Materials Letters 183 (2016) 105–108

Contents lists available at ScienceDirect

Materials Letters

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

Combined far-field, near-field and topographic imaging of nano-engineered polyelectrolyte capsules

Stefan G. Stanciu [a,*], Denis E. Tranca [a], Carmelina Ruggiero [b], George A. Stanciu [a], Elena Dellacasa [b], Alexei Antipov [c], Radu Hristu [a], Laura Pastorino [b]

[a] Center for Microscopy-Microanalysis and Information Processing, University Politehnica of Bucharest, Romania
[b] Department of Informatics, Bioengineering, Robotics and Systems Engineering, University of Genova, Italy
[c] PlasmaChem GmbH, Berlin, Germany

ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form
4 July 2016
Accepted 6 July 2016
Available online 7 July 2016

Keywords:
Nano-engineered polyelectrolyte capsules
Layer-by-layer self-assembly
CLSM
s-SNOM
AFM

ABSTRACT

Nano-engineered polyelectrolyte capsules (NPCs) were investigated using a multimodal approach based on Confocal Laser Scanning Microscopy, scattering-type Scanning Near-Field Optical Microscopy and Atomic Force Microscopy. Our experiment demonstrates that correlating optical information collected with far-field and near-field techniques and topographic data collected with scanning probe variants holds significant potential for gaining new insights on the structure of NPCs assembled with layer-by-layer strategies.

1. Introduction

The design and fabrication of micro- and nano-sized systems capable to carry bioactive substances at targeted sites within the human body and to release these at controlled time intervals, or under specific external or internal stimuli, are currently regarded as high-priority topics. With design and testing stages being highly demanding in terms of time and financial resources, a strong focus of attention has been placed lately on developing such delivery systems that can be easily assigned different functionalities, depending on the application at hand. Among various solutions that have been proposed over the past decades in the aforementioned context, the Nano-engineered polyelectrolyte capsules (NPCs) have gained a strong focus of attention, [1–3]. The fabrication of NPCs is based on the layer-by-layer (LbL) adsorption of oppositely charged macromolecules, mainly polyelectrolytes, on colloidal particles [4], which confers significant flexibility to this architecture. For example, the layers that constitute the NPC, as well as the colloidal particle at the core, can be based on different types of constituents, thus allowing a wide range of applications. Moreover, the shell thickness of NPCs can be easily controlled as this property is determined by the number of layers that are consecutively deposited around the inner core. Furthermore, the inner core of an NPC can be dissolved after the shell formation, resulting in a hollow capsule that can be refilled with substances or items of interest, e.g. drugs, reporters, nano-robots etc. The properties of the shell layers, and also of the cores, can be tailored in order for the capsules to react in specific ways under diverse internal or external stimuli, such as pH values, interactions with various proteins or antibodies, optical signals, magnetic signals, etc. [2]. With respect to design and testing stages, the in-depth understanding of the structural, functional and physicochemical properties of NPCs is crucial. Among different characterization approaches that are needed for this purpose, imaging methods represent tools of the utmost importance for understanding NPCs and their interactions with endogenous and exogenous elements. So far, light microscopy techniques have been demonstrated as valuable tools for elucidating various NPC-related aspects [5], but, to the best of our knowledge, optical techniques capable of resolving details below the diffraction limit have not yet been used for investigating this interesting class of materials.

In our experiment NPCs assembled from layers of polystyrene sulfonate (PSS) and polyallylamine hydrochloride (PAH) were imaged by three techniques: scattering-type Scanning Near-Field Optical Microscopy (s-SNOM), tapping-mode Atomic Force Microscopy (AFM) and Confocal Laser Scanning Microscopy (CLSM) operating in reflectance and fluorescence workmodes. With

* Corresponding author.
E-mail address: stefan.stanciu@cmmip-upb.org (S.G. Stanciu).

http://dx.doi.org/10.1016/j.matlet.2016.07.029
0167-577X/

Please cite this article as: S.G. Stanciu, et al., Combined far-field, near-field and topographic imaging of nano-engineered polyelectrolyte capsules, Mater Lett (2016), http://dx.doi.org/10.1016/j.matlet.2016.07.029

s-SNOM we collected nanoscale optical information in a label-free manner by recording light scattered elastically from the apex of a metallic tip scanned across the sample, while with CLSM we collected microscale optical information on fluorescent and reflective properties of the addressed $(PSS/PAH)_4$ NPCs. AFM was used to place optical information collected with s-SNOM and CLSM into a topographic context. All the investigation techniques used in our experiment for characterizing the considered NPCs were available in a multimodal imaging system recently developed at the Center for Microscopy-Microanalysis and Information Processing of University Politehnica of Bucharest. In contrast to traditional correlative approaches where the identification of corresponding sample regions of interest is cumbersome when transferring the samples of interest between independent imaging systems that rely on different contrast mechanisms, and which operate at different scales, multimodal imaging with an ‘all-in-one’ system resolves the problems of sample alignment, region/feature identification and image registration. Such multimodal imaging approaches not only provide massive advantages in terms of experiment speed, but also help avoid problems related to erroneous matching of corresponding sample regions and enable the accurate investigation of samples with properties that rapidly evolve with time that need to be characterized simultaneously (or at least at very close time points) with all the imaging systems of interest. Thus, the proposed imaging approach can provide new perspectives for the in-depth characterization of NPCs and for their accurate understanding, which could play important roles with respect to developing novel design and fabrication strategies and for optimizing existing ones.

