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Peptide hydrogels as mucoadhesives for local drug delivery

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ABSTRACT

We have investigated the possibility of using self-assembling peptide-based viscous solutions and hydrogels as mucoadhesives for the improved delivery of drugs to local mucosal surfaces. The stability of the samples under flow after deposition on a mucosal surface mimic was studied using a simplified *in vitro* model. Subsequently lidocaine and flurbiprofen, two commercial drugs, were incorporated into the viscous solutions and hydrogels and their release properties investigated using the same model. Peptide-based hydrogels showed a good resistance to erosion under flow conditions. Addition of the soluble drug (lidocaine at low pH) resulted in a stiffening of the samples but did not affect the overall peptide release. Although for this drug the conditions were not favourable, improved retention of the drug was observed for the stiffest samples tested. In the case of the insoluble drug (flurbiprofen) the samples mechanical properties were not altered when the drug was incorporated, however the sample stability and peptide release were. For mechanically weaker samples the presence of the drug as insoluble small particles resulted in an increase in their susceptibility to physically erode when a flow of medium was applied over its surface. On the other hand mechanically stronger samples showed an improved resistance to erosion, which resulted in enhanced drug retention.

1. Introduction

In the last two decades, significant efforts have been made to develop soft materials based on the self-assembly of peptide for biomedical applications with a focus on tissue regeneration (Collier et al., 2010; Gough et al., 2011). Under specific conditions various peptide-based systems can self-assemble into fibrillar structures, which in turn associate and/or entangle to form hydrogels. These structures are stabilised through a combination of non-covalent forces such as van der Waals, hydrogen bonding, hydrophobic effects, electrostatic interactions or π-stacking of aromatic moieties. By bringing together building blocks from the variety of amino acids available and the distinct physical properties associated with them, a wide range of self-assembling peptides with tuneable properties can be designed (Hamley, 2007; Mart et al., 2006).

Based on previous work by Zhang et al., 1993 we recently developed a range of self-assembling β-sheet forming peptides, typically 8 amino acids long and based on the alternation of hydrophobic and hydrophilic amino acids, allowing the design of hydrogels with tailored properties. In a recent article we described how these peptides can be used to create hydrogels for drug delivery (Roberts et al., 2012).

Based on our previous work and the unique properties of these hydrogels we decided to investigate the possibility of using these materials as mucoadhesives for local drug delivery. Mucosas are membrane that lines body cavities or passages that are open to the external environment such as in the buccal, nasal or vaginal areas. They are typically coated with a gelatinous mixture of proteins and carbohydrates (mucus). They offer a practical route for efficient topical delivery of drug as they are relatively permeable.

In this work we focussed on the octapeptide FEFEFKFK (F: phenylalanine; E: glutamic acid; K: lysine – Fig. 1A) which is well known to self-assemble into antiparallel β-sheet rich fibres and forms hydrogels (Fig. 1D) for concentrations in peptide greater than ~20 mg mL^{-1} (in water). First the adhesive properties of the peptide were investigated, subsequently two common drugs – lidocaine (Fig. 1B) and flurbiprofen (Fig. 1C) – were incorporated into the hydrogels and their release investigated. For the purpose of this proof-of-concept work a simplistic *in vitro* model based on the one developed by Smart and co-workers (Young and Smart, 1998; Kockisch et al., 2003; Riley et al., 2002) and comprising a Visking membrane was used. Visking membrane is a dialysis membrane made of cellulose (polysaccharide), which displays the elastic and soft properties of a gel when wet with water, similar to

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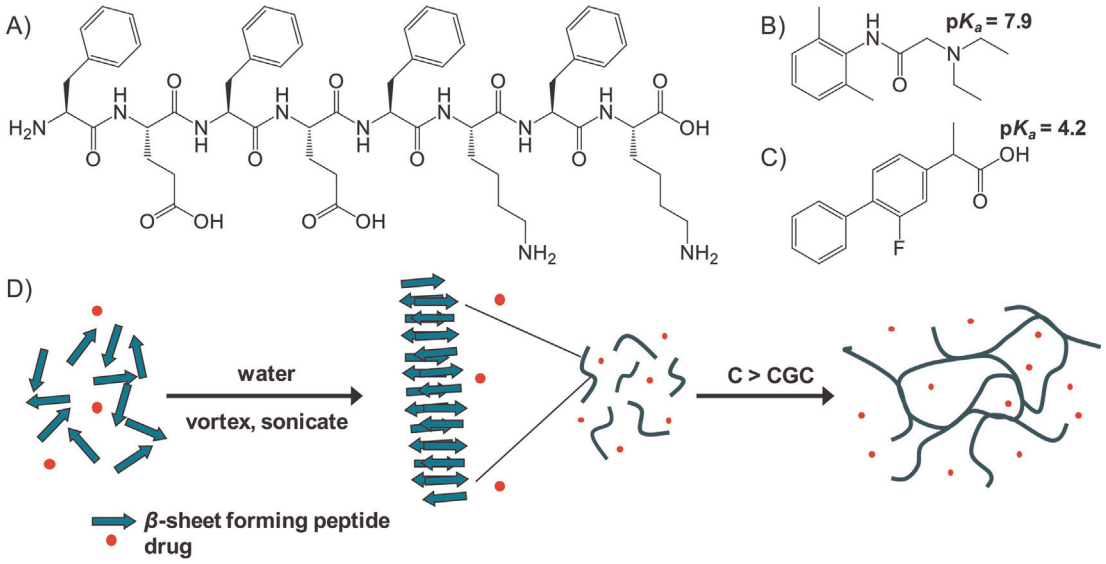

