DOI:
10.1080/17425247.2018.1504017

Document Version
Peer reviewed version

Recent advances in oral delivery of biologics: nanomedicine and physical modes of delivery

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Funding
This paper was not funded

Declaration of Interest
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Peer reviewer declaration of interest:
Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Abstract

Introduction: Research into oral delivery of biologics has a long and rich history but has not produced technologies used in the clinic. The area has evolved in terms of strategies to promote oral biologics delivery from early chemical absorption enhancers to nanomedicine to devices. Continued activity in this area is justifiable considering the remarkable proliferation of biologics.

Areas covered: The article discusses some physiological barriers to oral delivery of biologics, with a special focus on less characterised barriers such as the basement membrane. Recent progress in oral delivery of biologics via nanomedicine is subsequently covered. Finally, the emerging field of device-mediated gastrointestinal delivery of biotherapeutics is discussed.

Expert opinion: Oral delivery of biologics is considered a 'panacea' in drug delivery. Almost century-old approaches of utilising chemical absorption enhancers have not produced clinically translated technologies. Nanomedicine for oral biologics delivery has demonstrated potential, but the field is relatively new, and technologies have not progress to the clinic. Device-mediated oral biologics delivery (e.g. ultrasound or microneedles) is in its infancy. However, this space is likely to intensify owing to advances in electronics and materials, as well as the challenges and history related to clinical translation of alternative approaches.

Keywords:

Absorption enhancement, Biologics, Microneedle capsule, Nanomedicine, Oral delivery of biologics, Ultrasound

1. Introduction

Administration of biologics, such as peptides, therapeutic proteins and antibodies, is limited to injection (with a few exceptions). This is explained by the very poor bioavailability of most biotherapeutics following oral administration (in unformulated form) of less than 1% [1]. Oral administration is preferred over injections due to convenience [2, 3, 4, 5]. The oral administration route offers additional advantages over invasive routes. For example, oral insulin more closely mimics the physiology of endogenous insulin secreted by the pancreas, offering decreased levels of systemic insulin, hence less hypoglycaemic episodes and weight gain problems [6]. Furthermore, oral administration reduces needle-related complications and cost. With respect to the latter, it is difficult to calculate the cost-savings achieved with a switch from injection-based to oral therapy (as this depends on the individual therapeutics, patient numbers, dose, cost of oral delivery alternative, etc.). However, the reduction in healthcare costs associated with the switch from injection to oral administration of vitamin B_{12}, estimated to amount to 37-64% [7], highlights that the potential reduction in healthcare costs can be significant. This is an important consideration taking into account the increasing availability and likely future routine use of biotherapeutics not only for life-threatening acute conditions but also chronic illnesses of an aging population.

It must be noted that the field of oral delivery of biologics is nearly a century old, with oral administration of insulin examined as early as 1923-1924 [8, 9, 10]. The proliferation of biological therapeutics has intensified research efforts into non-invasive delivery approaches, with oral delivery in particular attracting significant attention. The field has evolved from chemical absorption enhancers with the ability to increase epithelial membrane fluidity, such as surfactants [11, 12, 13, 14, 15, 16], those that open epithelial tight junctions (TJs) [17, 18, 19, 20, 21] and mucoadhesive polymers for prolonging drug residence in the intestinal mucosa [22, 23, 24, 25, 26]. The application of such absorption enhancers as means to promote oral delivery of biologics has been reviewed elsewhere [27, 28] and therefore will only be briefly discussed here. Similarly, recent commercial activity and

progress of technologies based on these more conventional approaches of absorption enhancement has also been subject to recent reviews [29, 30].

More modern approaches explored for enabling oral delivery of biologics utilise nanotechnology as means to deliver biotherapeutic payloads across the intestinal mucosal barrier. Another recently-emerging activity in the area is related to devices, such as those utilising ultrasound or microneedles. This article will initially discuss the properties of key physiological barriers to biologics delivery, including the less well-characterised barrier of the basement membrane (BM), and subsequently focus on emerging technologies for promoting oral absorption of biotherapeutics.

2. Physiological barriers to oral delivery of biologics

The components of the gastrointestinal tract (GIT) that present barriers limiting the systemic bioavailability of biologics following oral delivery include acid, proteolytic enzymes in the gut lumen and at the brush border membrane, the mucus layer, the bacterial gut flora and the epithelium. The GIT mucosa is therefore organised to act as a selective barrier and minimise the entry of macromolecules and particulate matter from the external environment into the body. Although a restriction on the penetration of toxic materials and harmful pathogens is imposed, the mucosal surface is not completely impenetrable. The absorption of macromolecules and particulate matter is facilitated by a variety of mechanisms [31, 32], the understanding of which is important if biological transport mechanisms are to be exploited for oral delivery of biologics.

Although several physiological barriers to effective oral delivery of biologics exist, here we will only refer to mucus and the BM, with the latter being a relatively uncharacterised barrier which is less commonly discussed in the literature.

2.1 Mucus layer

The intestine is protected by a mucus layer, which ranges from 10 to 100-200 μm thick (jejunum to colon) [33], forming a single layer in the small intestine and a double layer in the colon, with the inner mucus layer firmly attached to the epithelium [34]. Mucus is a thick substance composed of water, proteins and lipids [35, 36], with the main structural component being mucin [13]. Mucin is a highly glycosylated protein with oligosaccharide side chains including sulphate residues that give an overall negative charge [37, 38]. Mucin has extensive intermolecular interactions forming a mesh-like structure (average pore size 5-500 nm) and is responsible for the viscoelastic nature of mucus [38]. These characteristics allow mucus to act as a natural barrier against certain material diffusing to the underlying epithelium [39].

