

ability of both cell types to heal wounds after the challenge with *H. pylori* components by monitoring cell migration in association with metabolic activity, proliferation, cell cycle distribution and damage to cell nuclei.

Results: We showed that UreA, CagA and GE promoted the epithelial cell renewal. However, in vivo their excessive activity may pose an increased risk of gastric neoplasia. In contrast, *H. pylori* LPS in a higher (25 ng/mL) but not lower (1 ng/mL) concentration showed cytotoxic and anti-proliferative activities associated with destruction of cell nuclei.

Conclusions: *Helicobacter pylori* LPS in vivo might lead to the maintenance of chronic inflammatory response and cause pathologies on the level of the gastric mucosal barrier.

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P04.06 | Critical role of AlpA and AlpB for biofilm formation and cell adhesion of *Helicobacter pylori*

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The human gastric pathogen *Helicobacter pylori* forms biofilms in vitro and in vivo. It was previously demonstrated that strain TK1402, which was isolated from a Japanese patient with duodenal and gastric ulcers, has a high biofilm forming ability in vitro and outer membrane vesicles (OMV) released by TK1402 play an important role in biofilm formation. The purpose of this study was to analyze which protein(s) in the OMV contributes to biofilm formation. A spontaneous mutant derived from TK1402 lacking biofilm forming ability was obtained. AlpB, an outer membrane protein in the OMV of the mutant was markedly decreased compared to that of wild-type. Complementation of TK1402 *alpB* to the mutant fully recovered the ability to form biofilm, on the other hand, complementation with *alpB* from other strains demonstrated incomplete recovery of biofilm formation ability. It was shown that the variable region of *alpB* (amino acid position 120-156) was involved in TK1402 biofilm formation. Moreover, *alpA* locating upstream of *alpB* is associated with biofilm formation directly or indirectly. In addition, diversification of *alpA* and *alpB* sequences was shown to affect the ability of the strain to adhere to human gastric AGS cells. These results demonstrated that AlpA and AlpB play an important role for biofilm formation as well as cell adhesion.

P04.07 | In vitro Evaluation of Bioactive Chitosan Microspheres for Eradicating *Helicobacter pylori* Biofilm

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Background: Certain *H. pylori* strains can form biofilm both inside and outside human host to protect itself under environmental stress. Biofilm contributes to development of antimicrobial resistance by some kind of mechanisms like providing a barrier for diffusion and allowing resistance gene expression.

Aim: To investigate the effect of cinnamon bark oil loaded chitosan microspheres on the inhibition of biofilm formation by *H. pylori* NCTC 11637.

Methods: The strain was cultured based on standard microbiological procedure. Suspension of bacteria (McFarland 2.0) was prepared in Brucella Broth supplemented with 5% FBS and inoculated per well into a 12-well plate containing sterile glass coverslips. For evaluation of antibiofilm ability of microspheres, *H. pylori* was incubated under microaerophilic conditions at 37°C for 72 h with shaking. Chitosan and cinnamon bark oil loaded chitosan microspheres were added before (0 h) and after (72 h) biofilm formation. Mature biofilm were incubated with microspheres for 24 h. Biofilm formation was both evaluated by crystal violet (CV) quantitation assay (Yonezawa H et al. J Gastroenterol Hepatol, 2010) and SEM analysis.

Results: After adding microspheres on mature biofilm, the reduction was observed in the amount of formed biofilm compared to control (Broth and bacteria). Chitosan microspheres inhibited mature biofilm. Cinnamon bark oil loaded chitosan microspheres were more effective than unloaded microspheres for inhibiting mature biofilm.

Conclusion: Chitosan microspheres had significant antibiofilm activity on both biofilm formation and mature biofilm. These results showed that chitosan microspheres with oil incorporation decreased biofilm formation by inhibiting bacteria and amount of mature biofilm in vitro. The tested microspheres could be a potential biotherapeutic carrier to prevent and treat *H. pylori* biofilm.