanced oral peptide/protein bioavailability, Biomater. Sci. 8 (2020) 5804–5823, https://doi.org/10.1039/d0bm01151g.

[83] S.R. Falsafi, A.C. Karaca, L. Deng, Y. Wang, H. Li, G. Askari, H. Rostamabadi, Insights into whey protein-based carriers for targeted delivery and controlled release of bioactive components, Food Hydrocoll. 133 (2022), https://doi.org/10.1016/j.foodhyd.2022.108002.

[84] L.K. Vora, A.D. Gholap, K. Jetha, R.R.S. Thakur, H.K. Solanki, V.P. Chavda, Artificial intelligence in pharmaceutical technology and drug delivery design, Pharmaceutics 15 (2023), https://doi.org/10.3390/pharmaceutics15071916.

[85] G. Xie, S. Lin, F. Wu, J. Liu, Nanomaterial-based ophthalmic drug delivery, Adv. Drug Deliv. Rev. 200 (2023) 115004, https://doi.org/10.1016/j.addr.2023.115004.

[86] X. Kong, P. Gao, J. Wang, Y. Fang, K.C. Hwang, Advances of medical nanorobots for future cancer treatments, J. Hematol. Oncol. 16 (2023) 74, https://doi.org/10.1186/s13045-023-01463-z.

[87] I. Neundorf, Medical use of cell-penetrating peptides: how far have they come?, in: Peptide and Peptidomimetic Therapeutics, 2022, pp. 235–254.

[88] M. Zorko, S. Jones, Ü. Langel, Cell-penetrating peptides in protein mimicry and cancer therapeutics, Adv. Drug Deliv. Rev. 180 (2022) 114044, https://doi.org/10.1016/j.addr.2021.114044.

[89] Osdd.net, CPPsite 2.0: A Database of Cell Penetrating Peptide. http://crdd.osdd.net/raghava/cppsite/, 2023.

[90] L.B. De Martini, C. Sulmona, L. Brambilla, D. Rossi, Cell-penetrating peptides as valuable tools for nose-to-brain delivery of biological drugs, Cells 12 (2023), https://doi.org/10.3390/cells12121643.

[91] M. Korivi, Y.W. Huang, B.R. Liu, Cell-penetrating peptides as a potential drug delivery system for effective treatment of diabetes, Curr. Pharm. Des. 27 (2021) 816–825, https://doi.org/10.2174/1381612826666201019102640.

[92] C.C. Young, A. Vedadghavami, A.G. Bajpayee, Bioelectricity for drug delivery: the promise of cationic therapeutics, Bioelectricity 2 (2020) 68–81, https://doi.org/10.1089/bioe.2020.0012.

[93] A. Pantos, I. Tsogas, C.M. Paleos, Guanidinium group: a versatile moiety inducing transport and multicompartmentalization in complementary membranes, Biochim. Biophys. Acta 1778 (2008) 811–823, https://doi.org/10.1016/j.bbamem.2007.12.003.

[94] M. Vazdar, J. Heyda, P.E. Mason, G. Tesei, C. Allolio, M. Lund, P. Jungwirth, Arginine "magic": Guanidinium like-charge ion pairing from aqueous salts to cell penetrating peptides, Acc. Chem. Res. 51 (2018) 1455–1464, https://doi.org/10.1021/acs.accounts.8b00098.

[95] R. Bajracharya, J.G. Song, B.R. Patil, S.H. Lee, H.M. Noh, D.H. Kim, G.L. Kim, S. H. Seo, J.W. Park, S.H. Jeong, C.H. Lee, H.K. Han, Functional ligands for improving anticancer drug therapy: current status and applications to drug delivery systems, Drug Deliv. 29 (2022) 1959–1970, https://doi.org/10.1080/10717544.2022.2089296.

[96] M. Ma, R. Zhao, X. Li, M. Jing, R. Song, J. Fan, Biological properties of arginine-rich peptides and their application in cargo delivery to Cancer, Curr. Drug Deliv. (2023), https://doi.org/10.2174/1567201820666230417083350.

[97] S. Zhang, L. Gan, F. Cao, H. Wang, P. Gong, C. Ma, L. Ren, Y. Lin, X. Lin, The barrier and interface mechanisms of the brain barrier, and brain drug delivery, Brain Res. Bull. 190 (2022) 69–83, https://doi.org/10.1016/j.brainresbull.2022.09.017.

[98] S. Parrasia, I. Szabò, M. Zoratti, L. Biasutto, Peptides as pharmacological carriers to the brain: promises, shortcomings and challenges, Mol. Pharm. 19 (2022) 3700–3729, https://doi.org/10.1021/acs.molpharmaceut.2c00523.

[99] O.N. Shilova, E.S. Shilov, A. Lieber, S.M. Deyev, Disassembling a cancer puzzle: cell junctions and plasma membrane as targets for anticancer therapy, J. Control. Release 286 (2018) 125–136, https://doi.org/10.1016/j.jconrel.2018.07.030.