Mucus plays a key role in providing protection against invasion by foreign agents. In addition, the lubricating properties of mucus facilitate the passage of food through the digestive tract [40]. However, in terms of drug delivery, the organisation of mucus gel as linear, glycosylated mucin fibres entwined within a dense network [41, 42], can result in particle entrapment and restriction of their movement from the intestinal lumen to the underlying epithelium [43, 44].

Drug diffusion through mucus is influenced by its molecular size, together with the effective mesh size of the mucus gel and molecular interactions between the drug and mucus components [39]. Mucin fiber interactions with foreign molecules can result in severe inhibition or hindrance of their movement through the gel [43, 44]. However, in terms of biologics, it is generally accepted that most biologics fall below the molecular weight threshold for their diffusion to be severely hampered by the intestinal mucus. This is based on evidence from a number of studies, including work by Olmsted et al. which reported that most proteins (15-650 kDa) diffused as fast in fresh human cervical mucus as they did in water (D_{muc}/D_{pbs} = 0.84-1.1) and had > 93% recovery [45] and that of Cu et al. showing that antibodies diffuse easily through mucus [46]. This is thought to be because the mesh size of

mucin fibres within mucus is larger than most proteins, as demonstrated by Saltzman and co-workers [47]. However, it must be pointed out that there is a degree of conflicting reports in this area, with some studies demonstrating relatively significant hindrance of diffusion in mucus of peptides and proteins as low in molecular mass as 12.4 kDa [48, 49]. For a comparison of diffusion of a number or macromolecules through (human cervical) mucus the reader is referred to a study by Olmsted et al. [45].

2.2 Basement membrane

Basement membranes (BMs) are thin, specialised sheets of extracellular matrices (ECM) found between epithelia and connective tissue in the human body [50, 51, 52]. The composition of BMs includes laminins, type IV collagen, nidogen and heparan sulphate proteoglycans (HSPGs) [53]. Collagen, the main protein of ECM, is covalently linked by multiple bonds including disulphide and hydrogen bonding that gives tensile strength to BM [54, 55]. Alongside collagen, laminin which strongly associates to cell surface, provides additional organised structural support to BMs [56]. BMs play an essential role in controlling a variety of epithelial phenomena, including cell attachment, growth, migration and differentiation [57, 58, 59]. BMs also serve a filter function due to a selective passage of molecules across this barrier [60].

Prior work by Vllasaliu et al. reported that airway epithelium-synthesised BM significantly hindered the diffusion of macromolecules in a molecular size-dependent manner [61]. Specifically, diffusion across BM (obtained via decellularisation of BM-synthesising airway epithelial cells) was hindered for FD4 (1.3-fold), Human Serum Albumin (HSA) (1.6-fold) and FITC-IgG (4.1-fold difference). In a study by Alfano et al. the penetration of relatively small macromolecule through the BM region of non-keratinized oral mucosal epithelium, namely inulin of molecular weight 5 kDa, was impeded by BM, whilst the penetration of a 20kDa dextran was not affected [62]. This ‘molecular sieving’ behaviour of the BM barrier was attributed to the possibility of small molecules binding to the structural

proteins of the BM or becoming caught in the finer interstices of the structural proteins akin to the mechanism of gel permeation chromatography [62].

3. Established strategies for improving oral delivery of biologics

Various approaches have been investigated for their potential to improve mucosal delivery of biotherapeutics, particularly peptide and protein drugs (Figure 1). These include the use of systems that increase drug contact time with mucus-covered biological barrier (mucoadhesive polymers) [63, 64], materials that increase membrane fluidity and/or disrupt cell membranes (e.g. surfactants) [65, 66, 67] and compounds, such as chitosan, that promote transient opening of the epithelial TJs [68, 69]. Receptor-mediated transcytosis pathways have also been exploited for transmucosal delivery of biologics. Additionally, systems that break down mucus [70, 71, 72, 73], those that improve drug stability in the gut lumen (e.g. proteolytic enzyme inhibitors [74, 75]), and materials promoting dissociation of protein aggregates (e.g. cyclodextrins) [76] have also been utilised.

Figure 1.

Considering the established research strategies that have been widely explored for oral delivery of biologics, it is worth noting Novo Nordisk's recent announcement of the successful completion of the first Phase 3a trial with oral semaglutide – a long-acting glucagon-like peptide (GLP-1) analog – for the treatment of type 2 diabetes. This formulation uses Emisphere Technologies' proprietary Eligen Technology to promote intestinal absorption. The trial, a 703 person study, successfully achieved its primary objective, showing significant improvements in hemoglobin A_{1c} level for three orally-administered doses of semaglutide (3, 7 and 14 mg) compared to placebo [77]. The absorption-enhancing technology is based on the absorption-enhancing excipient N-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) – a small fatty acid derivative that promotes gastrointestinal absorption of semaglutide via effects on transcellular pathways. SNAC is claimed to form non-covalent

complexes with substrates, providing protection of the therapeutic from intestinal degradation and an increase in hydrophobicity so to enable passive permeation of the epithelium, after which the complex disassembles [29]. Novo Nordisk expects regulatory submission of oral semaglutide in 2019.

