UNIVERSITY OF COPENHAGEN

FACULTY OF HEALTH AND MEDICAL SCIENCES

Influence of proximity on the permeability enhancing effect of microcontainers for oral insulin delivery

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Introduction

Microcontainers have the potential of increasing oral insulin bioavailability by confining the effective intestinal absorptive area and improving co-localisation with excipients by unidirectional release (Fig. 1). The principle has previously increased oral bioavailability of small molecules[1-2].

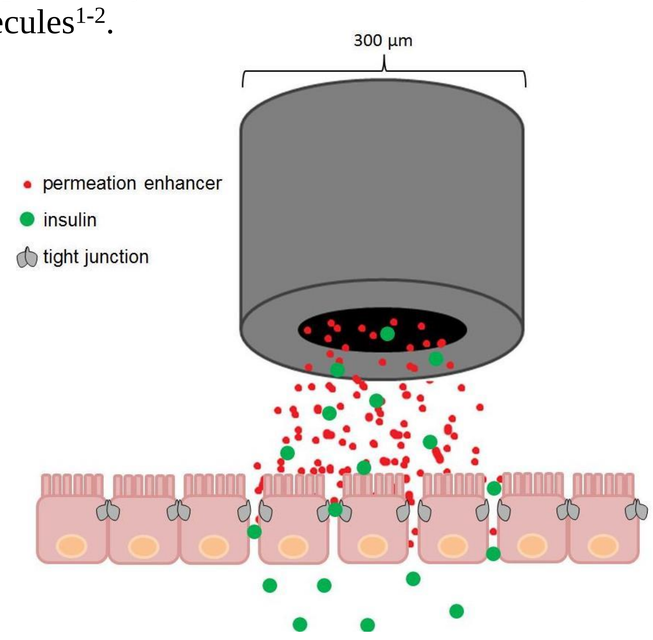


Fig 1. Concept of increasing insulin permeability by co-localisation with a permeation enhancer upon unidirectional release from a microcontainer.

Aims

- Gain *in vitro* proof-of-concept by studying the transport of insulin upon release in combination with sodium caprate (C_{10}) from microcontainers.
- Evaluate the influence of proximity by controlling the distance between microcontainers and the barrier.

Methods

Microcontainers were fabricated by photolithography in SU-8 on silicon wafers and cut into chips (12x12 mm), each holding 625 microcontainers. A powder mixture of human insulin and C_{10} (1:1 w/w) was then loaded by centrifugation (Fig. 2).

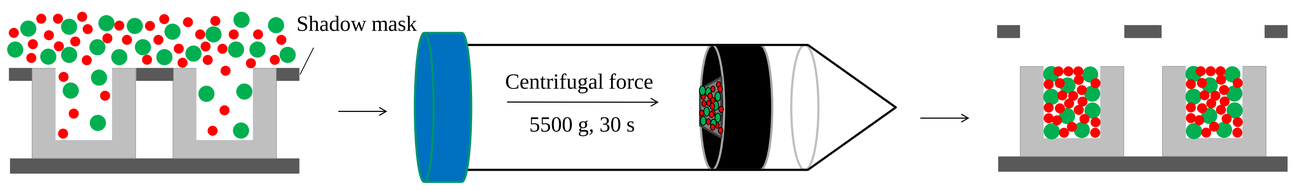


Fig 2. Loading process with insulin and C_{10}. A chip of 625 microcontainers was aligned with a shadow mask to avoid powder settlement between the microcontainers. Centrifugation in a swinging-bucket centrifuge facilitated the loading, followed by removal of the shadow mask.

Transport study conditions are given in Table 1. Loaded chips were placed with the openings of the microcontainers facing the barrier, either directly on top, or with elevation (0.2, 0.5 or 2.0 mm) obtained by polytetrafluoroethylene (PTFE) carvings (Fig. 3). Integrity assessments were done after 2 h transport or upon subsequent 24 h incubation in cell growth medium at 37 ºC and 5% CO_2.

