Buccal delivery of small molecules and biologics: of mucoadhesive polymers, films, and nanoparticles☆

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Buccal delivery of macromolecules (biologics) sets a great challenge for researchers. Although several niche small molecule products have been approved as simple sprays, tablets and oral films, it is not simply a case of adapting existing technologies to biologics. Buccal delivery of insulin has reached clinical trials with two approaches: oromucosal sprays of the peptide with permeation enhancers, and embedded gold nanoparticles in a dissolvable film. However, neither of these approaches have led to FDA approvals likely due to poor efficacy, submaximal peptide loading in the dosage form, and to wide intra-subject variability in pharmacokinetics and pharmacodynamics. It is likely however that printed film designs with lower molecular weight stable biotech payloads including lipophilic glucagon-like 1 (GLP-1) agonists and macrocycles with long half-lives will generate greater efficacy than was achieved to date for insulin.

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Introduction

The buccal route of administration has been investigated for decades as a site for drug absorption to reach systemic circulation as an alternative to the conventional oral or the more efficient intravenous routes [1•,2]. The buccal epithelium is located in the inner mucosal side of cheeks, and together with the sublingual epithelium, it is non-keratinized as opposed to other regions of the oral cavity. As a stratified epithelium with about 40–50 cell layers accounting for a 400–700 μm thick epithelium (variability due to invaginations) and a surface area of about 50 cm^2 [3,4], the buccal route allows permeation of conventional low molecular weight small molecules. Similar to other mucosal barriers in the body, absorption depends on the molecule's physicochemical properties, their interaction with cell membranes, and the selected delivery system or dosage form selected for administration [2,5]. The main permeability barrier identified in this tight junction-free epithelium is in the upper third cell layers, where a lipid-rich domain is found [6]. As such, small lipophilic molecules permeate faster than hydrophilic molecules, and markedly more so than biologics. It is generally understood that biologics including peptides and proteins permeate poorly across the buccal epithelium, with macromolecules only being absorbed in the presence of chemical- and electrical-based permeation enhancement approaches [7,8]. This review is a critique of the most recent advancements in strategies to enhance bioavailability of macromolecules delivered via the buccal route.

Mucoadhesive polymers for buccal drug delivery

In order to achieve systemic circulation, drug molecules permeating through the buccal epithelium need to remain located in the dosage form at the mucosa for extended time, which depends on the physicochemical properties of molecule, the dosage form, and the molecule's permeation kinetics across the buccal epithelium. Both the mechanical effects of the tongue and the salivary washout can quickly remove a dosage form from the buccal epithelium. In development of buccal dosage forms, mucoadhesive polymers have been used in formulations to achieve extended contact time and to enhance buccal bioavailability. Initial strategies in mucoadhesion included the use of hydrophilic polymers, especially the use of cationic ones, as the latter provide a favorable electrostatic interaction with the anionic groups of mucin [9]. First generation mucoadhesive polymers relied in non-covalent interactions with mucin via polymer chain entanglement and electrostatic pairing in order to achieve the required mucoadhesive bond. The next generation of mucoadhesive polymers further improved the strength of covalent bonding using thiol-derived

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☆ Focused theme issue: Drug-device combinations to solve unmet medical needs.

Figure 1
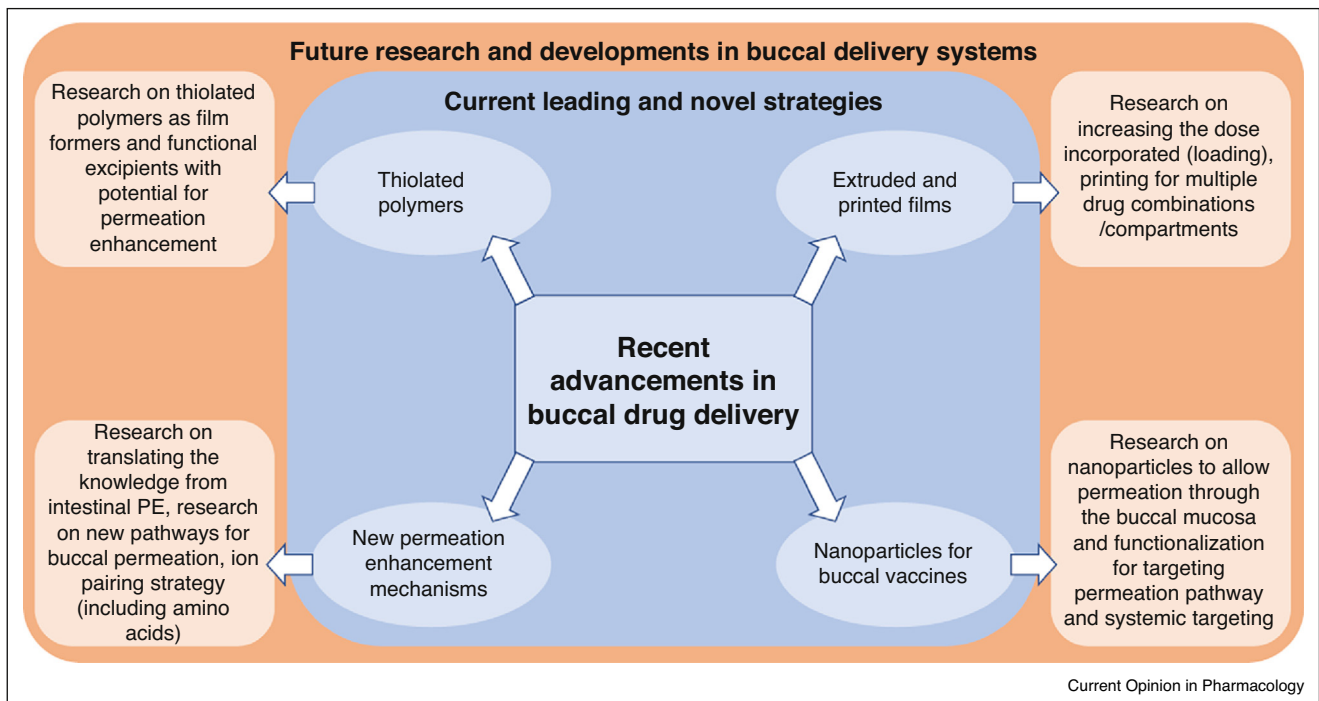
Current developments in buccal drug delivery and the direction of future research.