4. Nanomedicine-based strategies for oral delivery of biologics

Nanomedicine-based systems for oral delivery of biologics potentially offer a number of advantages, including protection of the biotherapeutic payload in the acid- and enzyme-rich environment of the GIT, targeted delivery and potentially improved penetration across the intestinal mucosa. Furthermore, the drug-loaded system can be targeted to various receptors on the surface of intestinal epithelial cells, enabling a more selective delivery of the therapeutic compared to absorption-enhancer approaches that non-selectively increase epithelial permeability. However, developing nanosystems for oral delivery of biologics is associated with a number of challenges, including, therapeutic loading and delivery capacity [78], modification of nanosystems in the highly complex GIT environment (discussed below), poor penetration of nanomaterials across the intestinal mucosa and finally, uncertainties associated with the safety of the nanodelivery approach.

Polymeric NPs in particular have attracted considerable attention for oral delivery of biologics. These have been reviewed extensively elsewhere [3, 33]. Here, we will focus on systems designed to permeate the intestinal mucosa via the biological transport process of transcytosis, followed by discussion of NP diffusion across the complex biological environments of mucus and the BM. We will also briefly discuss NP transformation via biocorona formation in the complex environment of the GIT.

4.1 Nanoparticles targeting intestinal epithelial transcytosis

To improve the inherently poor penetration of NPs across the intestinal epithelium, a common approach has been to utilise epithelial biological transport systems as potential

routes to shuttle nanoparticulates across the intestinal epithelium. To this end, biological transport processes responsible for transepithelial trafficking of IgG, vitamin B_{12} and folate have been explored. This work demonstrated that transepithelial delivery of model nanocarriers is possible in vitro following surface functionalisation (or adsorption) of model NPs with the fragment crystallisable (Fc) region of IgG (airway epithelium) [79], vitamin B_{12} (intestinal and airway epithelium [80] and [81], respectively), and folate (airway epithelium) [82].

Verma et al. investigated the potential of vitamin B_{12}-functionalised chitosan/calcium phosphate NPs (VitB12-Chi-CPNPs), fabricated via the layer-by-layer approach, for improved oral delivery of insulin [83]. The systems were tested in vitro in Caco-2 monolayers and in vivo in diabetic rats. The data showed higher uptake of VitB12-Chi-CPNPs in Caco-2 monolayer in comparison to non-functionalised systems (Chi-CPNPs), indicating increased uptake, presumably utilising the vitamin B_{12} internalisation route. Reduction in transepithelial electrical resistance (TEER) suggested paracellular insulin transport. In vivo intestinal uptake of FITC-labelled Vit-B12-Chi-CPNPs from different intestinal segments supported paracellular and receptor mediated uptake of VitB12-Chi-CPNPs. In diabetic rats the systems showed about four-fold increase in insulin bioavailability and sustained hypoglycaemic effects up to 12 h of administration in comparison to Chi-CPNPs [83] (Figure 2).

Figure 2.

In vivo studies investigating the potential usefulness of transcytosis-targeted NPs for oral delivery of biologics also include those directed towards the neonatal Fc receptor (FcRn) system. Fc-modified NPs demonstrated potential in animal studies for oral insulin [84]. Specifically, in mice, orally administered NPs targeted to FcRn, were found to cross the intestinal epithelium and reach the systemic circulation with a mean absorption efficiency of

13.7%*h compared with 1.2%*h for control (non-targeted) NPs. FcRn-targeted NPs containing a clinically relevant dose of insulin (1.1 U/kg) produced a prolonged hypoglycaemic response in wild-type mice. This effect was absent in FcRn knockout mice (hence signifying that increased NP transport was mediated by FcRn). In another study, polyethylene glycol-poly(lactic-co-glycolic acid) (PEG-PLGA) NPs modified with Fc were fabricated for oral delivery of exenatide [85]. Permeation of Fc-conjugated NPs in Caco-2 cells was reported to be enhanced compared to unmodified NPs. Oral administration of exenatide-loaded, Fc-modified PEG-PLGA NPs in mice resulted in prolonged hypoglycaemic effects compared with exenatide solution administered by subcutaneous injection. Additionally, fluorescently-labelled Fc-modified NPs remained in the GIT for a longer time compared to unmodified NPs.

While, the FcRn and vitamin B_{12} biological transport pathways have shown potential for oral delivery of NPs (as biologics carriers), additional biological transport targets are emerging. Park et al. described a novel bile acid-conjugated NP (heparin-deoxycholic acid and protamine nanocomplex) capable of traversing the intestinal epithelium by bile acid transporters [86]. NPs were internalised into the cytosol of intestinal epithelial Caco-2 cells. In animal studies (mice), bile acid conjugated nanosystems successfully interacted with bile acid transporters in the ileum, which resulted in uptake into epithelial cells and prolonged residence (several days) in this tissue.

4.2 Nanoparticle movement across the mucus

Human mucus strongly immobilises conventional synthetic NPs [45, 87, 88]. Attachment of polyethylene glycol (PEG) to NP surface has proven to be an effective strategy to enhance NP diffusion across mucus [87, 89]. Wang et al. [89] demonstrated that a shift in the overall surface charge of 220 nm negatively charged polystyrene NPs towards a more neutral charge, induced by surface modification by short-chain length PEG, promoted NP diffusion in cervicovaginal mucus. In another study investigating NP diffusion in cervical mucus, 170

nm diameter PLGA NPs were surface modified with different molecular weight PEG (2, 5 and 10 kDa) at a density range of 5-100% [90]. Surface decoration of NP with PEG promoted their diffusion in mucus (3- to 10-fold increase compared to unmodified particles), with the effect being dependent on the size of PEG molecule and coating density.