[100] C. Bechara, S. Sagan, Cell-penetrating peptides: 20 years later, where do we stand? FEBS Lett. 587 (2013) 1693–1702, https://doi.org/10.1016/j.febslet.2013.04.031.

[101] M.C. Shin, J. Zhang, K.A. Min, K. Lee, Y. Byun, A.E. David, H. He, V.C. Yang, Cell-penetrating peptides: achievements and challenges in application for cancer treatment, J. Biomed. Mater. Res. A 102 (2014) 575–587, https://doi.org/10.1002/jbm.a.34859.

[102] J. Xu, A.R. Khan, M. Fu, R. Wang, J. Ji, G. Zhai, Cell-penetrating peptide: a means of breaking through the physiological barriers of different tissues and organs, J. Control. Release 309 (2019) 106–124, https://doi.org/10.1016/j.jconrel.2019.07.020.

[103] C. Foged, H.M. Nielsen, Cell-penetrating peptides for drug delivery across membrane barriers, Expert Opin. Drug Deliv. 5 (2008) 105–117, https://doi.org/10.1517/17425247.5.1.105.

[104] M. Kristensen, D. Birch, H. Mørck Nielsen, Applications and challenges for use of cell-penetrating peptides as delivery vectors for peptide and protein cargos, Int. J. Mol. Sci. 17 (2016), https://doi.org/10.3390/ijms17020185.

[105] S. Futaki, I. Nakase, Cell-surface interactions on arginine-rich cell-penetrating peptides allow for multiplex modes of internalization, Acc. Chem. Res. 50 (2017) 2449–2456, https://doi.org/10.1021/acs.accounts.7b00221.

[106] K. Kurrikoff, B. Vunk, Ü. Langel, Status update in the use of cell-penetrating peptides for the delivery of macromolecular therapeutics, Expert. Opin. Biol. Ther. 21 (2021) 361–370, https://doi.org/10.1080/14712598.2021.1823368.

[107] J. Ye, E. Liu, Z. Yu, X. Pei, S. Chen, P. Zhang, M.C. Shin, J. Gong, H. He, V.C. Yang, CPP-assisted intracellular drug delivery, what is next? Int. J. Mol. Sci. 17 (2016) https://doi.org/10.3390/ijms17111892.

[108] J.S. Wadia, S.F. Dowdy, Transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer, Adv. Drug Deliv. Rev. 57 (2005) 579–596, https://doi.org/10.1016/j.addr.2004.10.005.

[109] B. Gupta, T.S. Levchenko, V.P. Torchilin, Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides, Adv. Drug Deliv. Rev. 57 (2005) 637–651, https://doi.org/10.1016/j.addr.2004.10.007.

[110] M. Rizzuti, M. Nizzardo, C. Zanetta, A. Ramirez, S. Corti, Therapeutic applications of the cell-penetrating HIV-1 tat peptide, Drug Discov. Today 20 (2015) 76–85, https://doi.org/10.1016/j.drudis.2014.09.017.

[111] E.J. Nielsen, S. Yoshida, N. Kamei, R. Iwamae, S. Khafagy el, J. Olsen, U. L. Rahbek, B.L. Pedersen, K. Takayama, M. Takeda-Morishita, In vivo proof of concept of oral insulin delivery based on a co-administration strategy with the cell-penetrating peptide penetratin, J. Control. Release 189 (2014) 19–24, https://doi.org/10.1016/j.jconrel.2014.06.022.

[112] M.M. El-Sayed Khafagy, Koichi Isowa, Jun Imai, Kozo Takayama, Effect of cell-penetrating peptides on the nasal absorption of insulin, J. Control. Release 133 (2009) 103–108.

[113] S. Fu, X. Xu, Y. Ma, S. Zhang, S. Zhang, RGD peptide-based non-viral gene delivery vectors targeting integrin α(v)β(3) for cancer therapy, J. Drug Target. 27 (2019) 1–11, https://doi.org/10.1080/1061186x.2018.1455841.

[114] K. Kardani, A. Milani, H.S. S, A. Bolhassani, Cell penetrating peptides: the potent multi-cargo intracellular carriers, Expert Opin. Drug Deliv. 16 (2019) 1227–1258, https://doi.org/10.1080/17425247.2019.1676720.

[115] M. Zorko, Ü. Langel, Studies of cell-penetrating peptides by biophysical methods, Q. Rev. Biophys. (2022) 1–55, https://doi.org/10.1017/s0033583522000026.

[116] J. Maeng, K. Lee, Systemic and brain delivery of antidiabetic peptides through nasal administration using cell-penetrating peptides, Front. Pharmacol. 13 (2022) 1068495, https://doi.org/10.3389/fphar.2022.1068495.

[117] S.J. Lee, H.K. Kang, Y.J. Choi, W.S. Eum, J. Park, S.Y. Choi, H.Y. Kwon, PEP-1-paraoxonase 1 fusion protein prevents cytokine-induced cell destruction and impaired insulin secretion in rat insulinoma cells, BMB Rep. 51 (2018) 538–543, https://doi.org/10.5483/BMBRep.2018.51.10.181.