polymer chains that attached to the cysteine groups of mucin resulting in thiolated polymers or "thiomers" [10]. Greater mucoadhesive efficacy in attaching to the buccal mucosa of thiomers has been confirmed compared with unmodified polymers [11–13]. Thiomers have therefore been used in formulations for buccal tablets, wafers, gels, and films, illustrating great versatility as excipients regardless of the dosage form (Figure 1) [14–16].

Recent advancements in films as mucoadhesive buccal delivery systems

The buccal drug delivery field has seen developments in dosage form design over the past decades including tablets, lozenge, sprays, mouthwashes, gels, and films [1•]. While bioadhesive tablets continue to be developed as buccal dosage forms due to established industrial standard manufacturing processes and high dose capacity, in parallel they also present conventional matrices for sustained drug release. In recent years however, much research has moved toward bioadhesive and biocompatible film development for buccal drug delivery. Films comprise several useful characteristics for the buccal route: 1) they are thin and flexible and can adjust to the oral mucosa contours and can cope with mechanical stress; 2) their administration is simple and, due to bioadhesion, they can remain in place for the duration of absorption; 3) due to the flexibility of the manufacturing process (either solvent casting, hot melt extrusion, and inkjet printing) several functions can be combined,

including incorporation of multiple drug layers and multiple release profile layers [17]. Due to the recognized safety of the excipient materials used to synthesize buccal films, (typically demonstrated *in vitro* [18]), they have been proposed as especially useful delivery systems for drugs used in pediatric therapy [19–21]. The main limitation of films is related to the relatively low concentration of the active phamaceutical ingredient (API) that can be formulated within a structure of limited dimensions, although recent research in inkjet printing and hot melt extrusion may resolve this [22•,23,24].

The use of inkjet printing to produce drug-loaded buccal films has brought about significant advances [17,22•], and there are now numerous printing strategies being researched for manufacture [23,25,26]. Although still at the pre-clinical research stage, printing could address the dose limitation issue by optimizing the printing process using droplet formation mechanisms in inkjet cartridges. The printing concept can also provide personalized dosage forms to patients (Figure 2) [27,28]. Aside from the potential of printing buccal films, solvent casting is typically the main method of manufacture, as it allows formulation of small and large molecules due to its flexibility [17]. As an alternative to solvent casting, hot melt extrusion is another method to manufacture films. Recently domperidone was incorporated in PEO (poly(ethylene oxide) N750 and Hydroxypropyl methylcellulose (HPMC) E5 LV matrices by hot melt extrusion and these
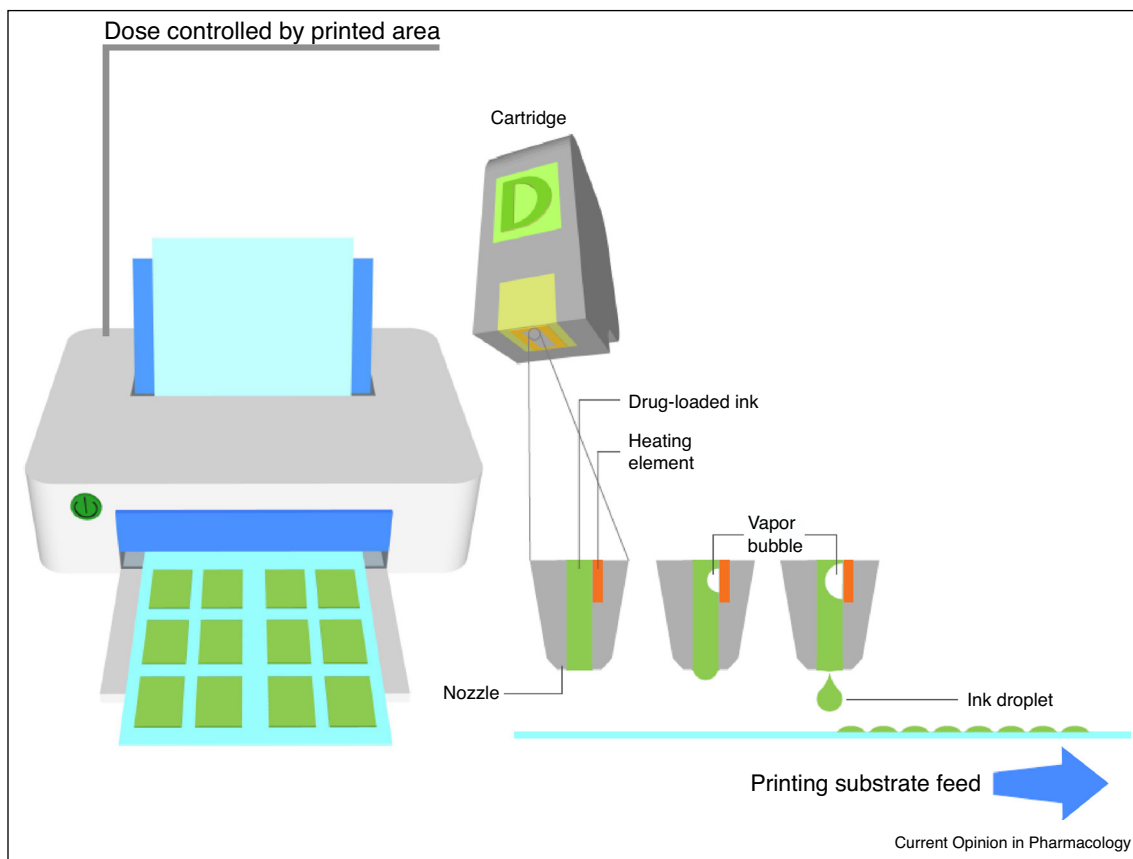
Figure 2

