PLGA-PEG multifunctional nanoparticles for simultaneous drug delivery and imaging by MRI and fluorescence microscopy

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ABSTRACT

This work deals with the synthesis of multifunctional nanoparticles based on biocompatible di-block copolymer (PLGA-PEG) via an emulsion-evaporation method. To enable their visualization, these nanoparticles can be loaded with iron oxide nanoparticles for Magnetic Resonance Imaging (MRI) and/or quantum dots for fluorescent microscopy. A therapeutic agent, Indomethacin, can also be loaded and released. The influence of synthesis parameters on nanoparticle size (in the range 70-150 nm) has been controlled to achieve specific cellular interactions avoiding possible immuno-response.

These multifunctional nanoparticles possess excellent photoemission properties for fluorescent microscopy and enhanced contrast efficiency for T_2 MRI imaging compared to available agents used today. In-vitro experiments confirm the low cytotoxicity of such nanoparticles and their excellent visualization properties by MRI and fluorescence microscopy in cells and biological tissues.

Keywords: PLGA-PEG, multifunctional nanoparticle, drug delivery, MRI, quantum dots

1 INTRODUCTION

In recent years, a lot of effort has been put to develop smart nanocarriers for biomedical applications, such as novel diagnostic tools [1], drug delivery [2], biomolecules separation [3] enhanced contrast imaging of different types [4-5]. Often polymeric coatings of inorganic nanoparticles are chosen as a preferred system to achieve targeted drug delivery due to their pharmacokinetic control[6], biodegradability and biocompatibility[7]. Still the main challenge is to integrate the therapeutic function, such as delivery of drug, with simultaneous diagnostic purposes, such as imaging of cells and tissue.[8]

This work deals with the synthesis of multifunctional nanoparticles based on biocompatible di-block copolymer poly(D,L-lactide-*co*-glycolide)-poly(ethyleneglycol) (PLGA -PEG) via an emulsion-evaporation method. [9] To enable tissue visualization for diagnostic purposes, these nanoparticles are loaded with superparamagnetic iron oxide nanoparticles (SPION) for Magnetic Resonance Imaging (MRI) [10] and/or Quantum Dots (QDs) in addition to the therapeutic agent Indomethacin (ICM). Since nanoparticles size is one of the crucial parameter to be controlled to achieve specific cellular interactions avoiding possible immuno-response, different synthesis parameters were varied to understand their influence on the size and distribution of such nanoparticles and drug release rate.

2 METHODS

2.1 Synthesis of multifunctional nanoparticles

First, PLGA-PEG copolymer was prepared from D,L-lactide/glycolide copolymer (PLGA by Purac) that was activated though dicyclohexylecarboiimide/N-hydroxy-succinimide mediated chemistry to activate the carboxylic acid groups to the semi stable amine reactive NHS-esters of PLGA. After removal of insoluble byproduct, the activated PLGA-NHS was reacted with diamino-PEG to obtain the desired PLGA-PEG-NH_2 copolymer. QDs with a CdSe core and a ZnS matrix were prepared according to the procedure described earlier[11]. SPIONs were obtained by a high temperature decomposition method.[2]

Multifunctional nanoparticles were synthesized through oil-in-water emulsification process, followed by organic solvent evaporation. In a typical experiment, 30 mg of prepared PLGA-PEG-NH_2 and 3 mg Indomethacin (IMC) were dissolved in 1 ml of dichloromethane. Then 50 µl of QDs suspension and 25 µl of SPION (2,4 mg/l) in chloroform, were added to the polymer-drug solution. 5 mL of polyvinyl alcohol (PVA) solution was added to the system as aqueous phase. The oil-in-water nanoemulsion was induced by a tip sonicator for 5 minutes, giving a total energy of ca 1000 J. Finally, 4 mL of distilled water were added to the emulsion, which was left to shake overnight to allow the evaporation of the organic solvent. PVA concentration and water to oil (W/O) ratio were changed in a series of experiments to study their influence on the nanoparticles size. Nanoparticles were washed twice by ultracentrifugation at 40000 rpm for 30 minutes, the supernatant was removed and water was added. Nanoparticles were freeze-dried before or after the washing procedure and stored for further use.