[118] B.L. Duivelshof, A. Murisier, J. Camperi, S. Fekete, A. Beck, D. Guillarme, V. D'Atri, Therapeutic fc-fusion proteins: current analytical strategies, J. Sep. Sci. 44 (2021) 35–62, https://doi.org/10.1002/jssc.202000765.

[119] T. Keum, G. Noh, J.E. Seo, S. Bashyal, D.H. Sohn, S. Lee, Examination of effective buccal absorption of Salmon calcitonin using cell-penetrating peptide-conjugated liposomal drug delivery system, Int. J. Nanomedicine 17 (2022) 697–710, https://doi.org/10.2147/ijn.S335774.

[120] K. Desale, K. Kuche, S. Jain, Cell-penetrating peptides (CPPs): an overview of applications for improving the potential of nanotherapeutics, Biomater. Sci. 9 (2021) 1153–1188, https://doi.org/10.1039/d0bm01755h.

[121] A. Shoari, R. Tooyserkani, M. Tahmasebi, D. Löwik, Delivery of various cargos into Cancer cells and tissues via cell-penetrating peptides: a review of the last decade, Pharmaceutics 13 (2021), https://doi.org/10.3390/pharmaceutics13091391.

[122] D. Khunt, M. Misra, Chapter-1 an overview of anatomical and physiological aspects of the nose and the brain, in: Direct Nose-to-Brain Drug Delivery, 2021, pp. 3–14.

[123] V.S. Prasad, P. Sudheer, K.B.C. Shetty, Current advances in nose to brain drug delivery, pharmaceutical sciences, Asia 50 (2023) 175–186, https://doi.org/10.29090/psa.2023.03.23.477.

[124] F. Sabbagh, R. Muhamad II, P.K. Niazmand, B.S. Kim Dikshit, Recent progress in polymeric non-invasive insulin delivery, Int. J. Biol. Macromol. 203 (2022) 222–243, https://doi.org/10.1016/j.ijbiomac.2022.01.134.

[125] M. Bayanati, A.G. Khosroshahi, M. Alvandi, M.M. Mahboobian, F. Sun, Fabrication of a thermosensitive in situ gel Nanoemulsion for nose to brain delivery of Temozolomide, J. Nanomater. 2021 (2021) 1–11, https://doi.org/10.1155/2021/1546798.

[126] Y.C. Wong, Z. Zuo, Intranasal delivery–modification of drug metabolism and brain disposition, Pharm. Res. 27 (2010) 1208–1223, https://doi.org/10.1007/s11095-010-0127-5.

[127] H. Kumar, G. Mishra, A.K. Sharma, A. Gothwal, P. Kesharwani, U. Gupta, Intranasal drug delivery: a non-invasive approach for the better delivery of Neurotherapeutics, Pharm. Nanotechnol. 5 (2017) 203–214, https://doi.org/10.2174/2211738505666170515113936.

[128] S.S. Hong, K.T. Oh, H.G. Choi, S.J. Lim, Liposomal formulations for nose-to-brain delivery: recent advances and future perspectives, Pharmaceutics 11 (2019), https://doi.org/10.3390/pharmaceutics11100540.

[129] L. Illum, Nasal drug delivery: new developments and strategies, Drug Discov. Today 7 (2002) 1184–1189, https://doi.org/10.1016/s1359-6446(02)02529-1.

[130] A.G. Beule, Physiology and pathophysiology of respiratory mucosa of the nose and the paranasal sinuses, GMS Curr. Top. Otorhinolaryngol. Head Neck Surg. 9 (2010), https://doi.org/10.3205/cto000071. Doc07.

[131] M. Agrawal, S. Saraf, S. Saraf, S.G. Antimisiaris, M.B. Chougule, S.A. Shoyele, A. Alexander, Nose-to-brain drug delivery: an update on clinical challenges and progress towards approval of anti-Alzheimer drugs, J. Control. Release 281 (2018) 139–177, https://doi.org/10.1016/j.jconrel.2018.05.011.

[132] BioProcess International, Ask the Expert: Translating Inhaled and Nasal Technologies for Biologics Delivery. https://ondrugdelivery.com/intranasal-delivery-of-biologic-therapeutics-and-vaccines/, 2024 (accessed 9 January 2024), in.

[133] K. Wanat, Biological barriers, and the influence of protein binding on the passage of drugs across them, Mol. Biol. Rep. 47 (2020) 3221–3231, https://doi.org/10.1007/s11033-020-05361-2.