Fig. 1. Chemical structure of (A) FEFEFKFK peptide, (B) lidocaine and (C) flurbiprofen. (D) Diagrammatic representation of the self-assembly and gelation processes of β-sheet forming peptides.

mucosa surface. This type of membrane was used successfully as a model for the oral mucosa in a number of adhesion studies (Bodde et al., 1990; Smart, 1993). The release profiles obtained were correlated to the viscous and viscoelastic properties of the different formulations studied. Infrared spectroscopy was also employed in order to ensure that the peptide conformation was not altered by the incorporation of drugs into the formulations.

2. Materials and methods

2.1. Materials

Peptides were purchased from Cambridge Research Biochemicals (UK) and used without further purification. The purity of the compounds was verified by HPLC (>90%) and mass spectrometry. Lidocaine, flurbiprofen and Visking membrane were purchased from Sigma–Aldrich (UK), Aesica Pharmaceuticals (UK) and Medicell International (UK) respectively and used as received. All salts, HPLC grade solvents, deuterated water (99.9 atom% D), acid and base solutions were purchased from Sigma–Aldrich (UK) and used as received.

2.2. Sample preparation

Depending on the desired concentration (20–40 mg mL^{-1}) the required amount of peptide was suspended in HPLC grade water. The sample was vortexed and sonicated (VWR ultrasonicator bath, 30 W) until the peptide was fully dissolved (typically for 5–30 min depending on the sample concentration). Due to the presence of residual trifluoroacetic acid (TFA) from the peptide synthesis samples with final pH values of ca. 2–3 were obtained. Drug loaded samples were prepared by mixing the drugs as powders with the freeze-dried peptide at molar ratios of 1.8:1.0 (20 mg mL^{-1}), 1.2:1.0 (30 mg mL^{-1}) and 0.9:1.0 (40 mg mL^{-1}) for samples with high loading and 0.9:1.0 (20 mg mL^{-1}), 0.6:1.0 (30 mg mL^{-1}) and 0.4:1.0 (40 mg mL^{-1}) for samples with low loading. The samples were then stored at room temperature for ~12 h (overnight) before experiments were performed.

2.3. Fourier transform infrared spectroscopy (FTIR)

Multiple bounce attenuated total reflectance (ATR) FTIR experiments were undertaken using samples prepared in deuterated water. Spectra were recorded on a Thermo Nicolet 5700 spectrometer equipped with a trough plate comprising of a zinc selenide crystal. The samples were spread directly on the surface of the trough plate. Spectra were acquired in the 4000–400 cm^{-1} range with a resolution of 4 cm^{-1} over 256 scans. The deuterated water spectrum was used as background and subtracted from all spectra (software used: Omnic version 7.2, Thermo Electron Corporation).

2.4. Dynamic shear rheometry

Viscous and viscoelastic properties were assessed using a stress-controlled rheometer (TA Instruments AR-G2) equipped with a Peltier plate to control temperature. A parallel plate geometry was used with a diameter of 20 mm. For viscosity measurements (viscous samples) the apparatus was used in a flow mode. The viscosity of the samples was measured as a function of shear rate in the range 0.01–1000 s^{-1}. For viscoelastic measurements (gel samples) the apparatus was used in an oscillatory mode. To ensure the measurements were made in the linear viscoelastic regime (LVR), amplitude sweeps were performed and showed no variation in G' and G'' up to a strain of 1%. The dynamic moduli of the hydrogels were therefore measured as a function of frequency in the range 0.1–100 rad s^{-1} at a strain of 0.1%. In both modes, all experiments were performed at 25 °C and repeated at least three times to ensure reproducibility. Unless stated otherwise all viscosities and moduli mentioned in the text are taken at a shear rate of 1 s^{-1} and an angular frequency of 10 rad s^{-1}.

2.5. Salt solution preparation

Salt solution was prepared according to the formulation described in reference (Diem and Lentner, 1972). Sodium hydrogen carbonate (0.42 g), sodium chloride (0.43 g), potassium chloride (1.49 g) and sodium dihydrogen phosphate dihydrate (1.03 g) were dissolved in HPLC grade water (1 L). Calcium chloride dihydrate (0.22 g) was then added to the solution, which was vigorously agitated until complete dissolution of the salt. The solution was stored at 4 °C. The final pH of the medium was 6.50 ± 0.05.

2.6. Stability in excess medium

The stability of the peptide formulations was assessed in static conditions. A glass slide was covered with a Visking (cellulose)