Materials Science and Engineering C 67 (2016) 231–236

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Uptake of silica covered Quantum Dots into living cells: Long term vitality and morphology study on hyaluronic acid biomaterials

Michele D'Amico [a,b,1], Calogero Fiorica [c,*], Fabio Salvatore Palumbo [c], Valeria Militello [b], Maurizio Leone [b], Benoit Dubertret [d], Giovanna Pitarresi [c], Gaetano Giammona [c]

[a] Dip. Biomedico di Medicina Interna e Specialistica, Universitá degli Studi di Palermo, Piazza delle Cliniche, 2, 90127 Palermo, Italy
[b] Dip. di Fisica e Chimica, Universitá degli Studi di Palermo, Viale delle Scienze, Ed. 18, 90128 Palermo, Italy
[c] Dip. di Scienze e Tecnologie Molecolari e Biomolecolari, Sezione di Chimica e Tecnologie Farmaceutiche, Universitá degli Studi di Palermo, Via Archirafi, 28, 90136 Palermo, Italy
[d] Laboratoire de Physique et d'Etude des Matèriaux, ESPCI-ParisTech, PSL Research University, Sorbonne Universitè UPMC Univ. Paris 06, CNRS, 10 rue Vauquelin, 75005 Paris, France

ARTICLE INFO

Article history:
Received 25 November 2015
Received in revised form 29 March 2016
Accepted 24 April 2016
Available online 05 May 2016

Keywords:
Fluorescence confocal microscopy
Silica Quantum Dots
Chondrocyte bovine cells
Hyaluronic acid
Labelling
Long term cell staining

ABSTRACT

Quantum Dots (QDs) are promising very bright and stable fluorescent probes for optical studies in the biological field but water solubility and possible metal bio-contamination need to be addressed. In this work, a simple silica-QD hybrid system is prepared and the uptake in bovine chondrocytes living cells without any functionalization of the external protective silica shield is demonstrated. Moreover, long term treated cells vitality (up to 14 days) and the transfer of silica-QDs to the next cell generations are here reported. Confocal fluorescence microscopy was also used to determine the morphology of the so labelled cells and the relative silica-QDs distribution. Finally, we employ silica-QD stained chondrocytes to characterize, as proof of concept, hydrogels obtained from an amphiphilic derivative of hyaluronic acid (HA-EDA-C_{18}) functionalized with different amounts of the RGD peptide.

1. Introduction

Quantum Dots are nanometric (2–30 nm) crystalline semiconductors particles which show unmatched optical features with bright photon emission [1,2]. After the optical absorption of a photon in the UV–visible range the so acquired exciton energy is mainly relaxed by emitting a fluorescence photon typical in the visible region of the electromagnetic spectrum. The key of the enormous growth of research studies on QDs in the last 30 years relies on the quantum confinement effect, which is due to comparable nanometric scale of both the Bohr exciton radius and the geometrical confining dimension of the system [1]. This effect explains the precise control which could be done on the discrete exciton energy levels and thus on the wavelength of main absorption and fluorescence peaks, ranging between ultraviolet and infrared regions as a function of dimension and relative quantum confinement of excitons [1,2]. The comprehension of the underlining physical mechanism and the ability in precise chemical synthesis is incredibly increased and now it is routine. In the last years, indeed, the direct colloidal synthesis of QDs in solution guarantees the formation of highly mono-disperse nanoparticles with well controlled optical features [2–4]. Moreover, over the dot nanocrystals, new shapes have been synthesized (rod, dot in rod, tetrapod, nanoplatelet) [1,2,5–12]. Beside the great control over the optical features and geometry, these systems show a higher brightness, larger absorption cross-section, broader absorption spectrum and higher resistance to photo-damage under continuous illumination, in respect to the more used conventional organic dyes [8,10,13]. These aspects are crucial to utilize these nanoparticles as a probe in biophysical systems [11] but some problems should be overcame: indeed, most of QDs are prepared using some toxic elements (like cadmium) which could in principle be delivered to the sample [13,14]. Moreover, the fluorescence of QDs is really sensible to the external environment. Indeed, the possible presence of electronic traps on QDs crystalline surface could act as fluorescence quencher and/or to give rise to a strong instability of the emission (blinking) [10]. One way to overcome these drawbacks is to create a protective shield around the dot in order to avoid both external fluorescence quenchers and to block any contact of toxic atoms with the external biological environment [12,13,15–17].

