CdTe QUANTUM DOTS APPLIED INTO THE CHICKEN TISSUE

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Abstract

CdTe Quantum dots (QDs) are nanoparticles (2 – 10 nm) made of semiconductor materials. QDs have got very good fluorescence properties, they have got high quantum yields, big Stokes shifts, high molar extinction coefficient and they are resistant to photobleaching and chemical degradation. These characteristics predispose them for use in fluorescent labelling. They can be excited by a broad spectrum of wavelengths and on the contrary, their emission spectra are narrow and size-tuneable (from the UV to the NIR regions). Using different emission filters (535 nm, 600 nm, 700 nm), it is possible to distinguish between different QDs. QDs of different colour were prepared by microwave synthesis (50 – 130°C; 20 minutes). QDs are potentially beneficial in the imaging of the biological processes in the living organisms either in the microscopic or macroscopic level.

The aim of this study was the application of QDs into the chicken muscle tissue and study of their behaviour in various depths under the surface (2, 5, and 7 mm). We investigated the differences between CdTe QDs with different emission maxima (550, 615 and 705 nm) and the limits of the usage as fluorescence probes for fluorescence in vivo imaging.

Keywords: Quantum dots, chicken muscle tissue

1. INTRODUCTION

Quantum dots (QDs) are inorganic semiconductor nanocrystals size of 2 – 10 nm, each dot consists of 100 up to 100 000 atoms. Quantum dots have excellent fluorescence properties; they have high quantum yields, broad absorption spectrum, narrow emission size tuneable spectrum, large Stokes shift, high molar extinction coefficient and are resistant to quenching and chemical degradation. The most common quantum dots are telluride, sulphide, selenide, arsenide with ions of cadmium, lead, zinc, indium, gallium and mercury. Properties of quantum dots depend on the way of the synthesis and surface modification. The conditions of microwave synthesis (temperature and reaction time) influence the size of quantum dots. At low temperatures and short reaction time, the smallest QDs are synthetized, which have a blue to green colour. On the contrary, at higher temperatures and longer heating times, the bigger particles are produced (orange to red colour) [2, 3, 5, 9, 13, 15, 16].

Quantum dots can be applied in bioanalytical chemistry and biology DNA probes, at the fluorescent labelling of microscopic preparations, the specific labelling of body tissues, antibodies, oligonucleotides, enzymes, aptamers, in FRET ( Förster resonance energy transfer), BRET (bioluminescence resonance energy transfer) and ECL (electrochemiluminiscence). The possibility of conjugation of quantum dots with biomolecules opens the way for their use in the biological labelling. Quantum dots can be used as markers in immunoassays. They can be used for the detection of small molecules, in medicine and in food safety control, in pharmaceutical and environmental monitoring. Quantum dots are a good alternative to the traditional organic dyes, enzyme labels, or isotopic labels [2, 6, 9, 11, 13]. Due to their properties, QDs can be used not only in vitro but also for in vivo imaging. Investigation of quantum dots is among the most
emerging field of nanotechnology [4]. In the future, we can expect the use of quantum dots in human medicine, since it is possible to visualize the use of transport of drugs.

In the area of basic research is the detection of therapeutics benefit especially in the development of targeted therapies and targeted drug delivery into the tumour tissues. Due to its small size, it is easy to get to the target organs. The biocompatibility of QDs is important for the usage of QDs in imaging and therapy [8]. A suitable modification of the surface of QDs reduces their toxicity and allows specific binding to a specific site in the body [1, 10]. A disadvantage of the in vivo imaging is the high autofluorescence of tissue at low wavelengths, it is preferable to study living organisms using near infrared radiation (NIR). Near infrared radiation better penetrate throw the tissue and can reaches a depth of several centimetres [7, 14].