Table 1. *In vitro* transport study

Barrier	Caco-2
Medium (HBSS, pH 7.4)	10 mM HEPES 0.05% BSA (w/v)
Conditions	pH 7.4, 37 ºC
Quantification	RP-HPLC
Integrity assessments	TEER, microscopy

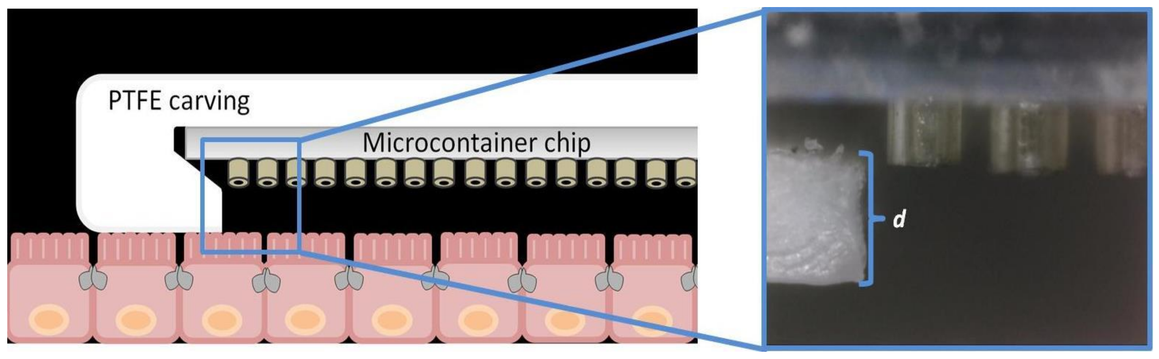


Fig 3. Left**: Illustration of the transport study setup using PTFE carvings for controlling the distance between the microcontainer chip and the barrier.** Right**: Micrograph of a microcontainer chip elevated by a PTFE carving, with depiction of dimension, *d*, ensuring exact microcontainer-monolayer distance.**

Results – Transport studies

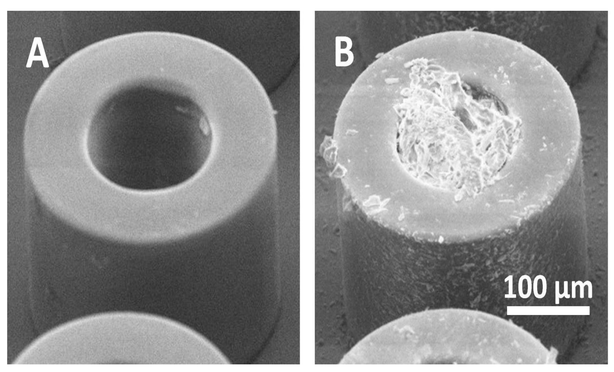
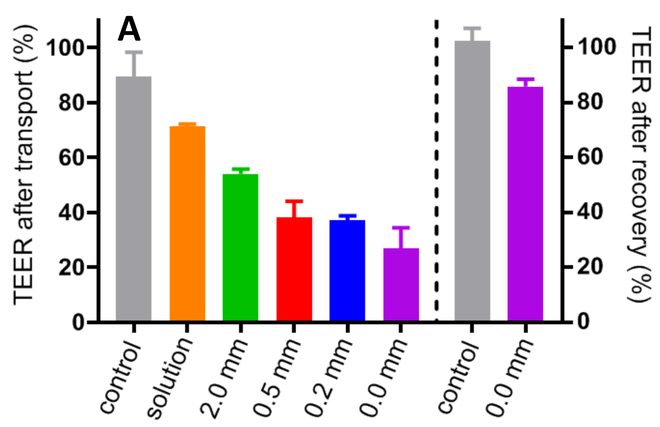


Fig 4. Representative SEM images of microcontainers, A: **empty,** B: **insulin + C_{10} (1:1 w/w).**



