Preparation and characterization of silica nanoparticles conjugated with a protein that specifically recognizes human cancer cells.

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BEL β-trefoil is a novel lectin purified from the fruiting bodies of king bolete mushrooms [1]. The lectin has potent anti-proliferative effects on human epithelial cancer cells which confers to it an interesting therapeutic potential as an antineoplastic agent. All the three potential binding sites present in the β-trefoil fold are able to selectively bind the T-antigen disaccharide (Thomsen Friedenreich antigen;Galβ1-3GalNAc) witch is specifically exposed in human carcinomas and other neoplastic tissues. Therefore BEL β-trefoil linked to nanoparticles could enable them to discriminate between healthy and neoplastic cells targeting nanoparticles specifically towards cancer cells. BEL β-trefoil is also available recombinantly expressed in E.coli fused with the GFP (Green Fluorescence Protein) that can be useful to immediately detect the presence and activity of the protein after the coupling reaction to nanoparticles.

RESULTS AND DISCUSSION

Nanoparticle preparation and characterization

The lectin was covalently linked to silica nanoparticles with mean diameter of 150 nm obtained by the Stober method [2] (Fig 1). The surface of the silica particles was modified in order to have on it aldehyde functional groups to allow covalent protein binding. The nanoparticles were then re-suspended in a carbonate buffer medium, pH=7.9 and bioconjugated with the recombinant protein or with the protein purified from its natural source, via Schiff base formation and reduction with sodium cyanoborohydride [3]. The particle suspensions were then washed and centrifuged several times with buffer prior to re-suspending them for further analysis. Particle size, zeta-potential and the polydispersity index (PDI) were analyzed and showed a unimodal particle size distribution with a mean diameter of 154 nm, a charge of -18 mV and a PDI of 0.4

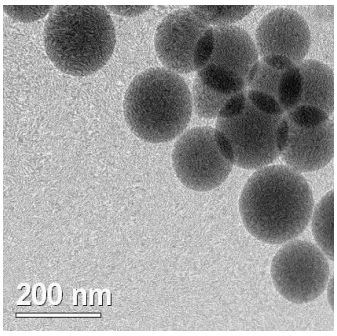

Figure 1 Silica nanoparticles

The presence of the protein bound to the nanoparticle surface was assessed by mixing 10 µl of NPs re-suspended at a concentration of 80 mg/ml in Phosphate buffer (PBS) and 90 µl of Coomassie Brilliant Blue (Fig2B). Figure 2A (left hand side) is the control experiment carried out with NPs not coupled to the protein.

Figure 2 Coomassie Brilliant Blue test

The concentration of the protein on the NP surface was determined via spectrofluorimetric experiments. The coupling efficiency was 8.72 nanomoles of protein per 1 mg of nanoparticles. The experiment was conducted exciting the protein tryptophanes at 280 nm and reading the fluorescence emission at 350 nm. In order to correct for the possible intrinsic fluorescence coming from the matrix, the signal of the uncoupled NPs was subtracted from that of the reading.

The activity of the protein was checked using a hemagglutination test: 20 mg of BEL conjugated NPs (Fig. 3B) and SiO_2 nanoparticles (Fig. 3A) were re-suspended in 250 µl PBS, 50 µl of PBS were mixed with 50 µl of the nanoparticle suspension in the first well and six serial dilutions were prepared. The last well was used as a negative control without NPs. 50 µl of red blood cells were added to all the samples. After 30-60 minutes incubation the cell agglutination becomes evident.

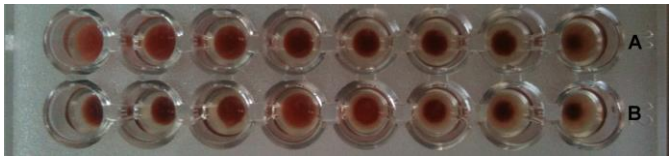

Figure 3 Hemagglutination test.

References

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