Development of microfluidic-based tools to mimic the human gastrointestinal tract

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Introduction: The tremendous growth of nanotechnology applications in the food and feed arena, requires suitable methodologies to ascertain their safety and efficiency. Digestion in the human gastrointestinal tract (GIT) poses a harsh environment to which orally-administrated bioactive compounds may not resist. Also, the intestinal epithelium constitutes an additional barrier, which needs to be overcome in order to achieve absorption to the systemic circulation[1]. Microfluidics offers the capacity to study valuable compounds in minute concentrations and small volumes (e.g., nanomaterials), thus paving the way to the fabrication of new platforms that will allow assessing the bioaccessibility and intestinal absorption of emerging nanoformulations. Here, we developed a modular microfluidic platform to simulate digestion in the human GIT and cell-based intestinal epithelial absorption (gut-chip). **Materials&Methods:** Microfluidic devices were designed using 2D and 3D CAD software and fabricated from PDMS following standard UV photolithography, rapid prototyping, and soft lithography techniques (Fig.1 A-B). On-chip digestion was quantified using a fluorescently-labelled casein derivative and compared to a standardised static digestion protocol[2]. Cell-based gut-chips were fabricated by bonding two parallel channels separated by a semi-permeable PDMS membrane fabricated in-house. The membrane was pre-treated, coated with a mixture of extracellular matrix (ECM) proteins (collagen/matrigel), and seeded with the human intestinal epithelial cell line, Caco-2, at $1x10^5$ cells/cm^2. Cell morphology and proliferation were assessed by immunocytochemistry of the tight junction protein occludin, with actin and nuclei counterstained using phalloidin and DAPI respectively. **Results&Discussion:** Fig. 2A shows that on-chip digestion was successfully achieved following a time-resolved kinetics profile in both gastric and intestinal phases. While the end-point fluorescence (Fig. 2B, green) was lower when compared to the current gold standard protocol, the results were consistent and reproducible, highlighting the potential of this miniaturised device to be used for *in vitro* studies of new nanomaterials. Gut-chips showed that Caco-2 cells were able to adhere and proliferate on the ECM-coated PDMS membranes (Fig. 1D). **Conclusion:** The present work contributed to the establishment of robust *in vitro* tools based on microfluidics to study the digestion and intestinal absorption of functional compounds designed for oral administration. Future work will focus on the introduction of biosensors to monitor relevant indicators (pH, temperature, transepithelial resistance) and on the development of a fully interfaced platform.

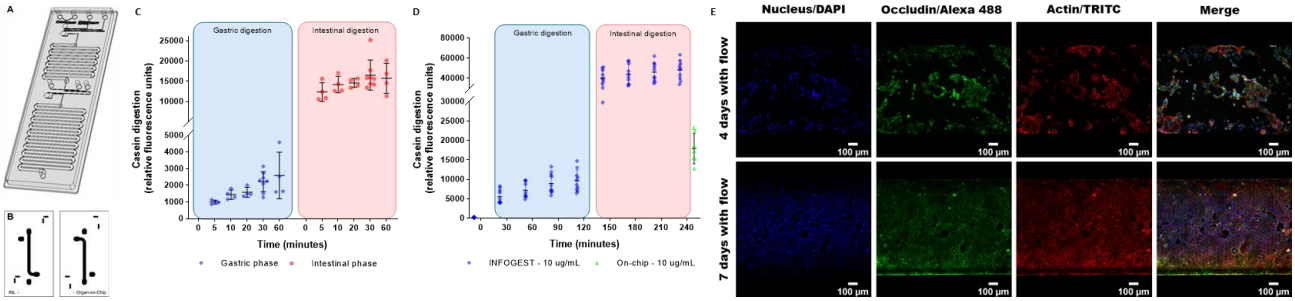

Figure 1: A-B. Representation of the digestion-chip design and gut-chip design (top and bottom slab), respectively; **C.** On-chip digestion of a casein reporter molecule (10 µg.mL^{-1}). Digestion on-chip profile kinetics obtained by varying the flow rates (12-1152 µL·h^{-1}) of the sample and simulated digestion fluids; **D**. Digestion following the static INFOGEST method of casein at 10 µg·mL-^{1} (blue) and its comparison with the end-point of on-chip digestions (green); **E.** Confocal microscopy images of Caco-2 cells cultured on top of ECM-coated PDMS membranes following 4 and 7 days of culture under continuous flow (30 µL·h^{-1}).

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