3 RESULTS & DISCUSSION

The comprehension of the influence of different synthesis parameters is essential for controlling and tailoring the size of nanoparticles in order to obtain specific biological interactions.

To confirm the diblock-copolymer structure prepared, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) have been performed. The TGA curve for the synthesized copolymer PLGA-PEG-NH_2 shows a

two-steps decomposition process, reflecting its di-block structure. The two components decompose at different temperature: the first step at 230 °C can be attributed to the decomposition of the PLGA chains while the second step at 325 °C is representing the decomposition of the PEG part of the prepared PLGA-PEG-NH_2 copolymer. From TGA data, the calculated ratio between PLGA and PEG segments is 76:20w%, in very good agreement with the feeding ratio between activated PLGA and diamino-PEG (75:25 w%). DSC analysis confirm the di-block structure of the obtained copolymer with a measured glass transition temperature T_g of 44 °C (lower than 53 °C measured for PLGA-NHS due to the plasticizer effect of the PEG chain).

SPION nanoparticles were synthesized according to our previous work [2] and the characterization results confirm the average diameter of 10 nm as measured from TEM images and the superparamagnetic behavior. Two kinds of QDs (green and red emitting) were also prepared and characterized according to reported procedures. [11] TEM analyses confirm the narrow size distribution and the core-shell structure while photoluminescence analyses prove the excellent emission properties of such nanocrystals at two distinct wavelengths (524 nm and 648 nm respectively).

As mentioned earlier, the control of the particle size is a critical issue for the further use of nanoparticles in medical applications. Different samples have been prepared by varying the PVA concentration and the ratio between the aqueous and organic phase (W/O). Dynamic light scattering measurements for different samples prepared by increasing the W/O ratio from 1,6 to 6 indicates that the mean hydrodynamic diameter of nanoparticles decreases from 157 ± 69 nm to 100 ± nm 39 nm. This is due to the fact that increasing the W/O ratio leads to higher amount of PVA in the system (total volume is kept constant), resulting in a reduction of the interfacial tension between the oil and the aqueous phases and therefore decreasing the emulsion droplet size, thus the nanoparticle size.

Another study has been performed to investigate the influence of the PVA concentration on the nanoparticle size. Samples prepared with PVA concentrations of 2, 4 and 6 % w/v present a mean hydrodynamic size of 102 ± 41 nm, 91 ± 34 nm and 71 ± 28 nm respectively. This is consistent with the fact that the higher the concentration, the more PVA can be oriented at organic solvent/water interface to reduce interfacial tension, promoting the formation of smaller emulsion droplets, thus nanoparticles. The amount of loaded QDs, SPION and IMC into multifunctional nanoparticles also influences their size. When W/O ratio of 4 and PVA concentration of 6% w/v are used, multifunctional nanoparticles containing QDs, SPION and IMC have a mean diameter of 107 ± 44 nm, larger than 71 ± 28 nm for particles without IMC, confirming the incorporation of drug inside.

In order to confirm the size analysis and morphology of nanoparticles and to verify the simultaneous loading of SPION and QDs inside the nanoparticles, TEM analysis has been performed.

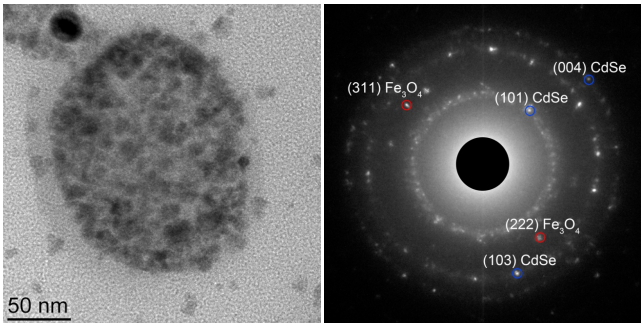

Figure 1: TEM image of multifunctional nanoparticles and selected area electron diffraction pattern.

