Journal of Colloid and Interface Science 516 (2018) 484–497

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Regular Article

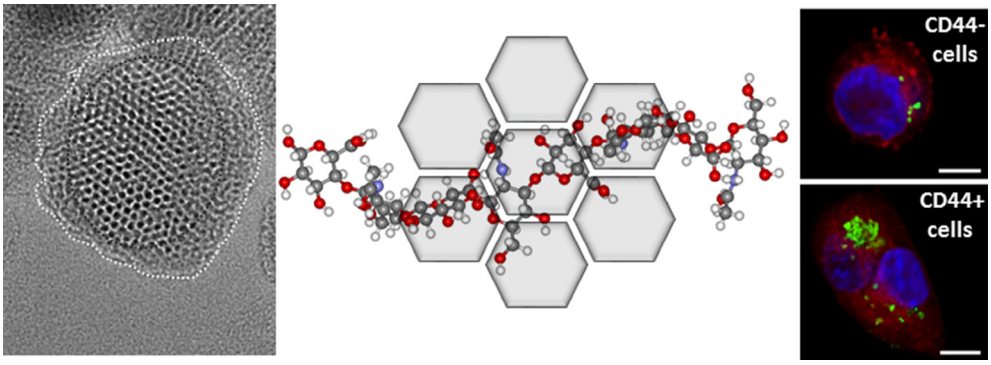
Hyaluronated mesoporous silica nanoparticles for active targeting: influence of conjugation method and hyaluronic acid molecular weight on the nanovector properties

Valentina Ricci [a], Daniele Zonari [b], Stefania Cannito [c], Alessandro Marengo [b], Maria Teresa Scupoli [d], Manuela Malatesta [e], Flavia Carton [e], Federico Boschi [f], Gloria Berlier [a,*], Silvia Arpicco [b,*]

[a] Department of Chemistry and NIS Centre, University of Torino, Via P. Giuria 7, Torino, Italy
[b] Department of Drug Science and Technology, University of Torino, Via P. Giuria 9, Torino, Italy
[c] Department of Clinical and Biological Sciences, University of Torino, Corso Raffaello 30, Torino, Italy
[d] Research Center LURM (University Laboratory of Medical Research), University of Verona, Piazzale L.A. Scuro 10, Verona, Italy
[e] Department of Neurosciences, Biomedicine and Movement Sciences, Anatomy and Histology Section, University of Verona, Strada le Grazie 8, Verona, Italy
[f] Department of Computer Science, University of Verona, Strada le Grazie 15, Verona, Italy

GRAPHICAL ABSTRACT

Hyaluronated mesoporous silica nanoparticles (MSN/HA) are preferentially internalized in CD44+ tumor cells.



ARTICLE INFO

Article history:
Received 25 October 2017
Revised 19 January 2018
Accepted 19 January 2018

Keywords:
Hyaluronic acid
Mesoporous silica nanoparticles
CD44 receptor
Active targeting

ABSTRACT

We have prepared and evaluated the physico-chemical and biological properties of four different hyaluronated mesoporous silica nanoparticles (MSNs) samples (MSN/HA). Hyaluronic acid (HA) with two different molecular weights (200 and 6.4 kDa) was used for the conjugation of aminopropyl-functionalized MSN (NH_2-MSN), following two different procedures. Namely, samples HA200A and HA6.4A were prepared by reacting activated HA with NH_2-MSN (method A), while samples HA200B and HA6.4B were obtained carrying out HA activation in the presence of the nanoparticles (method B). The four samples showed similar hydrophilicity, but clear differences in the HA loading, textural properties, surface charge and stability of the suspensions. More in detail, conjugation using low molecular weight HA with method A resulted in low HA loading, with consequent scarce effects on dispersity and stability in physiological media. The highest yield and corresponding best performances were obtained with method B using high

* Corresponding authors at: Department of Drug Science and Technology, University of Torino, Via P. Giuria 9, 10125 Torino, Italy (S. Arpicco); Department of Chemistry and NIS Centre, University of Torino, Via P. Giuria 7, 10125 Torino, Italy (G. Berlier).
E-mail addresses: gloria.berlier@unito.it (G. Berlier), silvia.arpicco@unito.it (S. Arpicco).

https://doi.org/10.1016/j.jcis.2018.01.072

Hydrophilicity
Infrared
Water adsorption
MSN

molecular weight HA. HA loading and molecular weight also influenced in a concerted way the biological response towards the MSNs of CD44 target cancer cells ($CD44^+$) and control cells ($CD44^-$): MDA-MB-231 and A2780, respectively. The absence of cytotoxicity was assessed. Moreover, the targeting ability of the best performing MSN/HA was confirmed by cellular uptake studies.

1. Introduction

Nanoparticles-based targeted therapy has emerged in recent years as an innovative strategy to maintain a drug therapeutic dose at the target site, while reducing systemic drug toxicity and adverse side effects to healthy tissues [1–3]. This approach is particularly important in relation to cancer therapy, where the differences in biochemistry between cancerous and normal tissues can be exploited for the selective targeting of over-expressed tumor specific receptors [4–7]. To this aim, nanomaterials are an ideal playground, thanks to their intrinsic properties such as high surface area, tuneable size and shape coupled to ease of synthesis and functionalization. Nanoparticles may be loaded with a plethora of bioactive molecules (e.g., small molecules, peptides, nucleic acids, etc.) to protect them from cleavage by external agents, thus the encapsulated drugs do not participate in the control over pharmacokinetic and biodistribution. Moreover, nanosize permits passive transport in biological fluids and to establish molecular interactions at the cellular and subcellular level [1].