Diagram of the process of buccal film manufacture using a thermal inkjet printer.
Reprinted with permission from [22•].

formulations were optimized for tensile strength, appropriate drug release profiles, and epithelial permeation *ex vivo* over a 6 hour period [24]. The optimized buccal extruded formulation exhibited 3.2 times higher bioavailability in comparison with orally-administered domperidone and achieved an *ex vivo–in vivo* type A correlation. Hot melt extruded dosage forms for buccal delivery have also been demonstrated to accommodate other small molecules for enhanced dissolution and bioavailability (Figure 1) [29,30]. Due to the levels of heat exposure during extrusion however, biologic-loaded matrices using the proteins, nisin and lysozyme, were degraded [17,31,32]. However, recent investigations in the optimization of extrusion conditions with plasticizers used have shown potential in formulating biologics in extruded polymer matrices [33] and this adaptation could increase use of extrusion for macromolecules.

Nanoparticles and microparticles enhance delivery across the buccal epithelium

Although it is a stratified epithelium with limited permeation for macromolecules, the buccal mucosa has been investigated as a delivery site for particulates. Among these studies, several describe the use of nanoparticles as means to formulate poorly water soluble molecules and achieve higher buccal bioavailability by increasing dissolution [34–37]. Similarly, others have focused on drug-releasing nanoparticles by quantifying drug permeation across the epithelium, although the mechanism for translocation has yet to be elucidated [38•,39–43]. Evidence of intact nanoparticle permeation across the buccal epithelium has been suggested from investigations using model nanoparticles. For example, studies of inorganic nanoparticles, including those made from silver (19 nm in diameter) [44] and titanium dioxide ($\sim$30–150 nm in diameter) [45], indicate a relationship between nanoparticle permeation and their physicochemical properties. Also pertaining to use of nanoparticles as drug carriers, polymeric nanoparticle permeation studies show that a combination of particle diameter and agglomeration properties influence particle permeability across the buccal epithelium [46,47]. Specifically, a study of 20 and 200 nm diameter anionic carboxyated-modified (20 and 200 nm diameter) and cationic amine-modified polystyrene nanoparticles (200 nm) revealed that the anionic versions were less efficient than cationic particles in permeating isolated

porcine buccal tissue in Franz Cell diffusion experiments [46]. While the larger diameter 200 nm anionic nanoparticles agglomerated and failed to permeate, the smaller 20 nm anionic particles permeated to the top third region of the buccal epithelium by the transcellular route. On the other hand, while the 200 nm cationic nanoparticles had a tendency to agglomerate, they had the capacity to permeate into lower regions of the buccal epithelium by endocytotic mechanisms [46]. A study examining the fate of neutral polystyrene nanoparticles highlighted the role of mucus and microplicae as barriers to nanoparticle permeation: 200 nm neutral nanoparticles penetrated faster and to deeper regions of the buccal epithelium in comparison to 25 and 50 nm particles in isolated porcine buccal mucosae [47].