2. Materials and methods

Cationic poly (allylamine hydrochloride) (PAH, Mw 70 kDa) and anionic poly (styrene sulfonate) (PSS, Mw 70 kDa) [Sigma-Aldrich, USA], were used for the shell assembly of the NPCs onto Docetaxel (DTX) loaded calcium carbonate microparticles (7 ± 2 μm in diameter) [6]. The NPCs were prepared using a well-established procedure [7]. Four (PSS/PAH) bilayers were deposited onto DTX loaded $CaCO_3$ particles; PAH labeled with Alexa Fluor® 488 [Molecular Probes, USA] according to a previous reported method [8], was used in the assembly of the third (PSS/PAH) bilayer. Ethylenediaminetetraacetic acid (EDTA) [Sigma-Aldrich, USA] was used as complexating agent for the removal of the $CaCO_3$ template.

The conducted investigations were performed using a multimodal imaging system developed at University Politehnica of Bucharest, Fig. 1. This system allows collecting optical data on overlapping field-of-views in micro- and nanoscale, in the far-field and near-field regimes, by several techniques; moreover, it allows topographic imaging using AFM. Nanoscale s-SNOM data was collected using a home-made module incorporated in this multimodal system, which was previously used in various experiments [9–11]. s-SNOM is a label-free imaging technique that exploits the fact that upon laser excitation a near-field electromagnetic interaction is induced between the nano-scaled apex of a metallic tip and the surface of the investigated sample, and that this interaction influences the radiation that is elastically scattered back into the far-field from the tip apex. In our configuration we used a Pt coated Ti probe with an apex radius of curvature of ~35 nm, NSC19/Ti-Pt [Mikromasch, USA]; same as in the case of AFM, in s-SNOM the maximum achievable resolution depends on the radius of curvature of the scanning tip's apex [12]. For s-SNOM excitation we used a 638 nm semiconductor laser, with 0.5 mW power and its focal spot was aligned with the position of the NSC19/Ti-Pt probe through a long working distance objective (50×, 0.42 N.A.). Near-field signals were collected using the same objective used for excitation and were directed onto a photodiode connected to a lock-in amplifier, locked at the cantilever's oscillation frequency, which allowed the optical signals of interest to be extracted from the intense background, thus enabling label free nanoscale imaging. Far-field information was collected from corresponding regions using the bottom module of the multimodal imaging system (Fig. 1), consisting in a custom-modified Nikon C2+ CLSM performing in fluorescence and reflectance workmodes using a 488 nm laser beam for illumination.

3. Results and discussion

This “sandwich” type architecture of the multimodal system that we have used, Fig. 1, allowed the in tandem investigation of the same NPCs with four imaging modalities relying on different contrast mechanisms, and hence providing complementary information.

In the top row of Fig. 2 we present AFM, s-SNOM, and fluorescence and reflectance CLSM images collected over a 20×20 μm

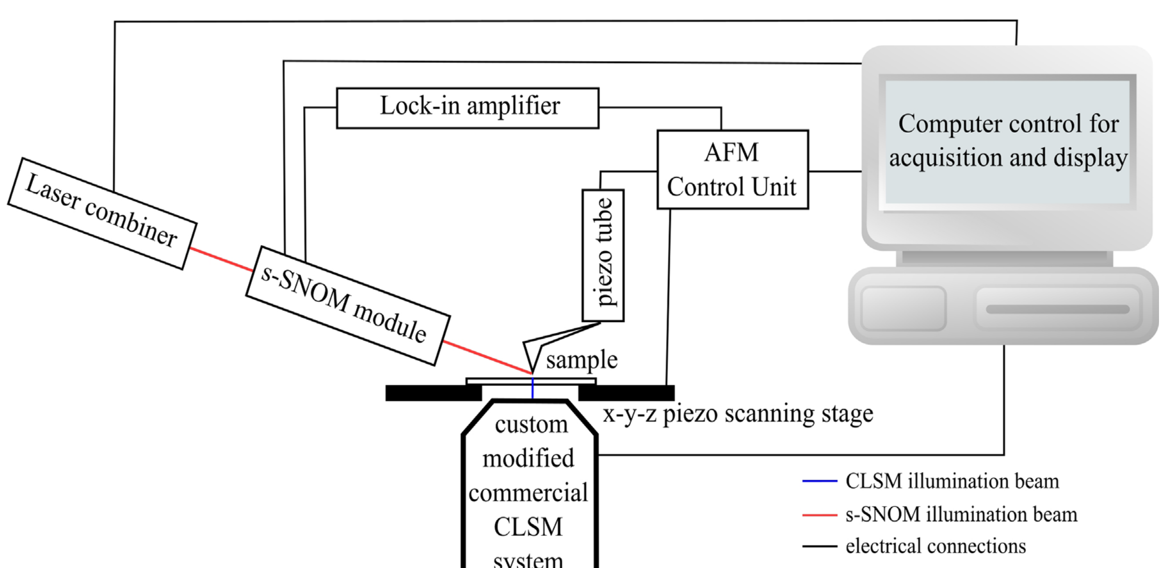

Fig. 1. Schematic diagram of the multimodal system used for combined far-field, near-field and topographic imaging of $(PSS/PAH)_4$ NPCs.