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RGDSK Peptide Functionalized Helical Rosette Nanotubes (RGDSK-HRNs) Inhibit *E. coli* Adherence to Jejunal Epithelium by Blocking Integrin αvβ3

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There is an ongoing effort to find ways to reduce the intestinal colonization of pathogens in both animals and humans. Integrin αvβ3, recognizing arginine-glycine-aspartic acid (RGD) sequences, has important functions in cell adhesion, signaling, and survival. However, the role of this protein in the adhesion of bacteria, particularly *E. coli* to the jejunum, remain elusive. Therefore, to explore the expression of integrin αvβ3 and its role in interaction with a novel treatment - RGDSK-HRNs in *E. coli* binding, we performed a series of experiments using porcine jejunum, intestinal porcine epithelial 1 cell line (IPEC1) and *E. coli* K88.

Immunohistochemistry staining results showed that the normal porcine jejunum strongly expressed integrin αvβ3 on the nucleus and apical surface of epithelium and gland cells. The expression of integrin αvβ3 decreased in the epithelium of the jejunum infected with *E. coli* or *E. coli* associated with *Salmonella*. Using immune-gold staining with the integrin αvβ3 antibody, we recognized that integrin αvβ3 was expressed on the plasma membrane, cytoplasm, and nucleus of IPEC1. In the porcine jejunum, integrin αvβ3 was also found in epithelial microvilli. Immunoprecipitation and western blot data showed that the expression of integrin αvβ3 on IPEC1 decreased at 15 minutes but returned to normal after 90 minutes of infection with *E. coli* K88 ($P<0.05$). We also found that the *E. coli* K88 had a protein-like integrin αvβ3.

In this study, we reported that dose-dependent RGDSK-HRNs mediated the attachment of *E. coli* to IPEC1 ($P<0.001$). Interestingly, RGDSK-HRNs slightly induced IPEC1 apoptosis compared to the normal untreated group but significantly enhanced the survival of IPEC1 upon *E. coli* infection compared to the *E. coli* infection group ($P<0.05$). Data from binding assays on 96-well plates showed that the number of *E. coli* binding on the integrin αvβ3 coated wells was significantly higher than that binding on uncoated ones with the same dose of *E. coli* ($P<0.05$). We then performed *ex-vivo* villus adhesion assays on scraped villi from porcine jejunum. Data showed that in F4 receptor positive villi, RGDSK-HRNs significantly reduced the number of adhering *E. coli* up to 12 hours compared with the *E. coli*-only challenging group ($P<0.05$). Both RGDSK peptide and monoclonal antibody anti integrin αvβ3 control groups remained effective in inhibiting the *E. coli* binding to villi up to 24 hours. Confocal images confirmed the binding of RGDSK-HRNs-FITC to both villi and *E. coli*.

These are the first data to show the role for the integrin αvβ3 in the adherence of *E. coli* to the intestinal epithelium, and that novel RGDSK-HRNs, a potential alternative to antibiotics, can inhibit the attachment of *E. coli* to the intestinal epithelium.

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