Lai et al. [87] had previously demonstrated that large PEGylated NPs can rapidly penetrate fresh human cervicovaginal mucus. The effective diffusion coefficients of 500 and 200 nm particles were only 4- and 6-fold lower in mucus than water, while the diffusion coefficients for uncoated particles were 2,400- to 40,000-fold lower in mucus compared to water. Larger PEGylated NPs of 200 nm and 500 nm produced a larger rate of diffusion in mucus, when compared with smaller 100 nm particles. This was attributed to the possibility of inadequate PEGylation of 100 nm NPs compared to 200 nm and 500 nm NPs (although the effectiveness of the PEG shield was similar for all particles) or size-exclusion chromatography principles, whereby smaller particles are able to access a larger number of small pores or pockets in the gel, increasing the tortuosity of their path and reducing the transport rate over long distances.

4.3 Nanoparticle movement across the basement membrane

The barrier characteristics of the BM component of the intestinal mucosa have not been as widely studied as other mucosal elements. In addition to being capable of negotiating the acid, enzymatic, mucus and epithelial barriers, nanocarriers must be able to avoid retention by the BM. To do so, NP diameters must be sufficiently small to pass through the BM pores. Additionally, particle surface properties also play a significant role in diffusion across the BM barrier and therefore must be carefully selected to minimise electrostatic and adhesive interactions with this barrier.

NP diffusion across the intestinal BM specifically has not been reported. However, the BM of nonkeratinized oral mucosal epithelium was reported to hinder the diffusion of a

bacterial endotoxin [91] and 2',3'-dideoxycytidine (MW 211 g/mol) [92]. In addition, BM dramatically limited the diffusion of HPV-16 pseudovirions (by approximately ~10,000 times) [93].

Studies of NP diffusion through ECM, a three-dimensional form of BM, may also shed some light into the potential barrier properties of the latter. ECM dramatically inhibits the diffusion of positively and negatively charged particles that are significantly smaller than its mesh size [94]. Other studies have demonstrated that this barrier to NP diffusion arises due to electrostatic interactions between charged components of the ECM and diffusing particle [95]. The same may therefore apply to the BM. Indeed, we previously reported that airway epithelium-produced BM dramatically suppressed the movement of model, negatively-charged polystyrene NPs in a size dependent manner [61], specifically 9.5-fold for 20 nm NPs and 31-fold for 50 nm NPs.

In terms of strategies to promote NP diffusion across the BM, Tomasetti et al. [96] described the diffusion of polyethylenimine-coated silica NPs of 120 nm, coated with PEG, in collagen I network and Matrigel (a BM matrix). PEGylation was achieved utilising PEG of different molecular weights – 2, 5 and 20 kDa – over a range of gradually increasing coating densities, namely 0.2, 2, 8 and 20 PEG/nm^2. In Matrigel, increases in both PEG density and PEG molecular weight improved the mobility of NPs in BM matrix. NPs demonstrated threshold zeta potentials due to PEGylation of 11.6 mV for PEG_{2kDa} and 13.8 mV for PEG_{5kDa}, below which particles were regarded as mobile. Regardless of PEG molecular weight, PEGylation density lower than 2 PEG/nm^2 gave rise to particles considered as immobile and PEGylation densities 8 and 20 PEG/nm^2 produced particles regarded as mobile.

4.4 Nanoparticle transformation in the gastrointestinal tract

When considering NP carriers as means to deliver biologics orally, the stability and potential transformation of NPs in the GIT biofluid via surface adsorption of biomolecules ('biocorona' formation) are critical. This is particularly important for systems surface-functionalised with ligands enabling transcytosis or targeted delivery. Different conditions reflecting both the GIT region and the effect of food on biofluid composition and therefore NP biomolecule corona formation must be considered. Unlike the study of NP biocorona formation in plasma, comprehensive characterisation of NP coronate in the GIT is presently lacking. Limited studies in this area have reported interesting findings. For example, digestion of Ag NPs with food compounds did not have an effect on the uptake by intestinal Caco-2 cells, while a decreased cell internalisation (to 60%) was seen following digestion in the absence of food [97]. Treatment of SiO_2 NPs with digestive solutions lowered their potential to generate reactive oxygen species, although cytotoxicity was not affected [98]. Another study investigating NP corona effects reported that coating of 20, 100, and 200 nm polystyrene NPs with bovine serum albumin and casein reduced their adhesion to Caco-2 cells, whereas coating with meat extract produced no such effect [99]. On the other hand, incubation of 20 nm and 200 nm particles in murine intestinal fluid increased their adherence to intestinal Caco-2 cells [99].

In addition to food, the effect of mechanical forces and a changing pH along the GIT on biomolecule corona formation also needs to be considered. In the stomach, contractions with pressures up to 150 mm Hg have been measured, but effects of this on NP corona formation, agglomeration and aggregation remain largely unknown [100].

5. Devices for oral delivery of biologics

The demand for non-invasive delivery of biologics, which so far have not been met by chemical absorption enhancer approaches, has recently intensified the focus on devices for drug delivery. This has been facilitated by modern advances in electronics and materials. Oral drug delivery devices resemble more conventional oral solid dosage forms (e.g.

capsules), but carry small electronic and/or mechanical elements [101]. According to MarketsandMarkets™, 'smart pills' market is expected to reach $8.98 billion in sales by 2024 [102].

5.1 Intestinal patch systems

Intestinal patch-based devices are potentially attractive for oral delivery of biologics considering their ability to prevent drug degradation in the GIT and promote intestinal absorption by forming a local drug depot adhered to the intestinal wall. These systems can be designed to provide unidirectional, controlled drug release while preventing luminal drug loss. Such patch-based devices are being developed for oral delivery of several biologics including insulin, exenatide, calcitonin, interferon-α, erythropoietin and human granulocyte colony-stimulating factor [103].