2. MATERIALS AND METHODS

2.1 Preparation of QDs

All chemicals were purchased from Sigma-Aldrich and used without further purification. Cadmium (II) acetate Cd(OAc)\(_2\) (10 ml; 5.32 g/ l) was dissolved in ACS water (25 ml). Mercaptosuccinic acid (MSA) (1 ml; 60 mg/ml) was slowly added to stirred solution. Afterwards, 1.8 ml NH\(_3\) (1 M) and 1.5 ml Na\(_2\)TeO\(_3\) (4.432 g/l) was added. NaBH\(_4\) (40 g) was poured into the solution under vigorous stirring. Subsequently the ACS water was added to the final volume of 100 ml, than the solution was pipetted (2 ml) into the vials, which were closed and put into the Microwave Reaction System (Multiwave 3000, Anton Paar, Graz, Austria). Microwave heating conditions: max. 300 W, temperature: 50 – 130 °C (QD1 – 50 °C, QD2 – 50 °C, QD3 – 60 °C, QD4 – 80 °C, QD5 – 90 °C, QD6 – 90 °C, QD7 – 100 °C, QD8 – 120 °C, QD9 – 130 °C, QD10 – 130 °C) 10 minutes rising of temperature, 10 minutes continuance and then cooling. Synthetized QDs were stored in dark at 4 °C. More details on QDs preparation in the conference proceeding paper by Melichar et al.

2.2 Fluorimetric analyses

Fluorescence spectrometer Tecan infinite M200 PRO (Grödig, Austria) was used for the fluorimetric analyses. Samples of volume of 100 µl were placed in a Nunc microplate MaxiSorp (Thermo Fisher Scientific, Roskilde Denmark). The absorbance spectrum was measured (300 nm – 1000 nm). The highest absorbance was set as excitation and the fluorescence spectrum of QDs was measured (430 - 850 nm). The parameters were as follows: number of flashes: 5; emission wavelength step size: 5 nm; gain: 50.

2.3 Highly sensitive CCD detector

The fluorescence properties were tested by Carestream In-Vivo Xtreme Imaging System (Carestream Health, Inc., Rochester, USA). The 4MP Camera is a cooled back-thinned, back illuminated camera designed for maximum sensitivity. The camera utilizes a two-stage thermo-electric cooler that cools down the CCD below -55 °C absolute. The camera collects the image data on a 2048 x 2048 pixel CCD. Single frame image data is digitized at 16-bits, and presented in software as a 32-bit floating point image. The images were processed by Carestream molecular imaging software (Carestream).

2.4 Application of doxorubicin into the muscle tissue

Quantum dots were applied directly into the chicken muscle tissue or into the tube (internal diameter of 2 mm) and it was inserted into the different depths of the tissue (0, 2, 5, 7 mm) and the fluorescence was detected. Finally three different colour QDs were applied into the chicken embryo. Fertilized eggs (ISA Brown) were incubated for 14 days in RCom 50 MAX incubator (Gyeongnam, Korea) with temperature (37.5°C) and humidity (45% rH) control and automatic egg rolling (every 2 hours). After the incubation the eggshell was removed and the QDs were applied into the embryo. The fluorescence of QDs was detected by Carestream In-Vivo Xtreme Imaging System (Carestream Health, Inc., Rochester, USA) using specific filters for a given QDs. The images were analysed by Carestream molecular imaging software (Carestream Health, Inc., Rochester, USA) and processed by software PhotoFiltre Studio X.
3. RESULTS AND DISCUSSION