[134] M.M. El-Sayed Khafagy, Noriyasu Kamei, Yoshimi Eda, Yohei Ikeno, Kozo Takayama, Efficiency of cell-penetrating peptides on the nasal and intestinal absorption of therapeutic peptides and proteins, Int. J. Pharm. 381 (2009) 49–55.
[135] M.M. El-Sayed Khafagy, Kozo Takayama, The role of intermolecular interactions with penetratin and its analogue on the enhancement of absorption of nasal therapeutic peptides, Int. J. Pharm. 388 (2010) 209–212.
[136] S. Gizurarson, The effect of cilia and the mucociliary clearance on successful drug delivery, Biol. Pharm. Bull. 38 (2015) 497–506, https://doi.org/10.1248/bpb.b14-00398.
[137] J.M. Heydel, P. Faure, F. Neiers, Nasal odorant metabolism: enzymes, activity and function in olfaction, Drug Metab. Rev. 51 (2019) 224–245, https://doi.org/10.1080/03602532.2019.1632890.
[138] M. Koparal, Y. Kapici, S. Aslan, S. Hepkarsi, M. Karataş, C. Yılmazer, Evaluation of nasal Mucociliary clearance as an Indicator of nasal function in obsessive-compulsive patients: a cross-sectional study, Indian, J. Otolaryngol. Head Neck Surg. 75 (2023) 3263–3267, https://doi.org/10.1007/s12070-023-03972-2.
[139] D. Cahn, G.A. Duncan, High-density branched PEGylation for nanoparticle drug delivery, Cell. Mol. Bioeng. 15 (2022) 355–366, https://doi.org/10.1007/s12195-022-00727-x.
[140] A. Froelich, T. Osmałek, B. Jadach, V. Puri, B. Michniak-Kohn, Microemulsion-based Media in Nose-to-Brain Drug Delivery, Pharmaceutics 13 (2021), https://doi.org/10.3390/pharmaceutics13020201.
[141] K. Kashyap, R. Shukla, Drug delivery and targeting to the brain through nasal route: mechanisms, applications and challenges, Curr. Drug Deliv. 16 (2019) 887–901, https://doi.org/10.2174/1567201816666191029122740.
[142] S. Ladel, P. Schlossbauer, J. Flamm, H. Luksch, B. Mizaikoff, K. Schindowski, Improved in vitro model for intranasal mucosal drug delivery: primary olfactory and respiratory epithelial cells compared with the permanent nasal cell line RPMI 2650, Pharmaceutics 11 (2019), https://doi.org/10.3390/pharmaceutics11080367.
[143] G. Patti, A. Ibba, G. Morana, F. Napoli, D. Fava, N. di Iorgi, M. Maghnie, Central diabetes insipidus in children: diagnosis and management, Best Pract. Res. Clin. Endocrinol. Metab. 34 (2020) 101440, https://doi.org/10.1016/j.beem.2020.101440.
[144] D.J. Kotak, P.V. Devarajan, Bone targeted delivery of salmon calcitonin hydroxyapatite nanoparticles for sublingual osteoporosis therapy (SLOT), Nanomedicine 24 (2020) 102153, https://doi.org/10.1016/j.nano.2020.102153.
[145] E.A. Eugster, Treatment of central precocious puberty, J. Endocr. Soc. 3 (2019) 965–972, https://doi.org/10.1210/js.2019-00036.
[146] T. Akita, Y. Oda, R. Kimura, M. Nagai, A. Tezuka, M. Shimamura, K. Washizu, J. I. Oka, C. Yamashita, Involvement of trigeminal axons in nose-to-brain delivery of glucagon-like peptide-2 derivative, J. Control. Release 351 (2022) 573–580, https://doi.org/10.1016/j.jconrel.2022.09.047.
[147] E.S. Von Zuben, J.O. Eloy, M.D. Inácio, V.H.S. Araujo, A.M. Baviera, M.P. D. Gremião, M. Chorilli, Hydroxyethylcellulose-based hydrogels containing liposomes functionalized with cell-penetrating peptides for nasal delivery of insulin in the treatment of diabetes, Pharmaceutics 14 (2022), https://doi.org/10.3390/pharmaceutics14112492.
[148] H.D. Bae, J. Lee, K.Y. Jun, Y. Kwon, K. Lee, Modification of translationally controlled tumor protein-derived protein transduction domain for improved intranasal delivery of insulin, Drug Deliv. 25 (2018) 1025–1032, https://doi.org/10.1080/10717544.2018.1464081.
[149] E.S. Khafagy, N. Kamei, Y. Fujiwara, H. Okumura, T. Yuasa, M. Kato, K. Arime, A. Nonomura, H. Ogino, S. Hirano, S. Sugano, M. Takeda-Morishita, Systemic and brain delivery of leptin via intranasal coadministration with cell-penetrating peptides and its therapeutic potential for obesity, J. Control. Release 319 (2020) 397–406, https://doi.org/10.1016/j.jconrel.2020.01.016.