In a recent study on mucoadhesive devices, Gupta et al. [104] evaluated devices prepared from a blend of FDA-approved polymers, carbopol, pectin and sodium carboxymethylcellulose, for the delivery of exenatide and insulin. When patches were surgically placed in rat jejunum a 42% decrease in blood glucose was observed, while this decrease was negligible in insulin solution treated group. The relative bioavailability of insulin and exenatide increased by 13 and 80-fold, respectively, compared to intestinal injections, with significant increase in half-lives and prolonged blood glucose reductions. The Desai group is developing micro/nanofabricated devices with planar, asymmetric geometries that readily adhere to the lining of the GIT for prolonged durations, while releasing the drug at high concentrations, unidirectionally toward epithelial tissue, thereby increasing drug permeation [105]. In a recent study, this group reported on enhancement of microdevice properties for oral drug delivery by incorporating nanostraw membrane caps [106]. The study demonstrated that the nanostraws facilitate facile drug loading and tuneable release (FITC-insulin), limit the influx of external molecules into the sealed drug reservoir and increase the adhesion of devices to epithelial tissue (Caco-2 cells and excised murine

intestinal tissue). This study is significant as nanostraws facilitate the transport of macromolecules, including nucleic acids, proteins and drugs into cells by piercing through membranes [107, 108].

While intestinal patches have demonstrated potential in vitro and in vivo, these systems must be comprehensively evaluated for safety upon chronic use. Potential challenges facing this technology include efficacy with larger biotherapeutics or those with low potency requiring high plasma concentration levels. It is also currently unclear how these systems behave in relation to intestinal mucus turnover, which can be as fast as 1h [109], or in diseases associated with mucus defects (e.g. inflammatory bowel disease). Furthermore, the effect of these systems on the mucus-associated microbiota and, importantly, the resulting interaction with the host, remains unknown.

5.2 Microneedle capsule

The concept of microneedle capsule technology for oral delivery of biologics is based on overcoming the intestinal mucosa barrier by penetration of hollow or solid microneedles directly into the mucosa [110]. Following this breach of the mucosal barrier, the biologic is released from a capsule reservoir or microneedles. This is depicted in Figure 3.

Figure 3.

The appeal of microneedles in oral delivery of biologics stems from: i) amenability to platform technology offering oral delivery of a wide range of therapeutics, ii) the insensate nature (absence of sharp-pain receptors) of the intestine leading to pain-free microinjection, iii) the capacity of the GIT to tolerate the passage of sharp objects and mucosal disruption [111]. This appeal has produced some activity in this area. However, presently this activity is more commercial than academic as evidenced by numerous issued and pending patent

applications in this space and limited number of published studies fully evaluating the safety and efficacy of this technology.

A leading player in the field of microneedles for oral drug delivery is Rani Therapeutics. Their technology, the ‘Rani Pill’ or ‘robotic pill’, remains in a capsule form in the stomach and once it reaches the small intestine, the change in pH causes an outer cellulose shell to dissolve. This triggers a carbon dioxide-producing reaction in a balloon, which in turn causes the balloon to inflate and force drug-loaded needles into the intestinal wall [112]. The needles are sugar-based and dissolvable. Rani’s self-reported pre-clinical studies have demonstrated greater than 50% bioavailability with insulin and adalimumab in swine [113]. Rani’s microneedle device has attracted significant attention by the Biopharma, enabling the company to raise $142 million, which includes investment from the Big Pharma, including Novartis and AstraZeneca, before testing the technology in humans [114].

A rare proof-of-concept study by the Langer group highlighted the potential of microneedle-based delivery of a model biologic, insulin, in pigs [115]. Delivery kinetics of insulin administered by microinjections at different sites in the GIT, namely stomach, duodenum, and the colon, was compared to subcutaneous administration. Onset time to hypoglycaemic effect was significantly reduced following microinjection-mediated administration of insulin in the stomach and duodenum compared to the skin and by almost 20 minutes compared to subcutaneous injection. The study also demonstrated the safe passage of microneedle-based devices from the GI tract.

It remains to be seen whether the microneedle capsule technology is successfully translated into the clinic and achieves wide adoption. Some of the challenges associated with this technology are inter-patient variability in terms of intraluminal intestinal pressure [110] and the effect of food on the performance of the device. A more obvious challenge is safety, as

well as cost, which would affect the approval and adoption of technology, particularly for biologics requiring frequent repeated dosing and chronic use.

5.3 Ultrasound

Localised, low-frequency ultrasound has been shown to significantly improve the delivery of biologics. Schoellhammer at al. examined the use of one-minute ultrasound treatments in porcine GI tissue ex vivo and in vivo [116]. Low-frequency ultrasound was reported to increase the absorption of model small molecular weight therapeutics 2–10-fold, as tested utilising ex vivo tissue. Ultrasound application resulted in penetration of 3- and 70-kD dextran throughout the colonic tissue ex vivo, while such permeation was not apparent with either dextran.

The same group also tested ultrasound in vivo, inserted rectally via a model device in Yorkshire pigs, alongside instillation of insulin enema [117]. The authors reported that one-minute treatment was found to be safe and well tolerated and the generation of transient cavitation was confirmed. A clear hypoglycaemic effect was achieved following co-administration of insulin enema in the colon with ultrasound (Figure 4), while no effect on blood-glucose was apparent in the absence of ultrasound.

Figure 4.