In this study, different CdTe capped by mercaptosuccinyl acid QDs were synthesised by microwave synthesis. By changing the temperature of the reaction, the different colour QDs were synthesised. In the low temperatures (50 °C), the QDs emitted low wavelength light (blue light), in comparison high temperatures (120, 130 °C) caused the synthesis of red colour QDs. The colour of QDs was observed in the transilluminator (Transilluminator Multiband TFX-35.MC, Torcy, France, excitation: 312 nm) and whole colour range of QDs was observed. The absorbance (Fig. 1A) and the emission spectra (Fig. 1B) were determined by fluorescence spectroscopy. All of the QDs absorbed the light of low wavelengths, so the QDs were excited by the light of 400 nm. The emission spectra of the QDs were covering the whole visible light spectrum (480 nm – 700 nm). The intensity of the fluorescence grew with the wavelength of the emitted light. Red QDs emitted light in the near infrared region (705 nm) and their intensity of the fluorescence was very high (26 300 a.u.). Near infrared QDs seems to be convenient fluorescence labels. Our QDs exhibited good fluorescence properties and had the potential to be used in the bioimaging. The usage of QDs in the living organisms is limited by the thickness of the tissue the light need to penetrate [12]. The determination of the limiting depth for the detection of QDs fluorescence in the tissue was one of the aims of this study. Therefore the QDs were analysed in different tissue depths (0, 2, 5 and 7 mm). The fluorescence of QDs was studied on the chicken muscle tissue. The QDs were applied by injection into the animal muscle tissue. But the direct application caused the uneven distribution in the tissue and the exact depth of the QDs in the tissue could not be determined. It is the reason, why we used tubes to prevent spreading of the dots in the tissue. QDs were applied into the tube (internal diameter of 2 mm) and it was inserted into the different depths of the tissue (Fig. 1C). The fluorescence of QDs was analysed by the Carestream In-vivo Xtreme Imaging System, which allows highly sensitive display of fluorescence. It allows not only excitation in the visible region, but also in the longer wavelengths (NIR) and the monitoring of fluorophores in a living organism. The device has high-quality camera software, which allows quantification of the intensity of radiation and separation of individual fluorophores. The images were analysed by Carestream molecular imaging software. The intensity of the fluorescence of different QDs (green, yellow and red) in tube in different tissue depths was analysed. The intensity of the fluorescence decreased with increasing depth of the tube in the tissue. It was found, that the red QDs has best fluorescence properties and it is possible to detect them in the depth of 10 mm. Green and yellow QDs can be detect only up to depth of 7 mm below the surface. Finally, all three types of QDs were applied into the chicken embryo body. The fertilized eggs were incubated for 14 days in the incubator RCom 50 MAX (37.5 °C; 45 % rH). After the incubation the embryo was removed and the three colour QDs (green, yellow and red) were applied (200 µl) by the injection into the muscle tissue, into the depth of 2 mm. The fluorescence of QDs was detected by Carestream In-Vivo Xtreme Imaging System (Carestream Health, Inc., Rochester, USA). Using different emission filters a multicolour analysis was enabled. All QDs were excited by the light of 480 nm and detected throw different emission filters. Green QDs were taken with the emission filter 535 nm, yellow QDs with the emission filter 600 nm and red QDs with the emission filter 700 nm. Finally the X-ray image was taken, than all four images were overlaid and combined together to one image (PhotoFiltre Studio X software, Version 10.4.0, France). The multicolour analysis enables the use of these QDs in the fluorescence labelling of different structures.
**Fig. 1** Fluorescence of quantum dots: **A**) Absorbance spectrum of synthesised quantum dots (350 – 800 nm). **B**) Fluorescence spectrum of synthesised quantum dots (440 – 840 nm); number of flashes: 5; emission wavelength step size: 5 nm; gain: 50. **C**) Fluorescence of the QDs in the tube in the different depth in the chicken muscle tissue, excitation: 650 nm, emission 700 nm, exposure time: 2 s, Field of View: 12, fStop: 1.1, Binning: 2 x 2 pixels (a) tube with red QDs in the depth of 2 mm, (b) tube with red QDs in the depth of 5 mm, (c) tube with red QDs in the depth of 2 mm, (d) The dependence of the fluorescence intensity on the depth of tube with QDs in the tissue (green, yellow and red QDs). **D**) Fluorescence of QDs applied into the chicken embryo (2 mm deep, 200 µL applied by direct injection) overlay of four images: X-ray, fluorescence of green, yellow and red QDs, excitation: 480 nm, emission: 535 nm (green QDs), 600 nm (yellow QDs), 700 nm (red QDs).

**4. CONCLUSION**

The quantum dots are great fluorescence labels, which can be used in fluorescence staining. Compared to organic dyes, QDs are stable and different colour QDs can be easy synthesised. Using the different emission filters, it is possible to distinguish between the different QDs in the tissue. It enables the use of QDs in simultaneously labelling of different structures in the body. Red QDs was possible to detect up to 10 mm deep, the reason is the high fluorescence intensity and good penetration of infrared radiation.

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LITERATURE


