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Foslip Loaded and CEA-Affimer Functionalised Silica Nanoparticles for Fluorescent Imaging of Colorectal Cancer Cells

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Abstract : Introduction: There is a need for real-time imaging of colorectal cancer (CRC) to allow tailored surgery to the disease stage. Fluorescence guided laparoscopic imaging of primary colorectal cancer and the draining lymphatics would potentially bring stratified surgery into clinical practice and realign future CRC management to the needs of patients. Fluorescent nanoparticles can offer many advantages in terms of intra-operative imaging and therapy (theranostic) in comparison with traditional soluble reagents. Nanoparticles can be functionalised with diverse reagents and then targeted to the correct tissue using an antibody or Affimer (artificial binding protein). We aimed to develop and test fluorescent silica nanoparticles and targeted against CRC using an anti-carcinoembryonic antigen (CEA) Affimer (Aff). Methods: Anti-CEA and control Myoglobin Affimer binders were subcloned into the expressing vector pET11 followed by transformation into BL21 Star™ (DE3) E.coli. The expression of Affimer binders was induced using 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Cells were harvested, lysed and purified using nickle chelating affinity chromatography. The photosensitiser Foslip (soluble analogue of 5,10,15,20-Tetra(m-hydroxyphenyl) chlorin) was incorporated into the core of silica nanoparticles using water-in-oil microemulsion technique. Anti-CEA or control Affs were conjugated to silica nanoparticles surface using sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo SMCC) chemical linker. Binding of CEA-Aff or control nanoparticles to colorectal cancer cells (LoVo, LS174T and HC116) was quantified in vitro using confocal microscopy. Results: The molecular weights of the obtained band of Affimers were ~12.5KDa while the diameter of functionalised silica nanoparticles was ~80nm. CEA-Affimer targeted nanoparticles demonstrated 9.4, 5.8 and 2.5 fold greater fluorescence than control in, LoVo, LS174T and HCT116 cells respectively ($p < 0.002$) for the single slice analysis. A similar pattern of successful CEA-targeted fluorescence was observed in the maximum image projection analysis, with CEA-targeted nanoparticles demonstrating 4.1, 2.9 and 2.4 fold greater fluorescence than control particles in LoVo, LS174T, and HCT116 cells respectively ($p < 0.0002$). There was no significant difference in fluorescence for CEA-Affimer vs. CEA-Antibody targeted nanoparticles. Conclusion: We are the first to demonstrate that Foslip-doped silica nanoparticles conjugated to anti-CEA Affimers via SMCC allowed tumour cell-specific fluorescent targeting in vitro, and had shown sufficient promise to justify testing in an animal model of colorectal cancer. CEA-Affimer appears to be a suitable targeting molecule to replace CEA-Antibody. Targeted silica nanoparticles loaded with Foslip photosensitiser is now being optimised to drive photodynamic killing, via reactive oxygen generation.

Keywords : colorectal cancer, silica nanoparticles, Affimers, antibodies, imaging
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