The buccal mucosa has also been examined as a potential site for vaccine administration. Compared to other mucosal sites, the buccal mucosa is an easily accessible mucosa [48], and is rich in antigen presenting cells that can mediate innate and adaptive immune responses to battle local and systemic infection [49]. In order to overcome the delivery barriers associated with rapid clearance by saliva and tongue movement, several strategies have been developed to achieve buccal vaccination, (typically in murine models) including supersaturation, nanoparticulates, nanofibers, iontophoresis, electroporation, and mucoadhesion (Figure 1) [50,51,52•]. Recently, a multi-layered mucoadhesive film including an electrospun nanofiber layer was developed in order to increase vaccine loading by exploiting the large surface area provided by the nanofibers. PEGylated liposomes and PLGA nanoparticles were used as delivery carriers and penetrated the porcine sublingual epithelium and were recognized by dendritic cells both *ex vivo* and *in vivo* [53]. In another attempt to increase contact time and exposure to delivery systems, Zhen *et al*. developed liposome-loaded microneedles for convenient and stable mucosal vaccination of mice [54]. After oral mucosal administration to mice, the microneedle system achieved robust systemic and mucosal immune responses against bovine serum albumin in comparison with conventional intradermal administration. This strategy was also used to develop a murine oral mucosa vaccine against hepatitis B virus using liposomes-loaded microneedles. The vaccine was stable for up to 3 days at 40 °C and able to elicit a strong systemic response comparable to intradermal and subcutaneous routes, where there was a much stronger mucosal response after oral mucosal administration with microneedles [55]. A caveat to some of these studies is that the physiological relevance of the murine oral mucosa buccal or sublingual epithelium to man is questionable due to differences in surface area and the degree of keratinization, however it is more practical to do immunology in mice compared to large animal models due to the availability of mouse-specific reagents.

Buccal permeation-enhancing strategies

The use of permeation (or penetration) enhancers (PE) to improve buccal bioavailability has been studied extensively due to both the barrier feature of the buccal epithelium and its capacity to tolerate permeation enhancers. While mucoadhesive systems have been described as a method for permeation enhancement, functional excipients that modify the physicochemical properties of the barrier are normally assigned the term "permeation enhancers", also described as "chemical enhancers". Surfactants, bile salts, and fatty acids have been used as PEs in buccal dosage form development with permeability increases seen both small drug molecules and biologics in a variety of bioassays [9,56,57]. More recently, amino acids have been studied as PEs for buccally-administered insulin (Figure 1). The cationic amino acids lysine and histidine, and the anionic amino acids glutamic acid and aspartic acid enhanced insulin permeation across human filter-grown TR146 buccal monolayers to different degrees, but without damaging the cell barrier or the insulin [58]. It was hypothesized that due to the ionic state of the amino acids and insulin, ion-pairing resulted in non-covalent complexes that could exploit the amino acid-mediated transport for enhanced insulin permeation through the epithelial model, while being non-cytotoxic at effective concentrations compared to the narrow window of permeability/cytotoxic concentrations seen with the bile salt, sodium deoxycholate [58]. It is important to note that comparison of permeability between the human TR146 model and isolated porcine buccal tissue mucosae using PEs is somewhat problematic, as the monolayers are less resilient and have higher basal permeability compared to the tissue mucosae. Iontophoresis is a method to enhance the permeation of molecules through biological barriers by applying an external electric potential and thus generating a flow of ionic hydrophilic molecules. This method has been successfully used in enhancing the permeation of small molecules and biologics through porcine model buccal and oesophageal epithelia as well as *in vivo* [8,59–61]. Ren *et al*. have shown that iontophoresis can enable drug transport even in keratinized palate regions of the oral cavity mucosa, highlighting its potential for permeation enhancement in periodontal disease [62].

Clinical translation of buccally-administered molecules

The initial products for the buccal route were developed for a local effect, and only more recently have small molecule products exploited the concept of buccal absorption aimed at systemic drug delivery [17]. While used successfully for marketed small drug molecules (fentanyl, nicotine, ondansetron, donepezil, risperidone, diphenhydramine, dextromethorphan, phenylephrine, buprenorphine, and naloxone) (Table 1), limited success with buccal delivery has been achieved to date for biologics. Amongst macromolecules, buccal delivery of

Table 1

List of pharmaceutical products for buccal drug delivery. Adapted with permission from ref. [17]

Drug and doses	Therapeutic use	Target	Manufacturer	Region of commercialization
Breakyl® (fentanyl; 200, 400, 600, 800 and 1200 μg)	Narcotic pain relief	Systemic	Lohmann Therapie-Systeme AG, Germany	Europe
Onsolis® (fentanyl; 200, 400, 600, 800 and 1200 μg)	Narcotic pain relief	Systemic		USA
Niquitin® (nicotine; 2.5 mg)	Smoking cessation	Systemic		Europe
Setofilm® (ondansetron; 4 and 8 mg)	Antiemetic	Systemic		Europe
Effentora® (fentanyl; 100, 200, 400, 600, and 800 μg)	Narcotic pain relief	Systemic	TEVA Pharma BV, Israel	Europe
Chloraseptic® (benzocaine; 2 mg)	Sore throat relief	Local	Prestige Brands Inc	USA
Donepezil Hexal® SF (donepezil; 5 mg and 10 mg)	Alzheimer's treatment	Systemic	Hexal AG, Germany	Europe
Gas-X® (simethicone; 62.5 mg)	Anti-flatulence	Local	GSK, USA	USA
Risperidone Hexal ®SF (risperidone; 0.5, 1, 2 and 3 mg)	Neuroleptic	Systemic	Hexal AG, Germany	Europe
Triaminic® or Theraflu® night time (diphenhydramine, phenylephrine; 12.5 mg/5 mg)	Cough and cold	Systemic	Novartis, Switzerland	USA/Europe
Triaminic® or Theraflu® day time (dextromethorphan, phenylephrine; 5 mg/2.5 mg)	Cough and cold	Systemic	Novartis, Switzerland	USA/Europe
Triaminic® or Theraflu® (diphenhydramine; 12.5 mg)	Allergy	Systemic	Novartis, Switzerland	USA/Europe
Suboxone® (buprenorphine, naloxone)	Opioid addiction	Systemic	Reckitt Benckiser, UK	USA

