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Abstract title	PREPARATION AND SURFACE MODIFICATION OF PROTEIN NANOCAPSULES FOR TARGETED DRUG DELIVERY
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1. Background

Specific targeting of malignant cells can improve efficacy of drugs and prevent damage of healthy cells and tissues. Therefore it is essential to modify the surface of drug delivery systems such as nanoparticles to introduce receptor or ligand molecules which can be recognized by target cells. Folic acid (FA) can be recognized by folate receptor beta (FRß) which is specifically expressed by chronically activated macrophages playing a key role in rheumatoid arthritis. Also antibodies (mAb) are often exploited to achieve targeting to malignant cells.

2. Aims

In this study we produced nanoparticles out of human serum albumin (HSA) and modified their surface with either FA to achieve a specific uptake by chronically activated macrophages or an antibody (mAb).

3. Methods

HSA nanocapsules were produced sonochemically without the use of crosslinking chemicals and emulsifiers. Several parameters like sets of time, amplitude control and pulsation were tested and optimized to achieve particles in the nano-range. A filtration step was introduced to remove bigger particles.

Confocal laser scanning microscopy (CLSM) was used to monitor the reaction conditions and allow three-dimensional analysis of HSA capsules after fluorescence labeling of HSA. The mean size of HSA nanocapsules, the size distribution and the zeta potential were determined by dynamic light scattering.

FA was linked site specific to amino groups of HSA-nanocapsules by a carbodiimide cross linker. Free FA was removed by filtration and the HSA/FA concentration was determined photometrically. Additionally the distribution of fluorescent labeled FA on the capsule surface was determined by CLSM analysis. Binding and uptake of HSA-nanocapsules by FRß positive/negative macrophages was assessed by flow cytometry. In another approach an antibody was covalently linked on the capsules surface using a polyethylene glycol spacer.

4. Results

In this study, optimization of energetic and time parameters in ultrasound based formation gave small nanocapsules with a diameter of 443.5 ± 9.0 nm and a narrow size distribution indicated by a polydispersity index of 0.066 ± 0.080. A filtration step was incorporated to eliminate the larger diameter particle population.

Capsules with two different concentrations of FA were produced to compare the cell uptake ability as a function of FA amount linked on the surface. The FA content on capsules was determined to be 0.38 and 6.42 molecules FA per molecule HSA respectively. The presence of mAb on HSA-nanocapsules was confirmed by SDS-PAGE.

With CLSM analysis it was verified that no aggregate formation occurred. The three dimensional reconstruction of a single capsule showed a shell of HSA which is surrounding a cavity into which drugs could be loaded. FA is clearly located on the surface of the HSA capsule (figure 1).

FRβ-expressing macrophages showed an increased binding and internalization for capsules with FA when compared to those without FA (figure 2).

5. Summary/Conclusion

We describe the sonochemical synthesis of HSA nanocapsules avoiding toxic cross-linkers or emulsifiers. The capsules were functionalized with folic acid for targeting of macrophages. Furthermore it was demonstrated that folate based nanocapsule are able to target folate receptor ß-positive macrophages.