[150] K.L. Hae-duck Bae, On employing a translationally controlled tumor protein-derived protein transduction domain analog for transmucosal delivery of drugs, J. Control. Release 170 (2013) 358–364.
[151] N.K. El-Sayed Khafagy, Ebbe Juel Bech Nielsen, Reiji Nishio, Mariko Takeda-Morishita, One-month subchronic toxicity study of cell-penetrating peptides for insulin nasal delivery in rats, Eur. J. Pharm. Biopharm. 85 (2013) 736–743.
[152] D.S. Southam, M. Dolovich, P.M. O'Byrne, M.D. Inman, Distribution of intranasal instillations in mice: effects of volume, time, body position, and anesthesia, Am. J. Phys. Lung Cell. Mol. Phys. 282 (2002) L833–L839, https://doi.org/10.1152/ajplung.00173.2001.
[153] S. Myou, X. Zhu, S. Myo, E. Boetticher, A.Y. Meliton, J. Liu, N.M. Munoz, A. R. Leff, Blockade of airway inflammation and hyperresponsiveness by HIV-TAT-dominant negative Ras, J. Immunol. 171 (2003) 4379–4384.
[154] J.L. Hae-Duck Bae, Kyu-Yeon Jun, Youngjoo Kwon, Kyunglim Lee, Modification of translationally controlled tumor protein-derived protein transduction domain for improved intranasal delivery of insulin, Drug Deliv. 25 (2018) 1025–1032.
[155] M.M. El-Sayed Khafagy, Nobuo Ida, Reiji Nishio, Koichi Isowa, Kozo Takayama, Structural requirements of penetratin absorption enhancement efficiency for insulin delivery, J. Control. Release 143 (2010) 302–310.
[156] N.J. Jiamin Wu, Nojoud A.L. Fayez, Po-Han Chao, Wu Angeline, Daniele Ribeiro, Elham de Araujo, Analisa Jia Rouhollahi, Shyh-Dar Li, Protamine-mediated efficient transcellular and transmucosal delivery of proteins, J. Control. Release 356 (2023) 373–385.
[157] M.K. Hae-Duck Bae, Joohyun Lee, Kyunglim Lee, Modified translationally controlled tumor protein-derived protein transduction domain enhances nasal delivery of exendin-4 as shown with insulin, Drug Deliv. 25 (2018) 1579–1584.
[158] R.K. Tomomi Akita, Saki Akaguma, Mio Nagai, Yusuke Nakao, Mamiko Tsugane, Hiroaki Suzuki, Jun-Ichiro Oka, Chikamasa Yamashita, Usefulness of cell-penetrating peptides and penetration accelerating sequence for nose-to-brain delivery of glucagon-like peptide-2, J. Control. Release 335 (2021) 575–583.
[159] J.L. David, D. Lo, A. Holly Eckelhoefe, M cell targeting by a Claudin 4 targeting peptide can enhance mucosal IgA responses, BMC Biotechnol. 12 (2012).
[160] N.K. El-Sayed Khafagy, Yui Fujiwara, Haruka Okumura, Teruyo Yuasa, Masahiro Kato, Kenji Arime, Anna Nonomura, Hideyuki Ogino, Serena Hirano, Sayaka Sugano, Mariko Takeda-Morishita, Systemic and brain delivery of leptin via intranasal coadministration with cell-penetrating peptides and its therapeutic potential for obesity, J. Control. Release 319 (2020) 397–406.
[161] A. Sánchez-Danés, C. Blanpain, Deciphering the cells of origin of squamous cell carcinomas, Nat. Rev. Cancer 18 (2018) 549–561, https://doi.org/10.1038/s41568-018-0024-5.
[162] F.J. Laine, W.R. Smoker, Oral cavity: anatomy and pathology, Semin. Ultrasound CT MR 16 (1995) 527–545, https://doi.org/10.1016/s0887-2171(06)80024-7.
[163] M.B.C. Maymone, R.O. Greer, L.K. Burdine, A. Dao-Cheng, S. Venkatesh, P. C. Sahitya, A.C. Maymone, J. Kesecker, N.A. Vashi, Benign oral mucosal lesions: clinical and pathological findings, J. Am. Acad. Dermatol. 81 (2019) 43–56, https://doi.org/10.1016/j.jaad.2018.09.061.
[164] J.O. Morales, P.R. Vuddanda, S. Velaga, Controlled drug delivery via the buccal and sublingual routes, in: Fundamentals of Drug Delivery, 2021, pp. 433–448.
[165] S. Hua, Advances in Nanoparticulate drug delivery approaches for sublingual and buccal administration, Front. Pharmacol. 10 (2019) 1328, https://doi.org/10.3389/fphar.2019.01328.
[166] Y.-G. Guo, A. Pratap Singh, Emerging strategies for enhancing buccal and sublingual administration of nutraceuticals and pharamaceuticals, J. Drug Deliv. Sci. Technol. 52 (2019) 440–451, https://doi.org/10.1016/j.jddst.2019.05.014.