In this work we prepared colloidal CdSe-CdS core-shell nanocrystals emitting in the red (@625 nm) and, by a micro-emulsion procedure of water in oil inverse micelles, we encapsulated each dot in a silica shell [12,16]. In this way the fluorescent nanoparticles became biocompatible,

* Corresponding author.
E-mail address: calogero.fiorica@unipa.it (C. Fiorica).
[1] Present address: Laboratoire de Physique et d'Etude des Matèriaux, ESPCI-ParisTech, PSL Research University, Sorbonne Universitè UPMC Univ. Paris 06, CNRS, 10 rue Vauquelin 75005 Paris, France.

http://dx.doi.org/10.1016/j.msec.2016.04.082
0928-4931/

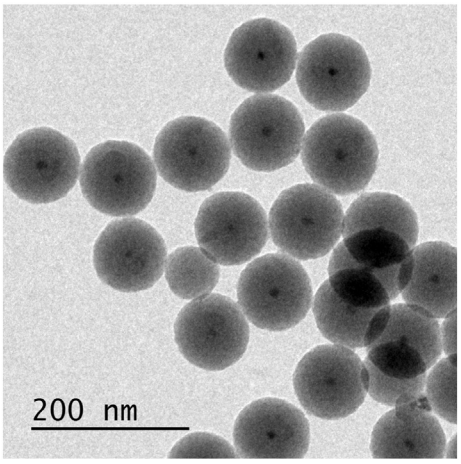

Fig. 1. Transmission Electron Microscopy image of the silica-QD nanoparticles.

are easy to prepare and could be also used in a water based environment (indeed the original nanocrystals are hydrophobic because of the presence of specific surface ligands which are needed for colloidal stability during the synthesis). Moreover, the silica shield is transparent to the excitation and emission wavelengths of interest. In some cases, it is observed a dot-fluorescence quenching by water after the silica growth [12,13,16,18]. However, in this work the silica layer was produced through a low temperature process that causes the increase in the water resistance eliminating almost completely the quenching phenomenon. The details of this procedure are out of the aim of this paper and will be discussed in another specific work. Here we want to demonstrate that it is possible to employ the silica-QDs obtained with this synthesis as a stable and non-toxic fluorescent probe for cell staining with the aim to use these fluorescent cells as a mean of characterization of different biomaterials without problems of probe quenching and toxicity. We thus measured the uptake time of such silica-QDs in bovine articular chondrocytes, employed as model cells and we tested long term cytocompatibility as well. Moreover, such silica-QDs stained chondrocytes have been deposited and observed on a hydrogel made by a derivative of hyaluronic acid (HA-EDA-C_{18}). HA is considered an excellent component of biomaterials [19] both in tissue engineering [20–22] and drug delivery fields [23,24]. However, HA is often mixed with other extra cellular matrix polymers because of its poor cell adhesivity. RGD is a recognition sequence for integrins that strongly increase the tendency of the cells to adhere onto various surfaces [25] and thus HA-EDA-C_{18} hydrogel have been functionalized with different RGD peptide amounts in order to evaluate the influence of this peptide on the adhesivity of live cells stained with the silica-QD nanoparticles.

2. Experimental section

2.1. Materials

Low molecular weight HA (Mw 240 kDa, polydispersity index 1.9) and the tetrabutylammonium salt of hyaluronic acid (HA-TBA) were produced as reported elsewhere [21]. Octadecylamine (C_{18}-NH_2), tetrabuthylammonium hydroxide (TBA-OH), bis(4-nitrophenyl) carbonate (4-NPBC), Triton X-100, 1-Hexanol, anhydrous dimethylsulfoxide (DMSOa), Ethylenediamine (EDA), tetrahydrofuran (THF), 4-Maleimidobutyric acid (MLB), *N*-Hydroxysuccinimide (NHS) and Tetraethyl Orthosilicate (TEOS) were purchased by Sigma-Aldrich. Dulbecco Modified Eagle Medium (DMEM) was purchased from EuroClone. N,N–Dicyclohexylcarbodiimide (DCC) was purchased from Alfa Aesar. Cyclo(-Arg-Gly-Asp-D-Phe-Cys) (CyRGD-SH) was purchased from Bachem.