The prepared multifunctional nanoparticles have a spherical morphology and their average size is in good agreement with the DLS measurements (ca 129 ± 60 nm by intensity distribution), considering the fact that particles are dried before TEM analysis. Furthermore, it is possible to observe different nanocrystals (dark contrast regions) inside the polymeric matrix of PLGA-PEG-NH_2 (not visible in TEM). These nanocrystals are SPION and QDs loaded during the synthesis process, as confirmed by Energy Dispersive x-ray analyses and electron diffraction pattern. The d-spacing of the different diffraction rings were indexed with the CdSe structure with strongest diffraction from (101) plane. The "isolated" diffraction spots can be indexed with the (222) and (311) planes of magnetite structure characteristic of SPION nanocrystals.

Photoluminescence studies confirm different emission peaks according to QDs size: green emission at 524 nm and red emission at 648 nm, proving the possibility to monitor the uptake of these nanoparticles by confocal microscopy.

The prepared multifunctional nanoparticles containing both QDs and SPION were also tested as T_2 contrast agent for Magnetic Resonance Imaging (MRI) in a 4.7 T scanner.

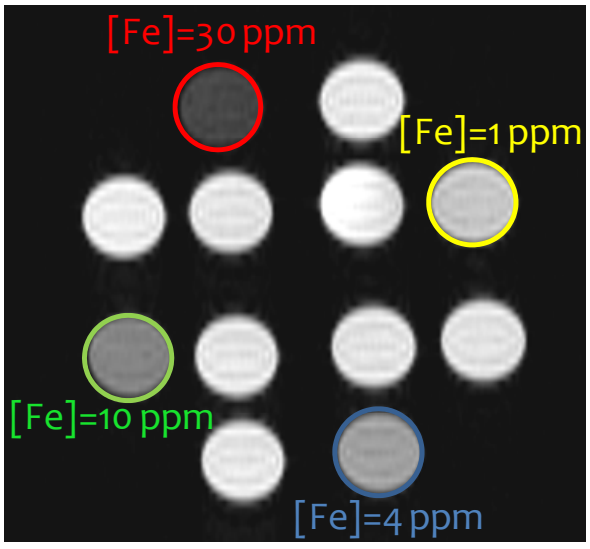

Figure 2: MRI T_2 image of phantom test tubes containing nanoparticles at different concentrations and water.

As it can be seen in Figure 2, test tubes containing the multifunctional nanoparticles are having a darker contrast compared to water (white) due to T_2 contrast enhancement. Different suspensions of multifunctional nanoparticles with different iron concentrations (from top, anti-clockwise: red circle: 30 ppm, green circle: 10 ppm, blue circle: 4 ppm and yellow circle: 1 ppm) were tested. The higher the concentration of nanoparticles, the more pronounced is the contrast effect, resulting in a darker image for that sample tube. Measurements of r_1 and r_2 MRI relaxivities have been performed and the calculated contrast efficiency for multifunctional nanoparticles is much higher than commercially available contrast agents (data not shown).

These results confirm the excellent T_2 contrast properties of multifunctional nanoparticles due to the presence of SPION.

Besides containing different agents for fluorescent visualization and MRI T_2 imaging, multifunctional nanoparticles loaded with IMC were tested to measure the drug release kinetic using a Franz cell at 37°C to mimic body conditions. In 9,5 h half of the drug loaded is released, while it takes 48h to release 98% of IMC. This indicates that hydrohpobic therapeutic compounds can be efficiently loaded into multifunctional nanoparticles during the synthesis process and slowly released.

Multifunctional nanoparticles has been tested for biocompatibility on different cell lines (PC12 and OC-k3) in view of their use in in-vivo experiments with the inner ear as target organ. PC12 cell line (model for neuronal differentiation) were incubated with multifunctional nanoparticles at different concentrations for 3, 6 and 24 h. Cell viability is larger than 90% for particle concentration in the range 0,15-1,5 μg/mL after 3 h exposure. At longer exposure time, the cell viability slightly decrease for high concentration of nanoparticles (0,75-1,5 μg/mL), but no significant morphological changes are noticed by light microscopy. FACS analysis confirm these results (data not shown).

In order to study the effect of multifunctional nanoparticles on the viability of OC-k3 cells (epithelial cells derived from the organ of Corti of transgenic mouse), the cells were incubated with different particle concentrations for 3 hrs and their viability was measured by MTS assay. Multifunctional nanoparticles induced decreases in MTS levels at 0,75 and 1,5 μg/mL and showed decrease viability down to 70% after 24h exposure.