[167] H. Kraan, H. Vrieling, C. Czerkinsky, W. Jiskoot, G. Kersten, J.P. Amorij, Buccal and sublingual vaccine delivery, J. Control. Release 190 (2014) 580–592, https://doi.org/10.1016/j.jconrel.2014.05.060.
[168] K.B. Christopher Squier, Human Oral Mucosa: Development, Structure and Function, 2010.
[169] J.A. Bartlett, K. van der Voort Maarschalk, Understanding the oral mucosal absorption and resulting clinical pharmacokinetics of asenapine, AAPS PharmSciTech 13 (2012) 1110–1115, https://doi.org/10.1208/s12249-012-9839-7.
[170] V. Trincado, R.P. Gala, J.O. Morales, Buccal and sublingual vaccines: a review on Oral mucosal immunization and delivery systems, Vaccines (Basel) 9 (2021), https://doi.org/10.3390/vaccines9101177.
[171] A. Talevi, The ADME Encyclopedia, 2022.
[172] S.G. Antimisiaris, A. Marazioti, M. Kannavou, E. Natsaridis, F. Gkartziou, G. Kogkos, S. Mourtas, Overcoming barriers by local drug delivery with liposomes, Adv. Drug Deliv. Rev. 174 (2021) 53–86, https://doi.org/10.1016/j.addr.2021.01.019.
[173] A.W. Lim, N.J. Talley, M.M. Walker, G. Storm, S. Hua, Current status and advances in esophageal drug delivery technology: influence of physiological, pathophysiological and pharmaceutical factors, Drug Deliv. 30 (2023) 2219423, https://doi.org/10.1080/10717544.2023.2219423.
[174] G. Iorgulescu, Saliva between normal and pathological. Important factors in determining systemic and oral health, J. Med. Life 2 (2009) 303–307.
[175] E. Kubala, P. Strzelecka, M. Grzegocka, D. Lietz-Kijak, H. Gronwald, P. Skomro, E. Kijak, A review of selected studies that determine the physical and chemical properties of saliva in the field of dental treatment, Biomed. Res. Int. 2018 (2018) 6572381, https://doi.org/10.1155/2018/6572381.
[176] L. Togni, M. Mascitti, A. Santarelli, M. Contaldo, A. Romano, R. Serpico, C. Rubini, Unusual conditions impairing saliva secretion: developmental anomalies of salivary glands, Front. Physiol. 10 (2019) 855, https://doi.org/10.3389/fphys.2019.00855.
[177] Y. Xu, X. Zhang, N. Wang, X. Pei, Y. Guo, J. Wang, S. Barth, F. Yu, S.J. Lee, H. He, V.C. Yang, Cell-penetrating peptide enhanced insulin buccal absorption, Int. J. Pharm. 584 (2020) 119469, https://doi.org/10.1016/j.ijpharm.2020.119469.
[178] H. Spleis, M. Sandmeier, V. Claus, A. Bernkop-Schnürch, Surface design of nanocarriers: key to more efficient oral drug delivery systems, Adv. Colloid Interf. Sci. 313 (2023) 102848, https://doi.org/10.1016/j.cis.2023.102848.
[179] T. Keum, G. Noh, J.E. Seo, S. Bashyal, S. Lee, In vitro and ex vivo evaluation of Penetratin as a non-invasive permeation enhancer in the penetration of Salmon calcitonin through TR146 buccal cells and porcine buccal tissues, Pharmaceuticals (Basel) 13 (2020), https://doi.org/10.3390/ph13110408.
[180] K.M. Van de Graaff, Anatomy and physiology of the gastrointestinal tract, Pediatr. Infect. Dis. 5 (1986) S11–S16, https://doi.org/10.1097/00006454-198601001-00005.
[181] Z. Vinarov, M. Abdallah, J.A.G. Agundez, K. Allegaert, A.W. Basit, M. Braeckmans, J. Ceulemans, M. Corsetti, B.T. Griffin, M. Grimm, D. Keszthelyi, M. Koziolek, C.M. Madla, C. Matthys, L.E. McCoubrey, A. Mitra, C. Reppas, J. Stappaerts, N. Steenackers, N.L. Trevaskis, T. Vanuytsel, M. Vertzoni, W. Weitschies, C. Wilson, P. Augustijns, Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review, Eur. J. Pharm. Sci. 162 (2021) 105812, https://doi.org/10.1016/j.ejps.2021.105812.
[182] S. Hua, Advances in oral drug delivery for regional targeting in the gastrointestinal tract - influence of physiological, pathophysiological and pharmaceutical factors, Front. Pharmacol. 11 (2020) 524, https://doi.org/10.3389/fphar.2020.00524.
[183] M. Müller, E.E. Canfora, E.E. Blaak, Gastrointestinal transit time, glucose homeostasis and metabolic health: modulation by dietary fibers, Nutrients 10 (2018), https://doi.org/10.3390/nu10030275.