Ultrasound was also investigated for local, colonic delivery of RNA in mice with dextran sodium sulphate (DSS)-induced colitis. Ultrasound delivery of unencapsulated small interfering RNA (siRNA) against anti-tumour necrosis factor *(Tnf)* messenger RNA *(*mRNA) reduced TNF protein levels in colon tissues, compared with delivery without ultrasound, in addition to reducing features of inflammation (statistically lower histology scores). In a separate experiment, colons of mice administered an mRNA (approximately 950 kDa) encoding firefly luciferase with ultrasound and the D-luciferin substrate had levels of

bioluminescence an order of magnitude higher than colons of mice given the mRNA alone [118].

The studies discussed above utilising low frequency ultrasound have clearly demonstrated the potential of ultrasound for oral delivery of biologics. Although the limited number of studies exploring ultrasound to permeabilise the intestinal mucosa for delivery of biologics have demonstrated the initial safety of this drug delivery modality, the safety of this approach following chronic administration should be evaluated comprehensively. Furthermore, while it is aimed that ultrasound-mediated tissue permeabilisation is in the future achieved by ingestible electronic devices, hence enabling oral administration, the cost of this technology and its suitability for repeated and long-term administration is currently unclear.

5.4 Microjet systems

Another recently described device technology for mucosal delivery of biologics relies on the generation of high-pressure liquid jet with sufficient velocity to penetrate the mucosa. The so called 'MucoJet' is a self-administered, two-compartment plastic device. A proof-of-concept study demonstrated its potential for vaccine delivery to the buccal mucosa [119].

Detailed description and mechanism of action of the MucoJet system has been described previously [119], but it is essentially a cylindrical plastic device (designed to be compatible with industry-scale thermoplastic fabrication methods) with an exterior compartment which is a water chamber and an interior compartment hosting separated propellant and vaccine reservoirs, a movable piston and a sealed delivery nozzle. Upon administration, water contact with the chemical propellant in the propellant reservoir triggers chemical generation of CO_2, increasing the pressure in the propellant chamber. This forces the piston toward the vaccine reservoir, breaking the nozzle membrane and ejecting a high-pressure liquid jet of vaccine.

In a proof-of-concept study [119], the MucoJet system was used to deliver fluorescein-labelled ovalbumin ex vivo in freshly prepared porcine buccal epithelium mounted in Transwell chambers. MucoJet produced a significant (eightfold) increase in the delivery of ovalbumin across the buccal tissue over three hours compared to dropwise application. In vivo experiments on New Zealand white rabbits showed that vaccination with MucoJet dramatically enhanced the immunogenicity of buccally administered antigens, as tested by blood and tissue (buccal, lymph node and Peyer's patch) specific antibody titres, relative to control groups, which received equivalent dosages of ovalbumin via a dropper at the buccal site. Specifically, antibody titres of IgG and IgA were three orders of magnitude higher in the MucoJet group compared to buccal administration of free ovalbumin.

6. Expert opinion

Oral delivery of biologics remains elusive despite research activity in the field for nearly a century. Approaches to improve oral bioavailability of biologics based on chemical absorption enhancers in general have not produced technologies that have successfully translated into the clinic and changed patients' lives. This highlights the scale of the challenge in promoting the stability and absorption of intact therapeutic biomolecules across the complex and multiple-barrier nature of the GIT.

The lack of success with chemical absorption enhancers can generally be attributed to their inability to successfully tackle the multiple and highly effective physiological barriers. This has meant that, for example, while many compounds have been shown to be effective permeation enhancers in intestinal epithelial models in vitro, they have failed (or are unlikely) to demonstrate sufficient effect on bioavailability in vivo if used alone. Indeed, the most commercially-advanced technologies for oral delivery of biologics employ materials with multiple absorption-enhancing mechanisms of action. However, these technologies fail to address additional issues, including luminal drug and absorption enhancer dilution and

narrow permeability enhancement versus toxicity window, so are therefore only pursued for relatively small peptides and proteins.

Nanomedicine is showing some potential as a tool for improving the oral delivery of biologics, but similarly to macromolecules, most nano-scale materials are not efficiently absorbed across the intestinal epithelium. Nanocarrier translocation across the intestinal epithelium can and has been promoted by designing systems that target and exploit the biological epithelial transport process of transcytosis. Such systems targeting the vitamin B_{12} and IgG biological transport machinery have shown promising results both in vitro and in vivo. However, this comes with its own issues of capacity (epithelial transcytosis is generally accepted to occur at a low rate) and potentially complete transformation of carefully designed nanosystems in the complex GIT biofluid and consequent loss of targeting and 'transport-enabling' capability of the system. Furthermore, nanocarrier-delivered biologics have to successfully transport across additional, non-epithelial barriers such as mucus, the BM and endothelium. Overall, the field of nanomedicine-mediated oral delivery of biologics is relatively young and technologies have not progressed beyond the preclinical and proof-of-concept phase. Furthermore, nanomedicine faces yet unaddressed challenges of translation in man, safety, scalability, cost and regulatory approval.

The area of oral delivery via 'smart' devices that for example may use ultrasound or microneedles is in its infancy and concept stage but has nevertheless significantly attracted the attention of the Biopharma industry. This points to signs of future growth in research activity in this area, also likely to be fuelled by advances in electronics, robotics and materials. It is highly likely that in the future we will see delivery strategies and technologies that combine devices with chemical or 'nano'-based absorption enhancer strategies. To determine the full potential of these technologies for routine use in the clinic it is critical that safety with chronic use is extensively evaluated, together with establishment of large-scale manufacture feasibility. Careful consideration must be given to marrying the biological drug

with the appropriate delivery technology, including dosage requirements, physiological and therapeutic advantages (or disadvantages) of oral delivery versus injection, and cost of the technology. With rapid innovation in associated technologies, the future development of clinically used devices for oral delivery of biologics looks promising.