insulin has been most widely investigated strategy in order to overcome effects of food on absorption that are expected to occur after oral administration [17,56,57]. Oral-lyn™ by Generex (Canada) is a micellar insulin solution buccal spray in combination with the PEs, bile salts and sodium caprate, and has been approved and commercialized in Ecuador and Lebanon, but has been discontinued in India pending more evidence of clinical efficacy evidence. It has been under review by the FDA since 2011 without gaining approval. Part of the problem is that it is reported to require up to 12 puffs to achieve delivery, and so is viewed as quite an inefficient system requiring a complicated administration protocol for patients. In another clinical development for buccal insulin, MonoSol Rx (USA) and Midatech (USA) collaborated to try and develop a buccal insulin delivery formulation, PharmaFilm®, in which recombinant human insulin was non-covalently bound to gold glycan-coated nanoparticles and then embedded in a film. After encouraging results in a Phase I clinical trial, the program was recently terminated after a Phase II trial that revealed low buccal insulin bioavailability [63•]. Current clinical trials for buccal

delivery of small molecules and macro molecules is summarized (Table 2). The challenge to deliver biologics across the buccal epithelium therefore remains as difficult as ever. Nonetheless, there is interest in testing the buccal route for formulated stable low molecular weight macromolecules with long half-lives, for example, lipophilic GLP-1 agonist analogues and macrocycles. Table 1 shows marketed and clinical phase small molecules and macromolecules.

Conclusions and future directions

Buccal delivery of macromolecules including peptides and proteins is one of the delivery routes less investigated compared to the oral or pulmonary routes. Successful approaches to formulating small molecules in biocompatible films involve solvent casting and so far to a lesser extent, hot melt extrusion and ink-jet printing. In terms of formulating biologics for buccal delivery, permeability and stability at the buccal mucosa seem to be increased compared to oral formulations, but the downside is the difficulty in achieving high loading of API in the low surface area bioadhesive systems. The drivers for buccal

Table 2

Completed and ongoing clinical trials (from 2008 to date) on formulations designed for systemic delivery following for buccal administration

Clinical trials	Indication	Phase	Identifier
Buccal prochlorperazine versus i.v. prochlorperazine for migraine, a RCT	Migraine	Phase III	NCT02779959
A comparison of sublingual and buccal misoprostol regimens after mifepristone for mid-trimester abortion	Legally-induced abortion	Phase IV	NCT02708446
Pharmacokinetics and pharmacodynamics of oral transmucosal dexmedetomidine	Sedation	Phase II, phase III	NCT03120247
Nasal fentanyl and buccal midazolam	Pain relief in terminal cancer	Phase IV	NCT02009306
The use of oxytocin, carbetocin and buccal misoprostol in patients undergoing elective cesarean section	Postpartum hemorrhage	Phase III	NCT02053922
Active comparator study of Generex's (Canada) Oral-lyn™ insulin spray with injected human insulin	Type 1 and 2 diabetes mellitus	Phase III	NCT00668850
Use of Generex's Oral-lyn™ insulin	Type 1 and 2 diabetes mellitus	Phase III	NCT00948493

delivery of peptides and proteins are the avoidance of the liver first pass effect, the potential for rapid delivery, and also the avoidance of potential food effects on pharmacokinetics. The current research approach for such formulations is to formulate the molecule in malleable biocompatible thin films, while iterations are to embed nanoparticles with payload and PEs in similar systems. Buccal delivery has also generated recent interest from transdermal delivery researchers, who are starting to leverage dissolvable microneedles for this route. As such, true progress in buccal delivery for macromolecules requires study of compatibility of drug-device combinations, and this requires collaboration between bioengineers, pharmaceutical formulators, and pharmacologists.

Conflict of interest statement
JOM reports no conflict of interest. DB consults for Pharma interested in non-injected peptide delivery. The authors alone are responsible for the content and writing of this article.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

1. Rathbone MJ, Pather I, Senel S: **Overview of oral mucosal**
• **delivery (Internet)**. In *Oral Mucosal Drug Delivery and Therapy*. Edited by Rathbone MJ, Senel S, Pather I. Springer US; 2015:17-29.
Seminal recent textbook on buccal delivery.