The treatments with multifunctional nanoparticles at different concentrations did not induce considerable toxic effects and the mortality rate was not significantly altered. Even after 24 h exposure, multifunctional nanoparticles had no major negative impact on cell viability.

Uptake of multifunctional nanoparticles by OC-k3 cells was also studied by fluorescence microscopy. Cells were incubated for 24h with multifunctional nanoparticles (1,5 μg/mL) containing QDs emitting either in the red region or in the green one. Microscopy images with 40 times magnification show a clear update of multifunctional nanoparticles that can be clearly visualized due to the bright specific emission of the QDs incorporated.

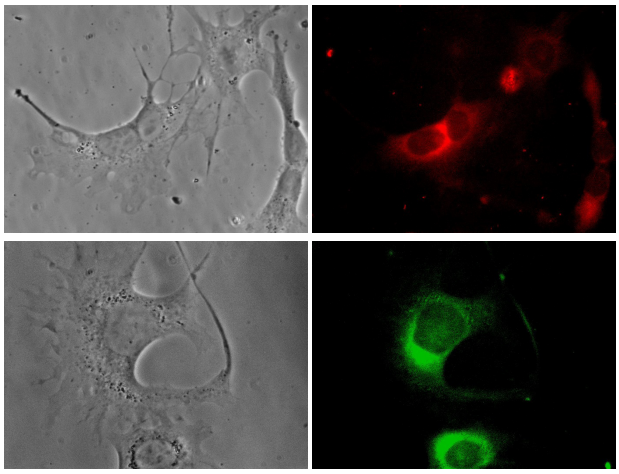

Figure 3: light and fluorescent microscopy images showing the uptake of multifunctional nanoparticles by OC-k3 cells after 24 h incubation.

Based on these studies, it is clear that multifunctional nanoparticles can be easily visualized by fluorescence microscopy to evaluate their uptake and localization in cells but also in biological tissues and eventually in ex-vivo samples.

A similar incubation procedure was used to prove the feasibility of using multifunctional nanoparticles for MRI contrast enhancement and for visualizing biological tissues. After 24 h incubation with different nanoparticle concentrations, cells were dissociated with trypsin, wash by PBS and fixed with 4% paraformaldehyde and mixed in 4% gelatin and filled in 500 ml eppendorf tube for MRI study. T_2 weighted images were recording with a 4.7 T MR scanner with bore diameter of 155 mm. A clear darker contrast is noticeable for cells incubated with higher concentrations, proving the uptake of multifunctional nanoparticles containing SPION by cells.

4 CONCLUSION

In this work we have shown the synthesis of multifunctional nanoparticles based on biocompatible di-block copolymer (PLGA-PEG) via an emulsion-evaporation method. Different synthesis parameters have been studied and adjusted to tailor the size of such multifunctional nanoparticles to a specific range (from 70 to 150 nm). To enable their visualization, these nanoparticles have been loaded with SPION for MRI imaging and quantum dots for fluorescent microscopy. A therapeutic agent, Indomethacin, can also be loaded and released. TEM analysis reveals the nanoparticles morphology and advanced electron diffraction analysis, together with energy dispersive x-ray spectroscopy, confirm the simultaneous loading of QDs and SPION inside the copolymer matrix.

These multifunctional nanoparticles possess excellent contrast properties for MRI T_2 contrast imaging as well as bright fluorescence and specific narrow emission when excited by UV-light.

In-vitro experiments with PC12 and OC-k3 cell lines confirm the high biocompatibility of such nanoparticles, even at high concentrations. Thanks to the excellent visualization properties, the update of multifunctional nanoparticles by OC-k3 cells has been studied by light and fluorescence microscopy as well as by MRI imaging.

Thus, this work demonstrates the possibility to design and synthesize multifunctional nanoparticles based on PLGA-PEG with a specific size and high biocompatibility. The detailed characterization shows how these nanostructures have high potential for drug delivery, fluorescent visualization and MRI contrast enhancement in biological tissues. Future in-vitro and in-vivo experiments will focus on the uptake mechanisms and biodistribution of these multifunctional nanoparticles in the inner ear to develop an efficient and multifunctional drug delivery system traceable via fluorescence microscopy and also capable of contrast enhancement for MRI tissues diagnosis.

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