2.2. Silica Quantum Dots synthesis

The preparation of 12 nm diameter core-shell CdSe-CdS Quantum Dots (of 1.5 nm core radius) was done accordingly to the high temperature protocol developed as in Ji et al. [12]. The formation of a silica shell around the QDs was performed by an inverse water-i*n*-oil microemulsion micelles method [12,16]. In particular, 1.9 g of the surfactant Triton X-100 and 1.5 g of 1-Hexanol as co-surfactant were mixed and dissolved in 15 ml of cyclohexane. Then, ~0.15 nmol of QDs dispersed in hexane were injected (typical volume of 60 μl) and after 10 min of stirring with a magnetic bar, 380 μl of water and 60 μl of ammonia (29% in water) were added. The solution should remain clear to ensure that nanometric water-in-oil micelles system was correctly formed. After thermalization at 4 °C in a cold room, 60 μl of TEOS were added to start the reaction of silica formation around the dots. After 6 h, another amount of 320 μl of TEOS was added to have final 100 nm diameter silica nanoparticles after 140 h of total time of growth (~40 nm silica thickness). As it is possible to observe in the TEM image of Fig. 1, the silica-QD nanoparticles show a high monodispersity (diameter 100 ± 4 nm), only one dot per silica particle and a few of empty silica beads. In comparison with previous SiO_2-QD nanoparticles prepared at room temperature [12], the formation of the silica layer at 4 °C increases their fluorescence resistance to water washing. This is probably due to a better formation and passivation of the QDs surface by hydrolyzed TEOS molecules thanks to a slower formation rate.

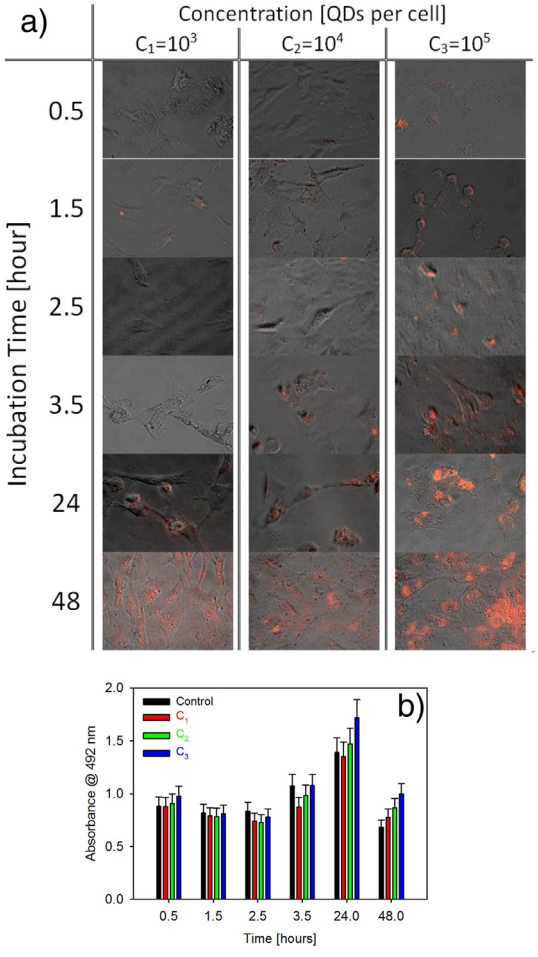

Fig. 2. Panel-a) Fluorescence microscope images (in red) of bovine chondrocytes at different times during the incubation in a SiO_2-QDs loaded cell medium at three different concentrations of fluorescent nanoparticles. The transmitted light gray-scale images are also superimposed. Panel-b) Relative MTS vitality test in comparison with a control cell sample (black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)