[184] S. Bodé, M. Dreyer, G. Greisen, Gastric emptying and small intestinal transit time in preterm infants: a scintigraphic method, J. Pediatr. Gastroenterol. Nutr. 39 (2004) 378–382, https://doi.org/10.1097/00005176-200410000-00014.
[185] Y. Zheng, F. Han, A. Ho, Y. Xue, Z. Wu, X. Chen, J.K. Sandberg, S. Ma, E. Leeansyah, Role of MAIT cells in gastrointestinal tract bacterial infections in humans: more than a gut feeling, Mucosal Immunol. 16 (2023) 740–752, https://doi.org/10.1016/j.mucimm.2023.06.005.
[186] Z. Davoudi, N. Peroutka-Bigus, B. Bellaire, A. Jergens, M. Wannemuehler, Q. Wang, Gut organoid as a new platform to study alginate and chitosan mediated PLGA nanoparticles for drug delivery, Mar. Drugs 19 (2021), https://doi.org/10.3390/md19050282.
[187] H.F. Helander, L. Fändriks, Surface area of the digestive tract - revisited, Scand. J. Gastroenterol. 49 (2014) 681–689, https://doi.org/10.3109/00365521.2014.898326.
[188] A. Elsayed, M. Al-Remawi, N. Jaber, K.M. Abu-Salah, Advances in buccal and oral delivery of insulin, Int. J. Pharm. 633 (2023) 122623, https://doi.org/10.1016/j.ijpharm.2023.122623.
[189] M. Durán-Lobato, Z. Niu, M.J. Alonso, Oral delivery of biologics for precision medicine, Adv. Mater. 32 (2020) e1901935, https://doi.org/10.1002/adma.201901935.
[190] S. Bashir, R. Fitaihi, H.E. Abdelhakim, Advances in formulation and manufacturing strategies for the delivery of therapeutic proteins and peptides in orally disintegrating dosage forms, Eur. J. Pharm. Sci. 182 (2023) 106374, https://doi.org/10.1016/j.ejps.2023.106374.
[191] H. Panwar, N. Rokana, S.V. Aparna, J. Kaur, A. Singh, J. Singh, K.S. Singh, V. Chaudhary, A.K. Puniya, Gastrointestinal stress as innate defence against microbial attack, J. Appl. Microbiol. 130 (2021) 1035–1061, https://doi.org/10.1111/jam.14836.
[192] R. Wall, R.P. Ross, C.A. Ryan, S. Hussey, B. Murphy, G.F. Fitzgerald, C. Stanton, Role of gut microbiota in early infant development, Clin. Med. Pediatr. 3 (2009) 45–54, https://doi.org/10.4137/cmped.s2008.
[193] A. Awad, C.M. Madla, L.E. McCoubrey, F. Ferraro, F.K.H. Gavins, A. Buanz, S. Gaisford, M. Orlu, F. Siepmann, J. Siepmann, A.W. Basit, Clinical translation of advanced colonic drug delivery technologies, Adv. Drug Deliv. Rev. 181 (2022) 114076, https://doi.org/10.1016/j.addr.2021.114076.
[194] V.K. Pawar, J.G. Meher, Y. Singh, M. Chaurasia, B. Surendar Reddy, M. K. Chourasia, Targeting of gastrointestinal tract for amended delivery of protein/peptide therapeutics: strategies and industrial perspectives, J. Control. Release 196 (2014) 168–183, https://doi.org/10.1016/j.jconrel.2014.09.031.
[195] S. Bengmark, Gut microbiota, immune development and function, Pharmacol. Res. 69 (2013) 87–113, https://doi.org/10.1016/j.phrs.2012.09.002.
[196] H. Elzayat, G. Mesto, F. Al-Marzooq, Unraveling the impact of gut and Oral microbiome on gut health in inflammatory bowel diseases, Nutrients 15 (2023), https://doi.org/10.3390/nu15153377.
[197] X. Tan, Y. Zhang, Q. Wang, T. Ren, J. Gou, W. Guo, T. Yin, H. He, Y. Zhang, X. Tang, Cell-penetrating peptide together with PEG-modified mesostructured silica nanoparticles promotes mucous permeation and oral delivery of therapeutic proteins and peptides, Biomater. Sci. 7 (2019) 2934–2950, https://doi.org/10.1039/c9bm00274j.
[198] F. Guo, T. Ouyang, T. Peng, X. Zhang, B. Xie, X. Yang, D. Liang, H. Zhong, Enhanced oral absorption of insulin using colon-specific nanoparticles co-modified with amphiphilic chitosan derivatives and cell-penetrating peptides, Biomater. Sci. 7 (2019) 1493–1506, https://doi.org/10.1039/c8bm01485j.
[199] E.-S.K. Yu Fukuoka, Takahiro Goto, Noriyasu Kamei, Kozo Takayama, Nicholas A. Peppas, Mariko Takeda-Morishita, Combination Strategy with Complexation Hydrogels and Biological and Pharmaceutical Bulletin 41, 2018, pp. 811–814.
[200] L.Y. Lei Li, Manman Li, Liefeng Zhang, A cell-penetrating peptide mediated chitosan nanocarriers for improving intestinal insulin delivery, Carbohydr. Polym. 174 (2017) 182–189.
[201] H.H. Jianyong Sheng, Limei Han, Jing Qin, Sunhui Chen, Ru Ge, Ruixiang Li, Pei Yang, Jianxin Wang, Victor C. Yang, Enhancing insulin oral absorption by using mucoadhesive nanoparticles, J. Control. Release 233 (2016) 181–190.
