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Development of nanoparticle complexes as liquid biopsy candidates for circulating tumor cell detection

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1. Introduction

Early detection of diseases is known to improve significantly the prognosis of many diseases. Liquid biopsy tests are emerging as an interesting approach, as they make diagnosis results available in a fast and simple way by just using biological fluids obtained in a minimally invasive manner. Certain solid tumour types, such as breast cancer, non-small cell lung cancer, colorectal cancer or prostate cancer, may be diagnosed by detecting circulating tumour cells (CTC) [1]. It is worth mentioning that the folate receptor is often highly expressed in these solid tumour types and may be considered as a biomarker. In this context, the use of nanoparticles may enhance the performance of liquid biopsy devices, through the specific recognition of disease biomarkers and enabling their detection in tiny volumes of body fluids. In the present research work, the potential of ethylcellulose nanoparticles as liquid biopsy nanodiagnostic tools has been preliminarily explored by using affordable materials and smooth preparation methods. For this purpose, the formation of positively charged ethylcellulose nanoparticles has been investigated using a low-energy emulsification method. Further, the ethylcellulose nanoparticles have been functionalized with gold nanoparticles or folate exploiting supramolecular interactions. The haemocompatibility of the latter complexes has been assessed as a key factor in diagnostic tests involving the analysis of blood samples.

2. Materials and methods

2.1. Materials

Ethylcellulose (abbreviated as EC10) was kindly donated by Colorcon, a distributor of the Dow Chemical Company. Ethyl acetate was from Merck. The nonionic surfactants Kolliphor® EL (abbreviated as CEL, HLB = 12-14) and Span 80® (sorbitan monooleate; Abbreviated as S80, HLB = 4.3) were from BASF and Sigma-Aldrich respectively. Varisoft RTM50 (abbreviated CatA) is a quaternized amidoamine surfactant from Evonik. Sodium dihydrogen phosphate monohydrate, di-sodium hydrogen phosphate dihydrate and sodium chloride used for phosphate buffered saline (PBS) solution preparation were from Merck. Folic acid, D-(+)-glucose and HEPES salt were from Sigma Aldrich. The latter was used to prepare the HEPES 20 mM buffer solution and HEPES buffered glucose (HBG) solution which were adjusted at a pH of 7.4. Water was deionized and MilliQ® filtered. For the synthesis of gold nanoparticles, choloroauric (III) acid trihydrate

from Sigma and sodium citrate from Merck were used.

2.2. Methods

Ethylcellulose nanoparticles were prepared by a low-energy emulsification and solvent evaporation method [2]. Gold nanoparticles (AuNP) were prepared by the Turkevich and Frens method [3]. Folate-nanoparticle complexes and AuNP-ethylcellulose hybrid nanomaterials were prepared by self-assembly of the preformed positively charged ethylcellulose nanoparticles with the negatively charged preformed AuNP or folate solution by mixing stock solutions at different ratios. The nanomaterials obtained were characterized by dynamic light scattering (DLS), zeta potential measurements, transmission electron microscopy and UV-vis spectroscopy. Haemocompatibility was assessed spectrophotometrically as described elsewhere [2].

3. Results and Discussion

O/W nano-emulsions have been obtained in aqueous solution / [CatA: nonionic surfactant] / ethylcellulose solution systems at room temperature by the phase inversion composition method. The nonionic surfactant used was either CEL or S80, while the aqueous component either phosphate buffered saline (PBS) or HEPES solution. Nano-emulsions with an oil-to-surfactant ratio of 70/30 and 95 % aqueous component were selected as templates for nanoparticle preparation by solvent evaporation. The nano-emulsions showed droplet sizes typically below 200 nm as determined by DLS. The ethylcellulose nanoparticles were obtained from the nano-emulsions by solvent evaporation under reduced pressure. The nanoparticles showed sizes smaller than those of the template nano-emulsion. Due to the presence of the cationic surfactant, the ethylcellulose nanoparticles showed a positive surface charge. As a proof of concept, the positively charged ethylcellulose nanoparticles were complexed with citrate-coated spherical gold nanoparticles (size around 30 nm and zeta potential of -22 mV). The hybrid nanomaterial showed a size of about 190 nm and a transverse plasmon band at about 598 nm. On the other hand, the formation of stable folate-ethylcellulose complexes was also investigated as a putative targeted platform towards circulating tumour cells. Stable complexes were formed as evidenced by both, particle size and zeta potential measurements. The folate-ethylcellulose complexes prepared showed excellent haemocompatibility suggesting that they may be suitable for being used in blood liquid biopsy procedures.

4. Conclusions

Positively charged ethylcellulose nanoparticles have been obtained by a low-energy emulsification and solvent evaporation method. These nanoparticles were successfully assembled with gold nanoparticles to form hybrid nanomaterials with promising optical properties for detection purposes in diagnostic tests. Further, ethylcellulose nanoparticles were also successfully complexed with folate, showing an excellent haemocompatibility. These preliminary results encourage the investigation of these complexes for use in liquid biopsy tests involving blood.

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