

Supporting Information

A once-a-day dosage form for the delivery of insulin through the nasal route: *in vitro* assessment and *in vivo* evaluation

H. Nazar, ^{*a1} P. Caliceti,^b B. Carpenter,^a A.I. El-Mallah,^c D.G. Fatouros,^d M. Roldo,^a S.M. van der Merwe,^a J. Tsibouklis.^a

^aSchool of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, Hampshire, PO1 2DT, UK.

^{a1}Present address: Department of Pharmacy, Health and Wellbeing, University of Sunderland, Sunderland, Tyne and Wear, SR1 3SD, UK.

^b Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Padua, Italy

^c Department of Pharmacology, Faculty of Pharmacy, Beirut Arab University, Lebanon

^d Department of Pharmaceutical Technology, School of Pharmacy, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece.

*Corresponding author: hamde.nazar@sunderland.ac.uk, (004) 1915152097

The Transwell[®] inserts used for the transport studies were also used for CLSM visualisation. Following the transport study, the monolayers were rinsed apically and basolaterally with sterile PBS, and a solution of propidium iodide (30 µg/mL, in Media 199) was applied apically for 3 min. The solution was then removed, and the support filter cut from the plastic insert and placed between two glass coverslips. The cells were visualised using a Zeiss LSM 510 Meta (Germany) instrument, with propidium iodide excitation at 514 nm. Cells that had been free from dye were decreed viable; an image of damaged cells (incubated with 0.1 % SDS for 2 h) was used as viability comparator. All experiments were carried out in triplicate.