Images

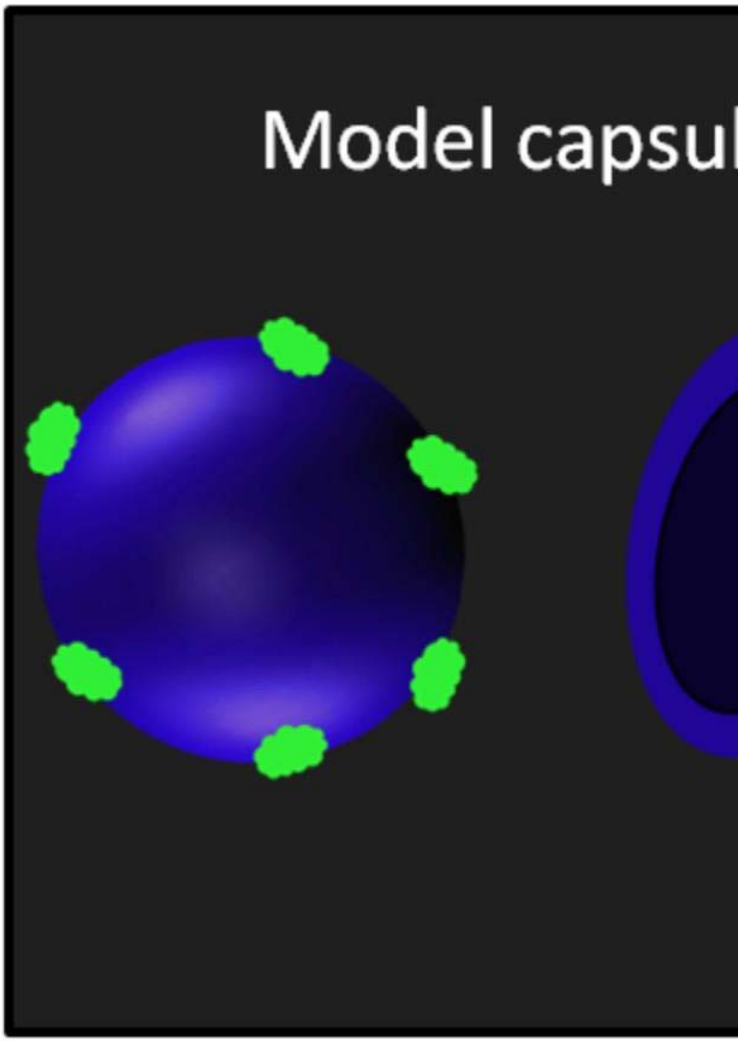

figure 1: Model of FA-HSA nanocapsule, CLSM image and 3-D reconstruction of FA-HSA nanocapsules

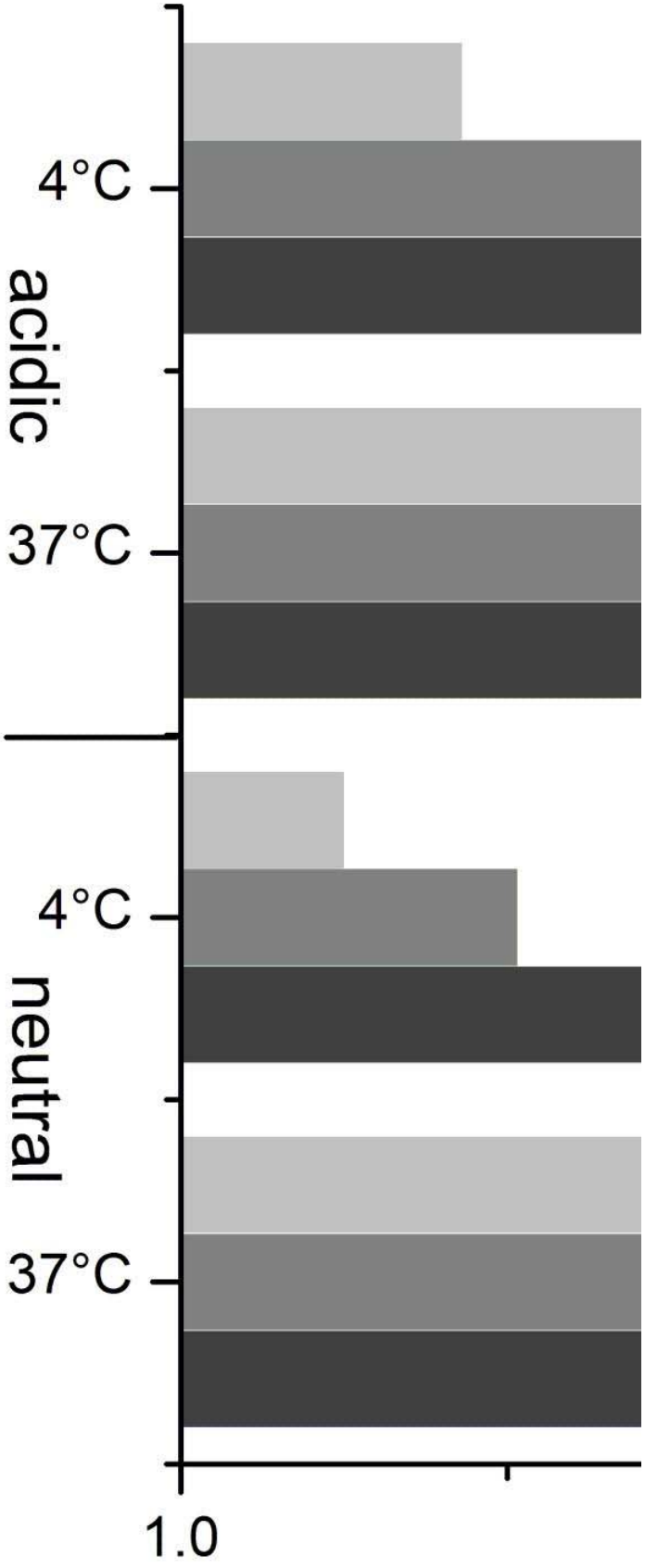
4°C
acidic
37°C
4°C
neutral
37°C
1.0

Figure 2: Binding/uptake of FITC-conjugated HSA nanocapsules by macrophages

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