Abstract

Systemic delivery by oral administration of macromolecular active pharmaceutical ingredients (APIs), e.g. peptides and proteins, is challenging due to their physical and chemical instability in the gastrointestinal (GI) fluids and their low membrane permeability. Common formulation strategies include the addition of excipients, such as protease inhibitors (PIs) and permeation enhancers (PEs), to overcome both, the enzymatic and absorptive barriers. Recently, clinical trials confirmed the advantageous use of PE-based oral dosage forms to achieve therapeutically relevant bioavailability of both insulin and a glucagon-like peptide-1 receptor agonist. Yet, the fraction of intact peptide reaching the systemic circulation is still relatively low (≈ 1%), necessitating high drug loads, leading to costly and potentially commercially unviable dosage forms. Upon oral administration, dissolution and diffusion of peptide and excipients along the GI-tract will decrease the respective local concentrations at the sites of absorption and reduce their co-localisation at the epithelium. This PhD thesis hypothesizes the possibility of increasing oral bioavailability of peptides by facilitating unidirectional release of drug and excipients towards the intestinal mucosa and thereby improving peptide and excipient co-localisation.

The first part of this thesis focuses on an in vitro investigation of the hypothesis by the use of cylindrical unidirectionally releasing microcontainers (MCs). A significant increase in insulin permeation across Caco-2 and Caco-2/HT29-MTX-E12 co-culture monolayers was observed by securing optimally directed release of insulin and the PE, sodium caprate (C10). With close contact between MCs and the Caco-2 cell monolayer, a 35-fold increase in insulin permeation was obtained compared to the equivalent amount of insulin and C10 in solution. Investigating the effect of distance between the optimally oriented MCs and the Caco-2 cell monolayer on insulin permeation was enabled by the use of custom-made sample holders for collective elevation of the MCs. This part of the study revealed the importance of proximity between the MCs and the absorptive barrier as a 50% decrease in insulin permeation was observed for every distance increase of 0.13 mm. A combination of confocal laser-scanning microscopy and transepithelial electrical resistance measurements uncovered the main mechanism of permeation enhancement as being a reversible local deterioration of the Caco-2 cell monolayer rather than opening of tight junctions, when close proximity (0.0 – 0.2 mm) was facilitated between MCs and the monolayers.
The second part of the thesis revolves around in vitro and ex vivo based optimisation of the insulin formulation for loading into the MCs prior to in vivo studies. Expanding the Caco-2 cell permeation study showed a significant increase in insulin permeation with sodium dodecyl sulphate (SDS) as PE compared to C_{10} and lauroyl carnitine. Likewise, SDS also proved more efficient than C_{10} in an ex vivo Franz diffusion cell setup with porcine intestinal tissue as the barrier. Despite the Caco-2 cell monolayer deterioration, visualised in the in vitro permeation studies in the first part of the thesis, no increased risk of pathogen absorption was observed ex vivo, based on monitoring of barrier integrity by apical addition of 70 kDa fluorescein isothiocyanate-dextran as a pathogen marker. Including soybean trypsin inhibitor (STI) together with insulin and SDS in the MCs further improved permeation of intact insulin in a combined proteolysis and permeation in vitro setup with apical inclusion of α-chymotrypsin. However, in spite of the promising in vitro and ex vivo results, no absorption was detected upon oral administration of MCs loaded with insulin, SDS and STI to rats. Subsequent, microscopic investigation of the GI-tracts of the rats suggested lack of intestinal retention of the MCs as a main obstacle counteracting the possible advantage of unidirectional release, and thus potential insulin absorption.

The third part of the thesis focuses on the development of an oral delivery device capable of ensuring optimal unidirectional release in close contact with the intestinal mucosa in vivo. The ability of elastomers to reversibly restore their original shapes upon structural alterations was utilised for drug delivery by the fabrication of a proof-of-concept polydimethylsiloxane foil with cavities for drug loading. A folded shape of the foil enabled insertion into a gelatine capsule, which was subsequently enteric coated. Capsule disintegration in the intestine would thereby result in complete unfolding of the foil and create close contact between the intestinal mucosa and the cavities of the foil loaded with insulin, PI and PE. The desired unfolding behaviour and gastric capsule protection were confirmed in vitro prior to in vivo studies in rats, which were conducted by external magnetic manoeuvring of capsules into the duodenum of anaesthetised rats. Administration of foils loaded with insulin, SDS and STI resulted in quantifiable plasma insulin concentrations for all rats, yet with a relatively low oral bioavailability of 0.12 ± 0.07% compared to subcutaneous injection. Nevertheless, the developed delivery device solved the direct challenge of MCs in terms of their orientation in vivo, forming an interesting platform for further development towards oral administration of peptides.