2. Morales JO, Fathe KR, Brunaugh A, Ferrati S, Li S, Montenegro-Nicolini M, Mousavikhamene Z, McConville JT, Prausnitz MR, Smyth HDC: **Challenges and future prospects for the delivery of biologics: oral mucosal, pulmonary, and transdermal routes**. *AAPS J* 2017 http://dx.doi.org/10.1208/s12248-017-0054-z.

3. Squier CA, Wertz PW: **Structure and function of the oral mucosa and implications for drug delivery**. In *Oral Mucosal Drug Delivery*. Edited by Rathbone MJ. Informa Health Care; 1996.

4. Gandhi RB, Robinson JR: **Oral cavity as a site for bioadhesive drug delivery**. *Adv Drug Deliv Rev* 1994, **13**:43-74.

5. Sattar M, Sayed OM, Lane ME: **Oral transmucosal drug delivery – current status and future prospects**. *Int J Pharm* 2014, **471**:498-506.

6. Law S, Wertz PW, Swartzendruber DC, Squier CA: **Regional variation in content, composition and organization of porcine epithelial barrier lipids revealed by thin-layer chromatography and transmission electron microscopy**. *Arch Oral Biol* 1995, **40**:1085-1091.

7. Hoogstraate AJ, Senel S, Cullander C, Verhoef J, Junginger HE, Boddé HE: **Effects of bile salts on transport rates and routes of FITC-labelled compounds across porcine buccal epithelium in vitro**. *J Controlled Release* 1996, **40**:211-221.

8. Patel MP, Churchman ST, Cruchley AT, Braden M, Williams DM: **Electrically induced transport of macromolecules through oral buccal mucosa**. *Dent Mater* 2013, **29**:674-681.

9. Morales JO, McConville JT: **Manufacture and characterization of mucoadhesive buccal films**. *Eur J Pharm Biopharm* 2011, **77**:187-199.

10. Laffleur F: **Mucoadhesive polymers for buccal drug delivery**. *Drug Dev Ind Pharm* 2014, **40**:591-598.

11. Laffleur F, Wagner J, Barthelmes J: **A potential tailor-made hyaluronic acid buccal delivery system comprising rotigotine for Parkinson's disease?** *Future Med Chem* 2015, **7**:1225-1232.

12. Laffleur F, Leder N, Barthelmes J: **In vitro evaluation of thio-poly acrylic acid for intraoral delivery**. *Drug Deliv* 2016, **23**:2065-2073.

13. Laffleur F, Bacher L, Vanicek S, Menzel C, Muhammad I: **Next generation of buccadhesive excipient: preactivated carboxymethyl cellulose**. *Int J Pharm* 2016, **500**:120-127.

14. Wasnik MN, Godse RD, Nair HA: **Development and evaluation of buccoadhesive tablet for selegiline hydrochloride based on thiolated polycarbophil**. *Drug Dev Ind Pharm* 2014, **40**:632-638.

15. Boateng JS, Ayensu I: **Preparation and characterization of laminated thiolated chitosan-based freeze-dried wafers for potential buccal delivery of macromolecules**. *Drug Dev Ind Pharm* 2014, **40**:611-618.

16. Mortazavian E, Dorkoosh FA, Rafiee-Tehrani M: **Design, characterization and *ex vivo* evaluation of chitosan film integrating of insulin nanoparticles composed of thiolated chitosan derivative for buccal delivery of insulin**. *Drug Dev Ind Pharm* 2014, **40**:691-698.

17. Montenegro-Nicolini M, Morales JO: **Overview and future potential of buccal mucoadhesive films as drug delivery systems for biologics**. *AAPS PharmSciTech* 2016, **18**:3-14.

18. Khan S, Trivedi V, Boateng J: **Functional physico-chemical, ex vivo permeation and cell viability characterization of omeprazole loaded buccal films for paediatric drug delivery**. *Int J Pharm* 2016, **500**:217-226.

19. Montero-Padilla S, Velaga S, Morales JO: **Buccal dosage forms: general considerations for pediatric patients**. *AAPS PharmSciTech* 2017, **18**:273-282.

20. Trastullo R, Abruzzo A, Saladini B, Gallucci MC, Cerchiara T, Luppi B, Bigucci F: **Design and evaluation of buccal films as paediatric dosage form for transmucosal delivery of ondansetron**. *Eur J Pharm Biopharm* 2016, **105**:115-121.

21. Khan S, Boateng JS, Mitchell J, Trivedi V: **Formulation, characterisation and stabilisation of buccal films for paediatric drug delivery of omeprazole**. *AAPS PharmSciTech* 2015, **16**:800-810.

22. Montenegro-Nicolini M, Miranda V, Morales JO: **Inkjet printing of**
• **proteins: an experimental approach**. *AAPS J* 2017, **19**:234-243.
First description of the potential of inkjet printing of biologics for buccal delivery.

23. Genina N, Fors D, Palo M, Peltonen J, Sandler N: **Behavior of printable formulations of loperamide and caffeine on different substrates—effect of print density in inkjet printing**. *Int J Pharm* 2013, **453**:488-497.