7. Article highlights

- The absence of clinically-used technologies for oral delivery of biologics despite long research activity in the field highlights the scale of the challenge of overcoming the physiological barriers of the gastrointestinal tract for successful delivery.
- Many chemical absorption enhancer strategies for oral biologics delivery are limited to in vitro success.
- Some physiological barriers, such as the basement membrane, require further evaluation, particularly if nanomedicine approaches are utilised to improve delivery.
- Nanomedicine shows potential for oral biologics delivery but is associated with yet to be addressed issues of safety, delivery capacity and regulatory approval.
- Device-mediated oral delivery of biologics (e.g. ultrasound or microneedles) is in its infancy but shows promising signs and is attracting the attention of the Biopharma industry.
- Future growth in research activity on oral biologics delivery devices is likely and this will be facilitated by advances in electronics, robotics and materials, as well as the lack of success of alternative approaches.

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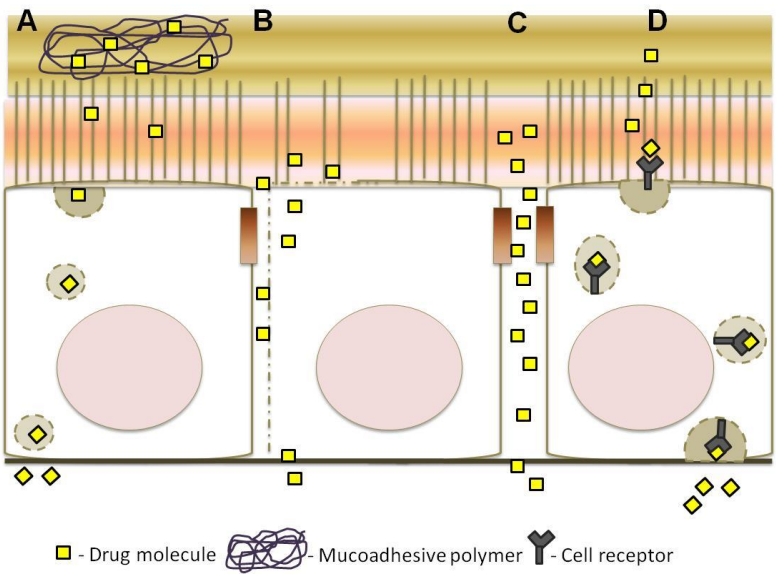

Figure 1. Commonly-researched strategies for improving mucosal absorption of biologics such as protein therapeutics. A) Mucoadhesive polymers prolonging the residence time of the complexed drug at the mucosal surface, B) Surface-active permeability enhancers compromising cell membrane integrity, whereby otherwise membrane-impermeable drug crosses the epithelial barrier, C) Tight junction opening where the paracellular space is increased, improving the paracellular access of macromolecules, and D) Exploiting epithelial transcytotic mechanisms by conjugating the drug (or a drug carrier nanoparticulate system) to a ligand (not shown) that is transported transepithelially via this route.

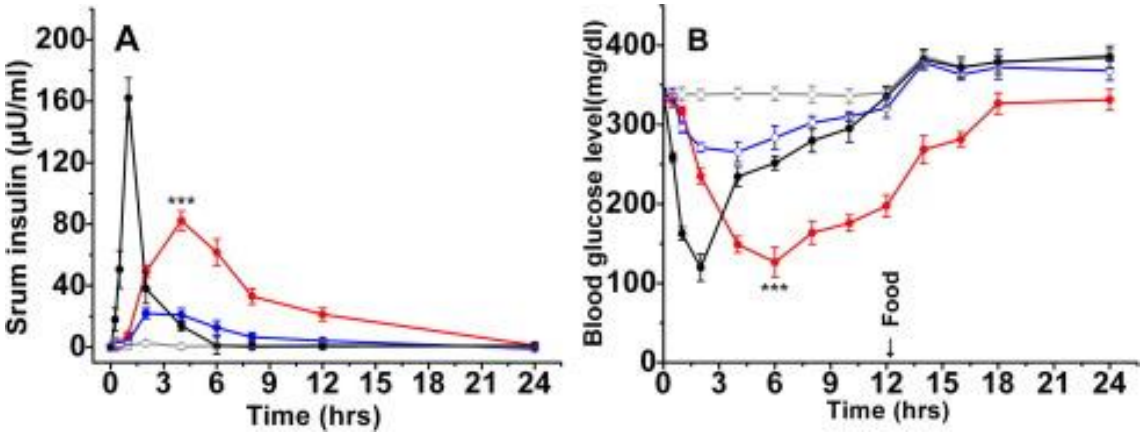

Figure 2. Effect of formulations and free insulin on (A) TEER and (B) transport of insulin through Caco-2 monolayer. (○) Plain insulin, (■) insulin loaded in CPNPs, (●) insulin loaded Chi-CPNPs and (●) insulin loaded VitB12-Chi-CPNPs. ∗Significant difference at $P < 0.05$ ($n = 6$, mean ± SD) VitB12-Chi-CPNPs vs Chi-CPNPs. Reproduced with permission from Verma et al. [81].