Liposomes [8–10] and biodegradable polymeric nanoparticles [11–13] are among the most versatile biocompatible systems to encapsulate active ingredients. Mesoporous silica nanoparticles (MSNs) can be considered as their inorganic counterparts, with intrinsic features such as a huge available inner volume, inertness and chemical stability [14–17]. The ease of surface functionalization makes them ideal materials to develop "pharmaceutically adapted platforms" [18,19], with great potentiality in relation to *stimuli responsive* applications [20–26]. One of the major drawbacks for the systemic application of these nanosystems is their poor dispersity in biological fluids, which can be however improved by appropriate surface functionalization [19]. Functionalization (often carried out to optimize the interaction with the guest drug molecules) can be obtained with a variety of organosilanes [27]. This has important consequences on the surface properties, such as charge and hydrophilicity [28], which can profoundly influence cytotoxicity [29], cellular uptake, transport and/or fate in biological fluids of the nanoparticles [30,31].

Surface modification of nanocarriers through macromolecules is particularly relevant in the field of cancer treatment [32], where the conjugation of cytotoxic drugs with macromolecules is designed to improve their pharmacokinetic profile, prolonging the distribution and elimination phases [33]. The most employed are N-(2-hydroxypropyl) methacrylamide (HPMA), polyglutamate, human serum albumin, dextrans, heparin, chitosan, dendrimers, multi-arm polyethylene glycol (PEG), and hyaluronic acid (HA) [32,33]. HA is a naturally-occurring glycosaminoglycan and a major component of the extracellular matrix. The HA receptor CD44 is overexpressed in many cancer cells, and in particular in tumor-initiating cells. HA has thus attracted considerable interest for the development of nanoplatforms for actively targeting drugs, genes, and diagnostic agents [34,35].

In recent years, HA-conjugated MSN systems have been proposed in the literature, with the double aim to improve dispersity and obtain a targeted delivery to CD44 overexpressing cancer cells [36–38]. Zhang et al. recently proposed biotin-modified HA coupled to MSN to enable controlled drug release at cancer cells expressing CD44 HA receptor [39]. Moreover, Chen et al. used HA as both capping and targeting agent. In their work, the entrapped guest molecules were released from the inner pores of MSNs upon HA degradation in response to hyaluronidase-1, after receptor-mediated endocytosis into targeted cancer cells [40]. The potentiality of HA-conjugated MSN systems was further investigated by developing *dual-stimuli responsive* systems. To this aim, the polysaccharide was conjugated to MSNs through a disulfide bond, which was cleaved in the presence of the high glutathione concentration characterizing cancer cells [23,24]. Additionally, multifunctional "theranostic" materials where developed, coupling the above-mentioned properties to those of a gadolinium based bovine serum albumin complex for simultaneous redox-responsive targeted drug delivery and magnetic resonance imaging [41].

In this work, hyaluronated MSNs (MSN/HA) have been developed with the double aim to improve their dispersion in physiological media and their targeting ability. For the first time we compare different synthetic approaches, and the use of HA of different molecular weights (6.4 kDa and 200 kDa), to identify the optimal strategy to obtain the better performances, both in terms of biological response and potential pharmaceutical application. A full physico-chemical description of the produced hybrid materials is given, including routine characterization, hydrophilicity assessment, molecular and quantitative analysis of the HA external shell. Finally, the biological effects in cultured cells were studied and the results correlated to the materials properties.

2. Experimental section

2.1. Materials

Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), sodium hydroxide (NaOH), (3-aminopropyl)-triethoxysilane (APTS) and all the other reagents and solvents were purchased from Sigma-Aldrich (Milan, Italy) and employed as received. Sodium hyaluronate (HA, of molecular weights MW 6.4 and 200 kDa) was purchased from Lifecore Biomedical (Chaska, MN). Fluorescein-5-isothiocyanate (FITC) was provided by Invitrogen (Life Technologies, Monza, Italy). MilliQ® water was used in all synthetic steps.

2.2. NH_2-MSN synthesis

MSN samples were prepared following a slightly modified literature procedure [42,43]. CTAB (1 g, 2.74 mmol) employed as Structure Directing Agent (SDA), was dissolved in 480 ml of water under stirring and heating. At the stable temperature of 80 °C, NaOH (2.0 M, 3.5 ml) was slowly added to the mixture. TEOS (5 ml, 22.4 mmol) was then added dropwise under vigorous stirring. After 2 h the milky reaction mixture was cooled to room temperature (RT) and the white precipitate was filtered off and washed with abundant water and methanol. The SDA was removed from the as-synthesized material by calcination at 550 °C, heating to the desired temperature under N_2 flow and switching to O_2 for a 6 h isotherm.

Aminopropyl-functionalized MSN (NH_2-MSN) sample was prepared with APTS by post-synthesis grafting with a procedure