Collagen-coated Magnetic Nanoparticles to Capture Pathogens

M. G. Sande[1], *L. Roque*[1], *M. Marques*[1], *D. Ferreira*[1], *J. L. Rodrigues*[1], *A. Braga*[1], *D. Ramada*[2], *C. J Silva*[2], *L. R. Rodrigues*[1]

[1]CEB-Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[2]CENTI-Center for Nanotechnology and Smart Materials, Rua Fernando Mesquita, 278, 4760-034 VNF, Portugal

Abstract

Background
The conventional method of diagnosing bacterial infections is based on microbial culture, however this method can take several hours or even days and lacks sensitivity. In the past few decades, the use of immunological and molecular techniques such as ELISA, PCR and mass spectrometry are used to diagnose infections. While these methods can be highly sensitive and reliable, they often require sample processing steps, complex equipment and skilled operators. Moreover, these factors contribute to the relatively high cost of such methods. Hence, there is a genuine need for point-of-care, simple and affordable diagnostics. The Covid-19 pandemic has made it evident that rapid testing outside the clinical environments is crucial. Enrichment of pathogens present in a patient's sample coupled with subsequent bacterial identification steps can serve as a simple and affordable point-of-care method for infection diagnosis.

Objectives
The goal of this study was to develop collagen-coated magnetic nanoparticles (Coll@MNPs) to capture *Escherichia coli* recombinantly expressing pathogenic adhesins YadA (adhesin from *Yersinia Enterocolitica*) and UspA2 (adhesin from *Moraxella Catarrhalis*).

Methods
The adhesins YadA and UspA2 are responsible for the adherence of the respective bacterial pathogen to the hosts collagen. Coll@MNPs were chemically synthesized and characterized by ATR-FTIR and DLS. Bacterial cloning was used to express YadA and green fluorescent protein (GFP) reporter in *E. coli*. Similarly, UspA2 and mcherry red fluorescent reporter were cloned in *E. coli.* In adhesion assays, Coll@MNPs were incubated with *E. coli* YadA GFP and *E. coli* UspA2 mcherry separately, as well as in a co-culture and images were captured using confocal fluorescent microscopy (CFM). The non-captured bacteria were recovered and used to calculate the capture efficiency.

Results

The ATR-FTIR spectra revealed characteristic bands of collagen thus confirming the successful collagen coating of the MNPs. Coll@MNPs with diameter 240.2 nm and a Zeta-potential of 18.2 mV were found to be stable and were selected for the following adhesion assays. CFM imaging clearly revealed the capture of *E. coli* YadA GFP and *E. coli* UspA2 mcherry by Coll@MNPs separately and in co-culture. Capture efficiencies of the Coll@MNPs for *E. coli* YadA GFP and *E. coli* UspA2 mcherry was 50% and 68%, respectively. In conclusion, collagen-coated magnetic nanoparticles were successfully used to capture with good capture efficiency bacteria expressing pathogenic adhesins YadA and UspA2.

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