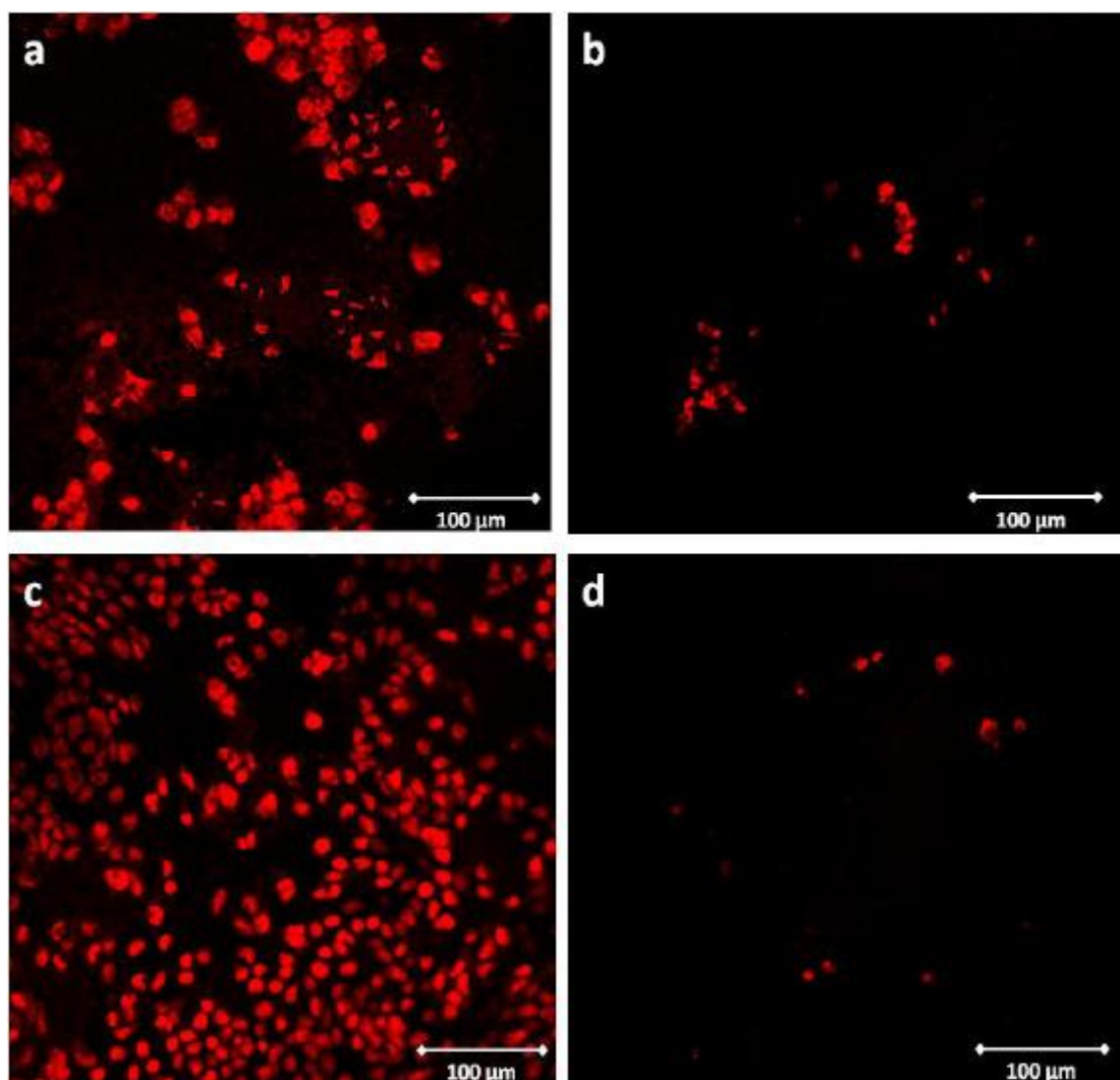


Fig. 6s. CLSM images of Calu-3 cells treated with (a) 3.6 % w/v TMC solution, (b) TMC-PEG-GP hydrogel, (c) 0.1 % SDS solution and (d) Media 199.

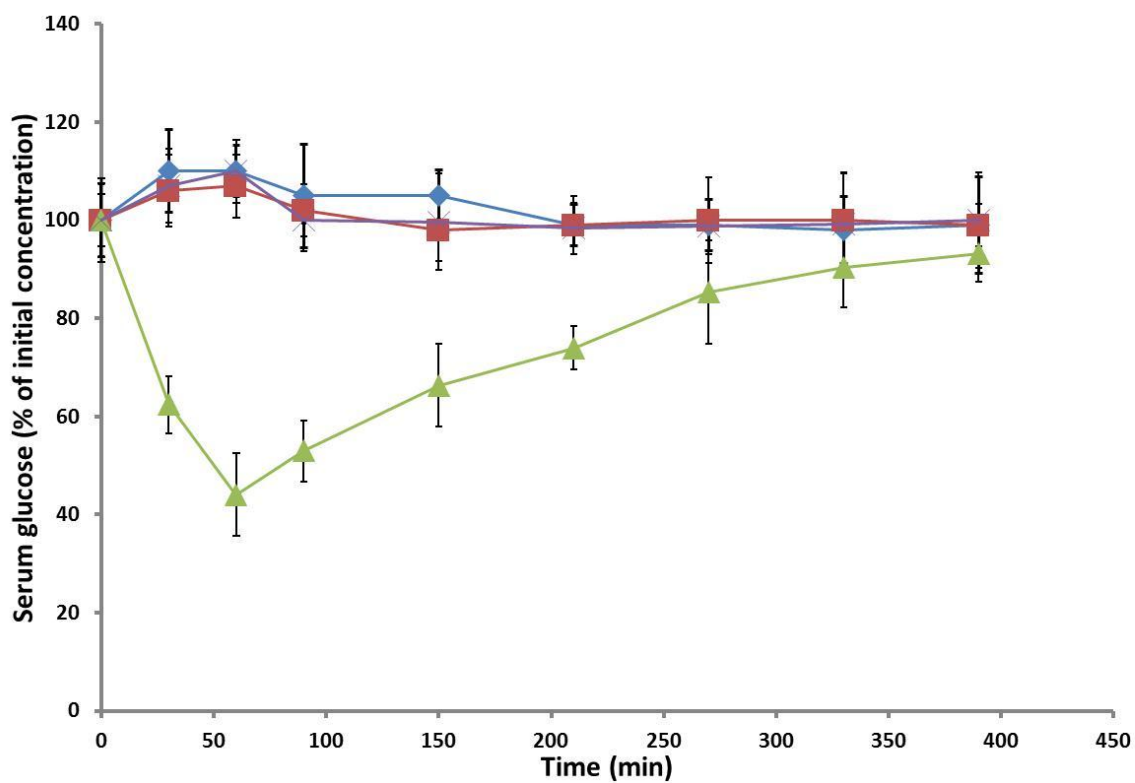


Figure 7s. Serum glucose levels in rats monitored over 6 hours following administration of: (■) TMC solution; (×) insulin solution; (◆) hydrogel, and (▲) insulin subcutaneous injection. Mean ± SD, n=5.