Chitosan microspheres for *Helicobacter pylori* infection treatment and prevention of gastric colonization

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**Introduction:** *Helicobacter pylori* (*H. pylori*) colonizes over 50% of the world’s population, causing gastric lesions, from gastritis to gastric cancer. The adhesion of *H. pylori* is mediated by adhesins on the bacterial surface which recognize glycoproteins expressed in mucins on the surface of epithelial gastric cells [1]. Chitosan has been investigated as a drug carrier to stomach because of its mucoadhesive properties [2], resulting from electrostatic interactions between its positively charged free amine and negatively charged gastric mucins. Chitosan is also known by its bacteriostatic properties due to electrostatic interactions of chitosan cationic amino groups with anions on the bacterial wall [3]. Despite these advantages, the high solubility of chitosan in low pH restricts its use for gastric drug delivery in the form of 3D structures, making chitosan crosslinking a requirement. This work aims to study the effect of chitosan microspheres crosslinked with genipin, a natural crosslinking agent, in preventing/removing *H. pylori* gastric cell colonization.

**Experimental methods:** Chitosan microspheres were produced in a high voltage electrostatic system by extruding chitosan droplets (1%w/v in acetic acid) into sodium triphosphate pentabasic solution. Microspheres were crosslinked in 1mM or 10mM genipin solutions over different reaction times. Microsphere size and morphology was visualized by scanning electron microscopy and optical microscopy. Chitosan crosslinking was accessed by time lapse fluorescence microscopy and its chemical structural changes monitored by infrared spectroscopy. Microspheres stability and swelling in simulated gastric fluid (SGF) with pepsin was evaluated by optical microscopy during 7 days. *H. pylori* strains expressing different adhesins (BabA and SabA) were 35S- or FITC-labeled and the gastric carcinoma cell line MKN45 (mainly expressing Sialyl-Leα, which binds SabA) was used. *H. pylori* adhesion to microspheres and/or cells was performed under pH 2.6 and 6.0 for 2h, at 37ºC, 120 rpm and four conditions were tested depending whether microspheres were incubated with gastric cells before or after *H. pylori* infection (Mic+*H.pylori*; Cells+*H.pylori*; Cells+*H.pylori*+mic and Cells+mic+*H.pylori*). FITC-*H. pylori* adherent to microspheres were detected by confocal microscopy and 35S-*H. pylori* adherent to gastric cells or microspheres were detected using a luminescence counter (Microbeta).

**Results and Discussion:** Chitosan microspheres with a mean diameter of 170±15μm were produced by ionotropic gelation. Genipin crosslinking was optimized since it occurs through the amine groups that are also necessary for maintaining chitosan mucoadhesive properties. Therefore, full crosslinking is not desirable, only the minimum to avoid microsphere disintegration in acidic conditions. Microspheres with 10mM/1h genipin were selected since they did not dissolve in SGF. Adhesion of all *H. pylori* strains to chitosan microspheres occurred under pH2.6 and pH6.0, despite being higher at pH6.0. SabA+ strain presented higher adhesion than SabA- strain to MKN45 cells, meaning that SabA+ *H. pylori* is recognizing the Sialyl-Leα receptor expressed by the cells, especially at lower pH’s. In both pH’s, the addition of chitosan microspheres to gastric cells before and after pre-incubation with *H. pylori* decreased the amount of *H. pylori* adhered to cells.

**Conclusion:** Microspheres crosslinked with 10mM/1h of genipin are stable under acidic conditions during at least 7 days, adhere different strains of *H. pylori* and prevent/remove *H. pylori* gastric cell colonization under different conditions. These findings reveal the potential of chitosan microspheres as alternative or complementary treatment for *H. pylori* gastric eradication or in the prevention of *H. pylori* colonization.


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**Keywords:** Biomaterials; chitosan microspheres; bacterial adhesion; *Helicobacter pylori*; gastric cancer.