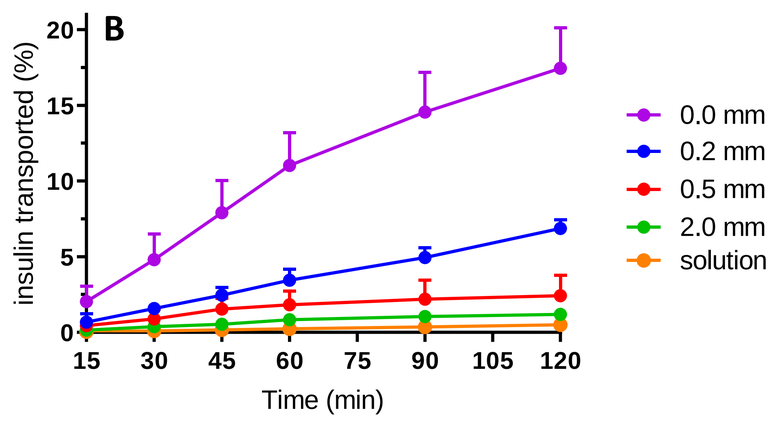


Fig 5. A: **TEER values of the monolayers, relative to initial values, after 2 h (left of dotted line) and after subsequent 24 h incubation (right of dotted line),** B: **Transport profiles obtained with different distances between microcontainers and the monolayer. Equivalent amounts of insulin (0.1 mM) and C_{10} (3 mM) were used for the solution. Mean + SD (n = 3).**

Results – Microscopy

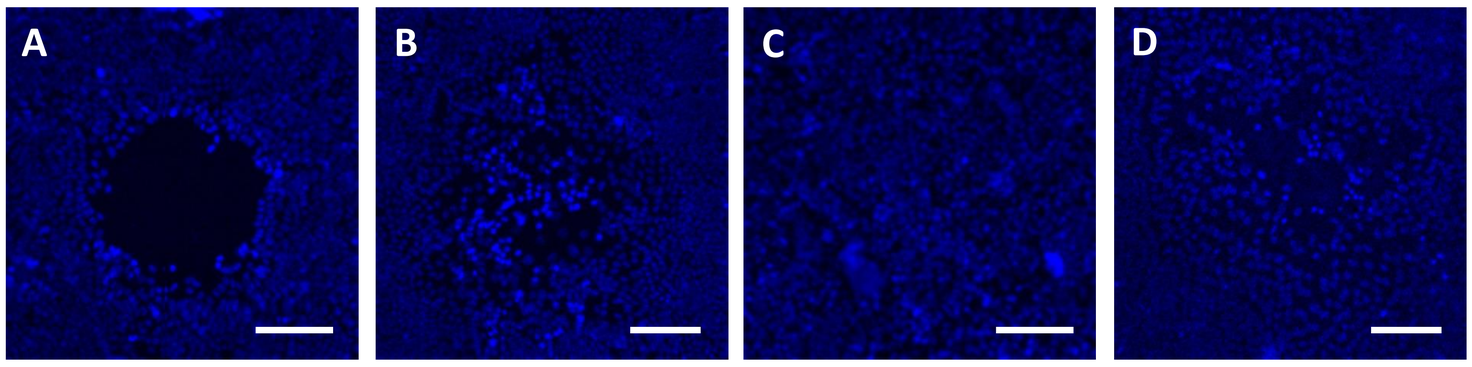


Fig 6. Representative confocal laser-scanning micrographs of Caco-2 monolayers with Hoechst 33342 nuclei staining. A: **After 2 h, *d* = 0.0 mm,** B: **After 2 h, *d* = 0.2 mm,** C: **After 2 h, *d* = 0.5 mm,** D: **after 24 h incubation, *d* = 0.0 mm. Scale bars represent 100 µm (n = 2).**

Conclusion

Confining the effective absorptive area and improving co-localisation of insulin and C_{10} significantly improved insulin permeability across Caco-2 monolayers, either by non-destructive paracellular permeation enhancement or local reversible deterioration of the barrier.

References

1. Nielsen, L.H. *et al.* Int. J. Pharm. **504**, 98–109 (2016)
2. Mazzoni, C. *et al.* J. Controlled Release **268**, 343–351 (2017).

Acknowledgements

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