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Novel fluorescent Labelled, Pegylated Prussian Blue Nanoparticles for *in vivo* Optical Imaging

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1. Introduction

Polyethylene glycols (PEGs) are widely used, non-toxic polymers in nanotechnology. These molecules are able to hide the nanoparticles from the immune cells, act as steric stabilizers and also, due to their hydrophilic character, enhance the clearance of the nanosystem. Our main goal was to successfully pegylate the Prussian Blue nanoparticles (PBNPs), using different PEGs. Furthermore, fluorescent labelling of PBNPs lets us follow the particles in vivo, thus providing information about the properties of the particles in the body.

2. Materials and methods

A modified method of Shokouhimehr was used to prepare biocompatible PBNPs [1,2]. After the PBNPs were synthesized, fluorescent modification of the particles was made. Two main approaches were tested, fluorescent labelling before and after the pegylation. The methylene blue (MB) was filtered and twofold diluted before use. PEGs (PEG 3000, 6000, 8000) were available in solid form, 10 m/m% solutions were made.

The absorbance of the samples was measured at 633 nm, by spectrophotometry. The surface charge and hydrodynamic diameter of the particles were determined using Dynamic light scattering (DLS) instrument, in backscattering mode. The samples were incubated at 37°C.

Morphological investigations of the NPs were carried out with transmission electron microscopy (TEM) and atomic force microscopy (AFM). AFM images were collected in noncontact mode. Structural measurements (X-Ray Diffraction & Fourier Transformation Infra Red spectroscopy) were also executed to determine the crystal structure.

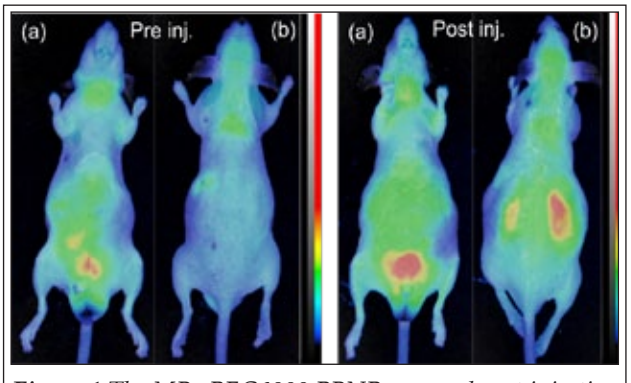

Figure 1 *The MB@PEG6000 PBNPs pre and post injection from dorsal (a) and ventral (b) plains, taken with FOBI*

In vivo and ex vivo imaging was carried out in C57BL/6 male mice (for FOBI scans; Fluorescence labeled Organism Bioimaging Instrument; red excitation, 1500 ms exposure time).

3. Results

Fluorescent labelling of Prussian Blue nanoparticles was successful. After that the synthesized nanoparticles remained stable for a 4-week period (confirmed by DLS). The best results were linked to PEG 6000. The AFM measurements showed a flat rectangular surface of the nanoparticles with a Z-average 10.01 ± 2.69 nm. Their aggregation-properties however changed over time. TEM images show slightly aggregated, cubical-shaped nanoparticles.

The pegylated, fluorescent PBNPs were injected into mice. Post injection, the particles showed enlarged signal intensity in the kidneys and urinary bladder. On the ex vivo images, taken 3 hours post injection, PBNP-MB@PEG6000 accumulation can be observed in the gastrointestinal tract, kidneys, spleen, liver and heart.

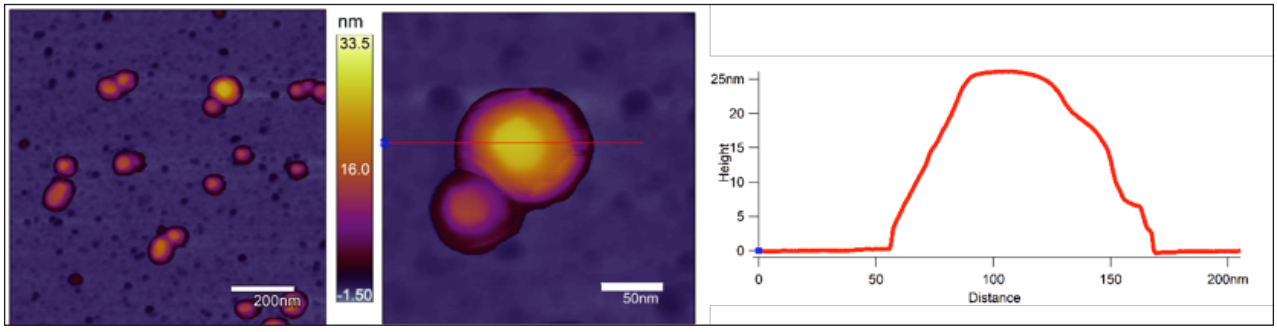

Figure 2 *The results of the AFM measurements of the PBNPs (Height Trace Map). Different magnifications (a-b) of the nanoparticles. Part (c) shows a particle with a height of 25 nm along with 60 nm diameter.*

4. Conclusions

We successfully pegylated the previously developed, citrate-coated Prussian Blue nanoparticles. Furthermore, our results suggest, that fluorescent labelling of the nanosystem is easy to perform. The quality control of pegylation and fluorescent labelling can be easily and nondestructively performed, using the FOBI instrument and different structure determination modalities, such as XRD and FTIR. AFM measurements showed cubical shaped nanoparticles with slightly rounded edges of an average height of 25 nm. DLS measurements revealed 24.45 ± **0**.75 nm along with a 0.3 PDI. XRD showed PB and PEG 6000 peaks, furthermore, unknown peaks were found at which are likely to be linked to MB. FTIR proves modified bonds, with PB, PEG and MB peaks, which suggest, that the molecules were successfully linked. Our results suggest, that PBNPs are versatile, multimodal platform for therapeutic and imaging uses.

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