Micro-ordering of luminescent nanoparticles by targeting of biomolecules

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Nowadays a great effort has been directed towards the early detection of pathogenic agents or cancer cells by optical methods. One of the most investigated approaches is the use of inorganic luminescent nanoparticles (quantum dots, gold nanorods and lanthanide doped nanoparticles), which have been used to obtain high resolution images of cancer cells in in-vitro and in-vivo assays. The specificy of bioassays can be improved by using the interactions between ligands found on cell surfaces and protein receptors as they are involved in several biological processes such as bacteria and virus adhesion, cancer diseases, inflammation, immunology, and cell-cell communications. Thus, it is of vital importance to develop nanoparticles exposing multiple copies of these ligands to be potentially used in highly sensitive assays for the detection of bacteria or virus infections, or cancer tumors.

As natural polysaccharide, chitosan (containing glucosamines groups) is used in many biological applications as it is nontoxic, biocompatible and biodegradable. It display multiple biochemical functions at its surface chitosan so could serve as support to bind luminescent nanoparticles with the above mentioned ligands, so it would be possible to develop luminescent markers for the detection of specific biomolecules. Thus in this study chitosan molecules were patterned at the surface of epoxy-silane modified slides and, it was demonstrated that the chitosan labeled at the surface of these modified-slides is able to strongly interact with water dispersible $NaGdF_4:Er^{3+},Yb^{3+}$ up-converting luminescent nanoparticles.

Chitosan molecules have been directly patterned on modified slides using microcontact printing, a method that has been very successfully adopted for the precise and gentle transfer of biomolecules (proteins, DNA and lipid bilayers) from stamp a to a substrate. This occurs in a few seconds and without loss of biological activity. The stamps were fabricated using Polydimethylsiloxane (PDMS) (Sylgard 184). PDMS was molded using a prefabricated master, consisting in a grating with 250 lines/mm. The stamp was then inked with a chitosan /acetic acid solution and pressed after drying in the silane coated slide allowing for a conformal contact between the stamp and the surface of the glass-slide. The transfer of the chitosan molecules from the stamp to the glass-slide could be observed by using an optical microscope under reflection illumination (see Figure 1(a)). The fabricated chitosan patterns were then submerged in a water solution of $NaGdF_4:Er^{3+},Yb^{3+}$ (1mg/mL) nanoparticles. These nanoparticles were synthesized by acidic treatment of the nanoparticles previously synthesized via the thermal decomposition procedure.

After removing the sample from the solution, it was abundantly washed with distilled water. To ensure the presence of the nanoparticles in the sample after the washing process, the sample was illuminated by a laser diode with 980 nm wavelength. A strong green signal was observed with the naked eye. The emission spectrum of the sample was then characterized, corresponding to the Er^{3+} emission spectrum. To ensure the binding of the nanoparticles to the patterns, the washing procedure was repeated 10 times. The intensity emitted by the nanoparticles was not observed to significantly decrease after the washing process, indicating a strong binding chitosan-nanoparticles.

The characterization of the samples has been carried out by two different optical techniques. By Near-field Scanning Optical Microscopy (NSOM) optical contrast could be observed between the lines with chitosan and the surrounding regions, the higher brightness corresponding to the chitosan lines. Then we use fluorescence microscopy to detect the distribution of the nanoparticles in the sample. For this purpose we focused a 980 nm wavelength laser beam by means of a 100X objective, and the variation of the luminescence intensity of the sample was mapped. Although we could detect Er^{3+} emission from the nanoparticles along the entire sample (see Figure 1(b)), the intensity of emission was higher along the chitosan lines, showing that the nanoparticles bind preferably to the chitosan molecules.

In conclusion, we have demonstrated that luminescent nanoparticles can bind to chitosan molecules. We have demonstrated this preference by the fabrication of chitosan micropatterns where the nanoparticles are preferently bound. In addition to the biological applications of the nanoparticles to targeting of specific biomolecules, the work describes a fast and easy technique for the fabrication of luminescent micro arrays, with applications in other fields of science and technology, as for instance biosensing, chromatography, diagnostic immunoassays, cell culturing, DNA microarrays, and other analytical procedures.

Figures

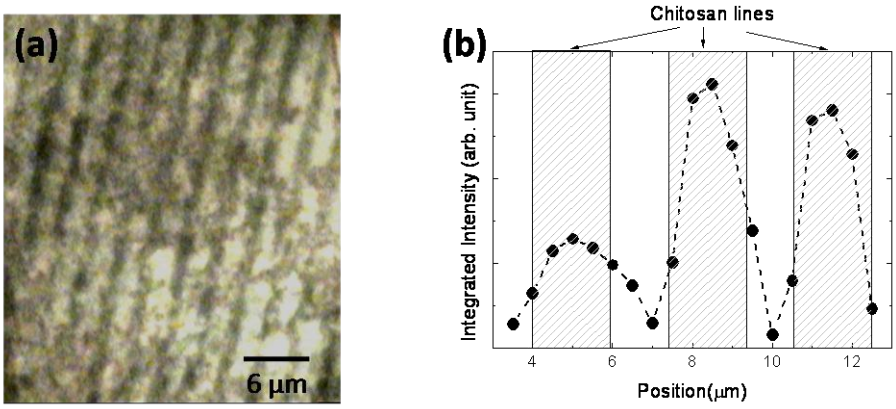

Figure 1 (a) Optical microscope image of the micropatterned sample, white ones correspond to chitosan; (b) Er^{3+} luminescence intensity across three chitosan lines showing the preferent binding of $NaYF_4$: Er^{3+}, Yb^{3+} nanoparticles to chitosan. Dotted lines are guides for the eyes.