[202] B. Hasannejad-Asl, F. Pooresmaeil, S. Takamoli, M. Dabiri, A. Bolhassani, Cell penetrating peptide: a potent delivery system in vaccine development, Front. Pharmacol. 13 (2022) 1072685, https://doi.org/10.3389/fphar.2022.1072685.
[203] S. Lim, J.H. Koo, J.M. Choi, Use of cell-penetrating peptides in dendritic cell-based vaccination, Immune Netw. 16 (2016) 33–43, https://doi.org/10.4110/in.2016.16.1.33.
[204] E. Koren, V.P. Torchilin, Cell-penetrating peptides: breaking through to the other side, Trends Mol. Med. 18 (2012) 385–393, https://doi.org/10.1016/j.molmed.2012.04.012.
[205] P. Zakeri-Milani, S. Najafi-Hajivar, M. Sarfraz, A. Nokhodchi, H. Mohammadi, S. Montazersaheb, M. Niazi, M. Hemmatzadeh, M. Soleymani-Goloujeh, B. Baradaran, J. Shahbazi Mojarrad, M. Farshbaf, T. Gholikhani, H. Valizadeh, Cytotoxicity and immunogenicity evaluation of synthetic cell-penetrating peptides for methotrexate delivery, Iran, J. Pharm. Res. 20 (2021) 506–515, https://doi.org/10.22037/ijpr.2021.114429.14842.
[206] C.M. Backlund, R.L. Holden, K.D. Moynihan, D. Garafola, C. Farquhar, N. K. Mehta, L. Maiorino, S. Pham, J.B. Iorgulescu, D.A. Reardon, C.J. Wu, B. L. Pentelute, D.J. Irvine, Cell-penetrating peptides enhance peptide vaccine accumulation and persistence in lymph nodes to drive immunogenicity, Proc. Natl. Acad. Sci. USA 119 (2022) e2204078119, https://doi.org/10.1073/pnas.2204078119.
[207] N.Q. Shi, X.R. Qi, B. Xiang, Y. Zhang, A survey on “Trojan horse” peptides: opportunities, issues and controlled entry to “Troy”, J. Control. Release 194 (2014) 53–70, https://doi.org/10.1016/j.jconrel.2014.08.014.
[208] W.P. Verdurmen, R. Brock, Biological responses towards cationic peptides and drug carriers, Trends Pharmacol. Sci. 32 (2011) 116–124, https://doi.org/10.1016/j.tips.2010.11.005.
[209] D. Kalafatovic, E. Giralt, Cell-penetrating peptides: design strategies beyond primary structure and Amphipathicity, Molecules 22 (2017), https://doi.org/10.3390/molecules22111929.
[210] Y. Lobaina, D. Urquiza, H. Garay, Y. Perera, K. Yang, Evaluation of cell-penetrating peptides as mucosal immune enhancers for nasal vaccination, Int. J. Pept. Res. Ther. 27 (2021) 2873–2882, https://doi.org/10.1007/s10989-021-10296-8.
[211] S.F. Dowdy, C.J. Gallagher, D. Vitarella, J. Brown, A technology evaluation of the atypical use of a CPP-containing peptide in the formulation and performance of a clinical botulinum toxin product, Expert Opin. Drug Deliv. 20 (2023) 1157–1166, https://doi.org/10.1080/17425247.2023.2251399.
[212] FDA Allows At-Home Dosing of Intranasal Foralumab for Multiple Sclerosis. https://www.neurologylive.com/view/fda-allows-home-dosing-intranasal-foralumab-ms, 2023.
[213] S. Niman, J. Hardy, R.F. Goldfaden, J. Reid, M. Sheikh-Ali, D. Sutton, R. Choksi, A review on the efficacy and safety of Oral Semaglutide, Drugs R D 21 (2021) 133–148, https://doi.org/10.1007/s40268-021-00341-8.
[214] N. Kamei, S. Kikuchi, M. Takeda-Morishita, Y. Terasawa, A. Yasuda, S. Yamamoto, N. Ida, R. Nishio, K. Takayama, Determination of the optimal cell-penetrating peptide sequence for intestinal insulin delivery based on molecular orbital analysis with self-organizing maps, J. Pharm. Sci. 102 (2013) 469–479, https://doi.org/10.1002/jps.23364.
[215] M. Kristensen, H.M. Nielsen, Cell-penetrating peptides as tools to enhance non-injectable delivery of biopharmaceuticals, Tissue Barri. 4 (2016) e1178369, https://doi.org/10.1080/21688370.2016.1178369.
[216] A. Erazo-Oliveras, K. Najjar, L. Dayani, T.Y. Wang, G.A. Johnson, J.P. Pellois, Protein delivery into live cells by incubation with an endosomolytic agent, Nat. Methods 11 (2014) 861–867, https://doi.org/10.1038/nmeth.2998.
[217] Y. Liu, Z. Zhao, M. Li, Overcoming the cellular barriers and beyond: recent progress on cell penetrating peptide modified nanomedicine in combating physiological and pathological barriers, Asian, J. Pharm. Sci. 17 (2022) 523–543, https://doi.org/10.1016/j.ajps.2022.05.002.
[218] G. Guidotti, L. Brambilla, D. Rossi, Cell-penetrating peptides: from basic research to clinics, Trends Pharmacol. Sci. 38 (2017) 406–424, https://doi.org/10.1016/j.tips.2017.01.003.
[219] CellGate Announces Phase IIa Study Results of PsorBan in the Treatment of Psoriasis. http://test.pharmabiz.com/news/cellgate-announces-phase-iia-study-results-of-psorban-in-the-treatment-of-psoriasis-15396, 2003.