Delivery of Therapeutic F(ab) antibody fragments to the Oral Mucosa Using Electrospun Patches

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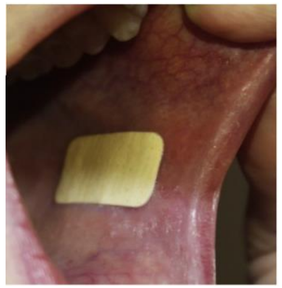
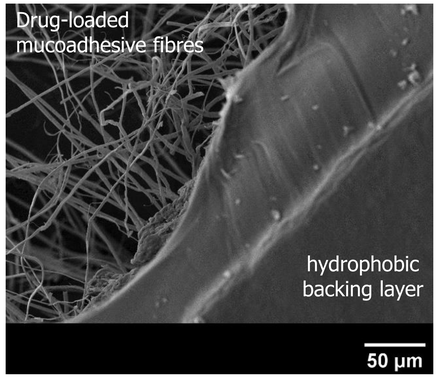
Background

We previously developed a biodegradable mucoadhesive drug-delivery patch for the oral mucosa[1]



Advantages:
- Site specific delivery
- High patient acceptability
- Long residence times
- Flexible
- Unobtrusive

Applications:
- Oral lichen planus
  - ✓ Stage II trial successful
- Oral candidiasis
- Pain relief
- Delivery of biologicals[2]



1. H. E. Colley et al. Pre-clinical evaluation of novel mucoadhesive bilayer patches for local delivery of clobetasol-17-propionate to the oral mucosa, *Biomaterials*, 2018, **178**, 134–146.
2. J. G. Edmans et al. Incorporation of lysozyme into a mucoadhesive electrospun patch for rapid protein delivery to the oral mucosa, *Mater. Sci. Eng. C*, 2020, **112**, 110917.

Review: J. G. Edmans et al. Mucoadhesive electrospun fibre-based technologies for oral medicine, *Pharmaceutics*, 2020, **12**, 1–21

Release of a model F(ab) fragment

A model biotinylated F(ab) fragment was electrospun into the existing formulation and released while maintaining antigen-binding activity

97% ethanol was the most effective electrospinning solvent for preserving F(ab) binding activity

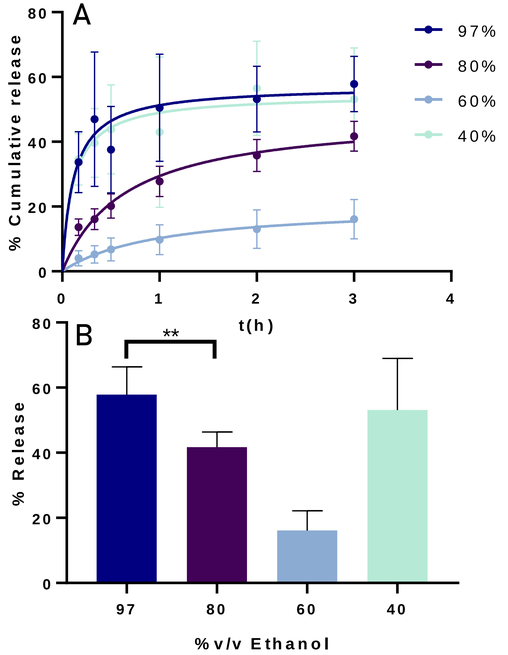


Figure 1. Cumulative release (**A**) over time and (**B**) after 3h of antigen-binding biotinylated goat anti-mouse F(ab) fragment from electrospun membranes prepared using different ethanol/water solvent mixtures, following release in simulated saliva at 37°C for 3h. Measured using direct ELISA with mouse IgG coated wells (mean +/- SD, N=3). Analysed using one-way ANOVA with post hoc Tukey tests. **, $p < 0.01$

Preparation of anti-TNFα F(ab)

A polyclonal rabbit anti-TNFα IgG (~150 kDa) was fragmented by papain degradation to produce antigen neutralising F(ab) fragments (~50 kDa). These F(ab) fragments will be used in this study as a model therapeutic, analogous to approved F(ab)s such as Certolizumab pegol.

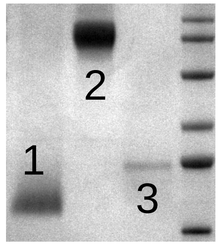


Figure 2. SDS-PAGE of papain degradation products and starting material.

1. F(ab) fragment product
2. IgG starting material
3. Fragment crystallisable region

The TNFα neutralising effect of the F(ab) can be measured in an oral keratinocyte cell line as a decrease in IL8 expression

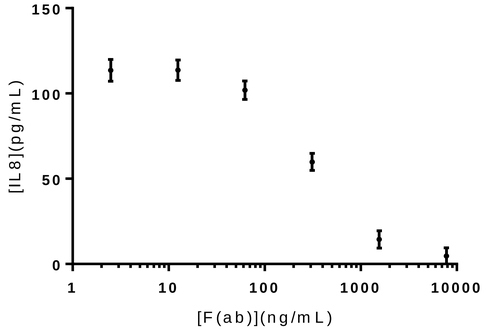


Figure 3. Dose response curve of FNB6 oral keratinocytes stimulated with TNFα (5 ng/mL) preincubated with varying concentrations of neutralising F(ab) fragment (1h, 37°C). Cells were treated overnight before measuring IL8 concentration using an ELISA kit as directed (mean +/- SD, N=3).

Activity of eluted anti-TNFα F(ab)

As a preliminary experiment the F(ab) was electrospun into membranes at a low dose (18.9 ng/mg). The F(ab) membrane eluate reduced IL8 expression to a greater extent than the placebo, showing that the F(ab) maintained neutralising activity following release. Interestingly the placebo also reduced IL8. This is likely related to poly(vinyl pyrrolidone) in the membrane eluate. It is expected that a higher dose could be used to achieve complete neutralisation.

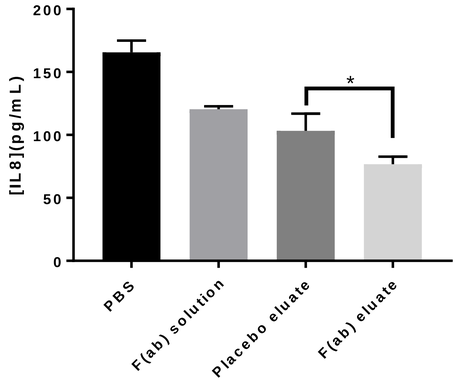


Figure 4. IL8 expression in FNB6 oral keratinocytes stimulated with TNFα (5 ng/mL) preincubated with membrane eluted neutralising F(ab). Membrane samples (20 mg) were eluted in simulated saliva (1mL) at 37°C for 3h. PBS and placebo membrane eluate were used as negative controls. F(ab) at an equivalent concentration to the calculated maximum release was used as a positive control. Cells were treated overnight before measuring IL8 concentration using an ELISA kit as directed (mean +/- SD, N=3). Analysed using one-way ANOVA with post hoc Tukey tests. *, $p < 0.05$

Future work

The delivery of Fab fragments to the oral mucosa will be investigated using fluorescence imaging in tissue engineered epithelial models

A final project aim will be to deliver anti-TNFα fragments topically to inflamed tissue engineered oral mucosal models and investigate inflammatory biomarkers and histology