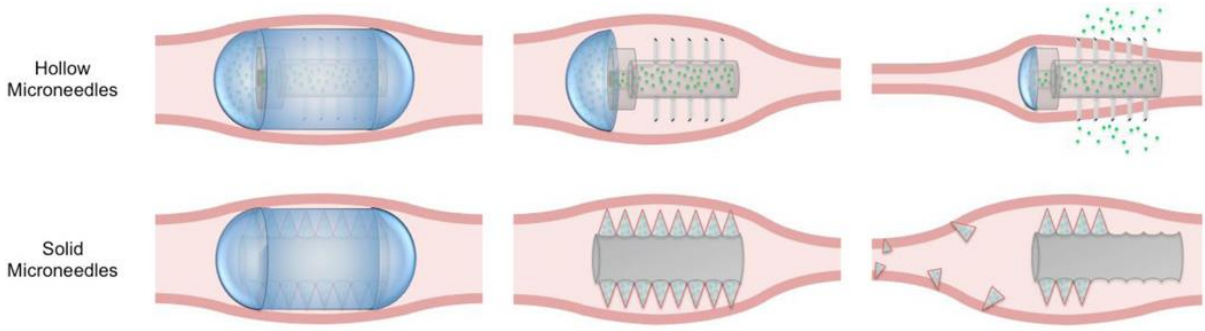

Figure 3. Therapeutic use concept of the microneedle pill. Both hollow and solid microneedles could be used. In both cases, the pill's needles are initially coated by a pH-responsive coating to aid in ingestion (left). When the pill has reached the desired location in the GI tract, the coating dissolves, revealing the microneedles (middle). In the case of hollow microneedles (top right), the drug reservoir is compressed through peristalsis, releasing the drug through the needles. In the case of solid microneedles (bottom right), the drug is formulated into the microneedles. The microneedles penetrate the tissue and break off of the pill, leaving the needle to release the drug in a controlled manner, based on the needle formulation. Reproduced from Traverso et al. [112].

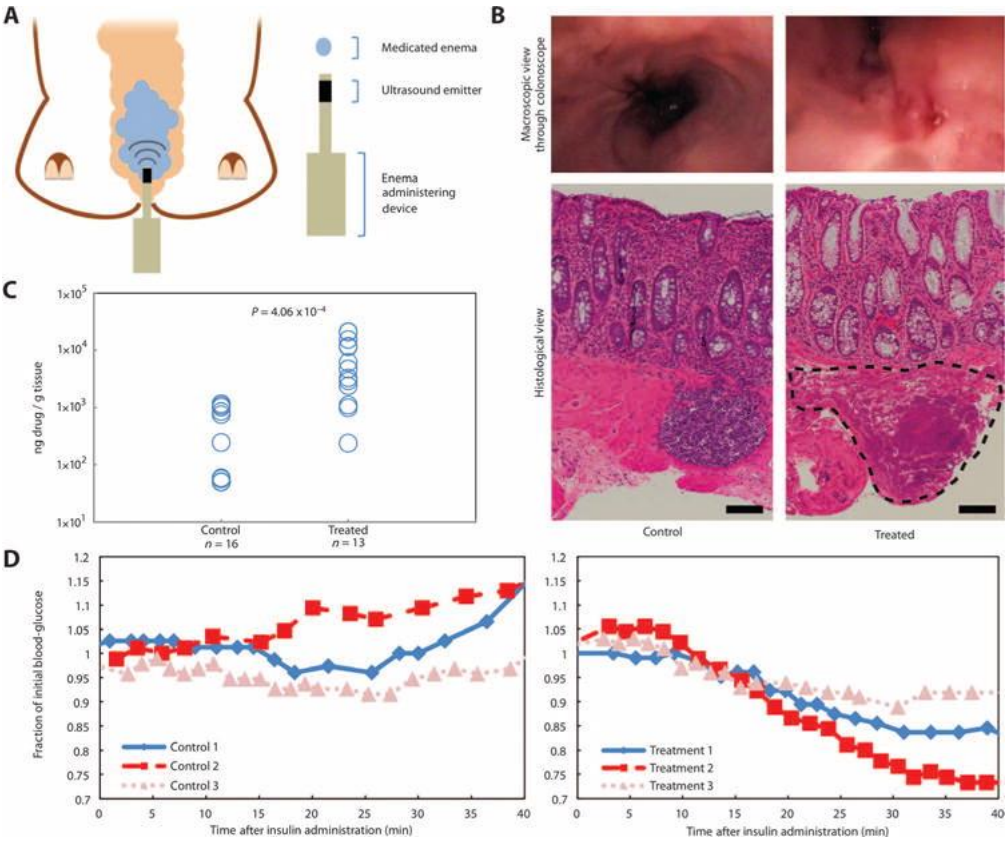

Figure 4. A) Experimental setup showing placement of a medicated enema and insertion of the 20-kHz ultrasound probe in the rectum of a pig. (**B**) Representative macroscopic (top) and histological (bottom) views of the rectum not treated with ultrasound (control) or a single administration of 20-kHz ultrasound. The outlined area indicates minor localized saponification of the muscularis in <5% of the treated area examined. Scale bars, 100 µm. (**C**) Mesalamine drug content in colon tissue biopsies normalized by the mass of the tissue biopsy without (control) and with (treated) a single administration of 20-kHz ultrasound. Each point represents one biological replicate. *P* value determined by two-tailed Student's *t* test. (**D**) Animals' blood glucose normalized to its initial value as a result of the placement of an enema containing 100 U of insulin without (left) or with (right) a single administration of 20-kHz ultrasound. Each individual curve is a biological repeat. Reproduced with permission from Schoellhammer et al. [114].