24. Palem CR, Dudhipala NR, Battu SK, Repka MA, Rao Yamsani M: **Development, optimization and in vivo characterization of domperidone-controlled release hot-melt-extruded films for buccal delivery**. *Drug Dev Ind Pharm* 2016, **42**:473-484.

25. Buanz ABM, Saunders MH, Basit AW, Gaisford S: **Preparation of personalized-dose salbutamol sulphate oral films with thermal ink-jet printing**. *Pharm Res* 2011, **28**:2386-2392.

26. Genina N, Janßen EM, Breitenbach A, Breitkreutz J, Sandler N: **Evaluation of different substrates for inkjet printing of rasagiline mesylate (Internet)**. *Eur J Pharm Biopharm* 2013 http://dx.doi.org/10.1016/j.ejpb.2013.03.017.

27. Alomari M, Mohamed FH, Basit AW, Gaisford S: **Personalised dosing: printing a dose of one's own medicine**. *Int J Pharm* 2015, **494**:568-577.

28. Sandler N, Preis M: **Printed drug-delivery systems for improved patient treatment**. *Trends Pharmacol Sci* 2016, **37**:1070-1080.

29. Palem CR, Dudhipala N, Battu SK, Goda S, Repka MA, Yamsani MR: **Combined dosage form of pioglitazone and felodipine as mucoadhesive pellets via hot melt extrusion for improved buccal delivery with application of quality by design approach**. *J Drug Deliv Sci Technol* 2015, **30**:209-219.

30. Alhijjaj M, Bouman J, Wellner N, Belton P, Qi S: **Creating drug solubilization compartments via phase separation in multicomponent buccal patches prepared by direct hot melt extrusion – injection molding**. *Mol Pharm* 2015, **12**:4349-4362.

31. Dawson PL, Hirt DE, Rieck JR, Acton JC, Sotthibandhu A: **Nisin release from films is affected by both protein type and film-forming method**. *Food Res Int* 2003, **36**:959-968.

32. Padgett T, Han IY, Dawson PL: **Incorporation of food-grade antimicrobial compounds into biodegradable packaging films**. *J Food Prot* 1998, **61**:1330-1335.

33. Cossé A, König C, Lamprecht A, Wagner KG: **Hot melt extrusion for sustained protein release: matrix erosion and in vitro release of PLGA-based implants**. *AAPS PharmSciTech* 2017, **18**:15-26.

34. Morales JO, Ross AC, McConville JT: **Protein-coated nanoparticles embedded in films as delivery platforms**. *J Pharm Pharmacol* 2013, **65**:827-838.

35. Morales JO, Huang S, Williams RO III, McConville JT: **Films loaded with insulin-coated nanoparticles (ICNP) as potential platforms for peptide buccal delivery**. *Colloids Surf B Biointerfaces* 2014, **122**:38-45.

36. Rao S, Song Y, Peddie F, Evans A: **Particle size reduction to the nanometer range: a promising approach to improve buccal absorption of poorly water-soluble drugs**. *Int J Nanomedicine* 2011 http://dx.doi.org/10.2147/IJN.S19151.

37. Baumgartner R, Teubl BJ, Tetyczka C, Roblegg E: **Rational design and characterization of a nanosuspension for intraoral administration considering physiological conditions**. *J Pharm Sci* 2016, **105**:257-267.

38. Mouftah S, Abdel-Mottaleb MMA, Lamprecht A: **Buccal delivery**
• **of low molecular weight heparin by cationic polymethacrylate nanoparticles**. *Int J Pharm* 2016, **515**:565-574.
One of the largest molecular weight molecules considered for buccal delivery.

39. Al-Dhubiab BE, Nair AB, Kumria R, Attimarad M, Harsha S: **Formulation and evaluation of nano based drug delivery system for the buccal delivery of acyclovir**. *Colloids Surf B Biointerfaces* 2015, **136**:878-884.

40. Mazzarino L, Borsali R, Lemos-Senna E: **Mucoadhesive films containing chitosan-coated nanoparticles: a new strategy for buccal curcumin release**. *J Pharm Sci* 2014, **103**:3764-3771.

41. Abd El Azim H, Nafee N, Ramadan A, Khalafallah N: **Liposomal buccal mucoadhesive film for improved delivery and permeation of water-soluble vitamins**. *Int J Pharm* 2015, **488**:78-85.

42. Giovino C, Ayensu I, Tetteh J, Boateng JS: **An integrated buccal delivery system combining chitosan films impregnated with peptide loaded PEG-b-PLA nanoparticles**. *Colloids Surf B Biointerfaces* 2013, **112**:9-15.

43. Lv Q, Shen C, Li X, Shen B, Yu C, Xu P, Xu H, Han J, Yuan H: **Mucoadhesive buccal films containing phospholipid-bile salts-mixed micelles as an effective carrier for Cucurbitacin B delivery**. *Drug Deliv* 2015, **22**:351-358.

44. Mauro M, Crosera M, Bianco C, Bellomo F, Bovenzi M, Adami G, Larese Filon F: **In vitro permeability of silver nanoparticles through porcine oromucosal membrane**. *Colloids Surf B Biointerfaces* 2015, **132**:10-16.

45. Teubl BJ, Leitinger G, Schneider M, Lehr C-M, Fröhlich E, Zimmer A, Roblegg E: **The buccal mucosa as a route for TiO$_2$ nanoparticle uptake**. *Nanotoxicology* 2015, **9**:253-261.

46. Roblegg E, Fröhlich E, Meindl C, Teubl B, Zaversky M, Zimmer A: **Evaluation of a physiological in vitro system to study the transport of nanoparticles through the buccal mucosa**. *Nanotoxicology* 2012, **6**:399-413.

47. Teubl BJ, Meindl C, Eitzlmayr A, Zimmer A, Fröhlich E, Roblegg E: **In-vitro permeability of neutral polystyrene particles via buccal mucosa**. *Small* 2013, **9**:457-466.

48. Kweon M-N: **Sublingual mucosa: a new vaccination route for systemic and mucosal immunity**. *Cytokine* 2011, **54**:1-5.

49. Upadhyay J, Upadhyay RB, Agrawal P, Jaitley S, Shekhar R *et al.*: **Langerhans cells and their role in oral mucosal diseases**. *North Am J Med Sci* 2013, **5**:505.

50. Wang N, Wang T, Zhang M, Chen R, Niu R, Deng Y: **Mannose derivative and lipid A dually decorated cationic liposomes as an effective cold chain free oral mucosal vaccine adjuvant-delivery system**. *Eur J Pharm Biopharm* 2014, **88**:194-206.

51. Wang N, Wang T, Zhang M, Chen R, Deng Y: **Using procedure of emulsification–lyophilization to form lipid A-incorporating cochleates as an effective oral mucosal vaccine adjuvant-delivery system (VADS)**. *Int J Pharm* 2014, **468**:39-49.

52. Baudner BC, O'Hagan DT: **Bioadhesive delivery systems for**
• **mucosal vaccine delivery**. *J Drug Target* 2010, **18**:752-770.
Realization that buccal delivery has potential for mucoadhesive vaccines.

53. Maek J, Lubasová D, Lukáč R, Turánek-Knotigová P, Kulich P, Plocková J, Maková E, Procházka L, Koudelka , Sasithorn N *et al.*: **Multi-layered nanofibrous mucoadhesive films for buccal and sublingual administration of drug-delivery and vaccination nanoparticles – important step towards effective mucosal vaccines**. *J Controlled Release* 2017, **249**:183-195.

54. Zhen Y, Wang N, Gao Z, Ma X, Wei B, Deng Y, Wang T: **Multifunctional liposomes constituting microneedles induced robust systemic and mucosal immunoresponses against the loaded antigens via oral mucosal vaccination**. *Vaccine* 2015, **33**:4330-4340.

55. Wang T, Zhen Y, Ma X, Wei B, Li S, Wang N: **Mannosylated and lipid A-incorporating cationic liposomes constituting microneedle arrays as an effective oral mucosal HBV vaccine applicable in the controlled temperature chain**. *Colloids Surf B Biointerfaces* 2015, **126**:520-530.

56. Morales JO, McConville JT: **Novel strategies for the buccal delivery of macromolecules**. *Drug Dev Ind Pharm* 2014, **40**:579-590.

57. Caon T, Jin L, Simões CMO, Norton RS, Nicolazzo JA: **Enhancing the buccal mucosal delivery of peptide and protein therapeutics**. *Pharm Res* 2014 http://dx.doi.org/10.1007/s11095-014-1485-1.

58. Iyire A, Alaayedi M, Mohammed AR: **Pre-formulation and systematic evaluation of amino acid assisted permeability of insulin across in vitro buccal cell layers**. *Sci Rep* 2016, **6**:32498.

59. Patel MP, Churchman ST, Cruchley AT, Braden M, Williams DM: **Delivery of macromolecules across oral mucosa from polymeric hydrogels is enhanced by electrophoresis (iontophoresis)**. *Dent Mater* 2013, **29**:e299-e307.

60. Oh D-H, Chun K-H, Jeon S-O, Kang J-W, Lee S: **Enhanced transbuccal salmon calcitonin (sCT) delivery: effect of chemical enhancers and electrical assistance on in vitro sCT buccal permeation**. *Eur J Pharm Biopharm* 2011, **79**:357-363.

61. Telò I, Tratta E, Guasconi B, Nicoli S, Pescina S, Govoni P, Santi P, Padula C: **In-vitro characterization of buccal iontophoresis: the case of sumatriptan succinate**. *Int J Pharm* 2016, **506**:420-428.

62. Ren W, Baig A, White DJ, Li SK: **Characterization of cornified oral mucosa for iontophoretically enhanced delivery of chlorhexidine**. *Eur J Pharm Biopharm* 2016, **99**:35-44.

63. **Midatech Pharma – news release**. http://www.midatechpharma.
• com/news/69/143/Midatech-Pharma-announces-clinical-pipeline-update.html (accessed: 26th April 2017).
Update on Midatech and Monosol pipeline and description of the gold nanoparticle-in-film system for insulin.