Gold Nanoparticles Encapsulated in a Polymeric Matrix of Sodium Alginate

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Abstract

Plasmonic nanoparticles can be used as building blocks for the design of multifunctional systems based on polymeric capsules. The use of functionalised particles in therapeutics and imaging and understanding their effect on the cell functions are among the current challenges in nanobiotechnology and nanomedicine. The aim of the study was to manufacture and characterize polymeric microstructures by encapsulating plasmonic gold nanoparticles in biocompatible matrix of sodium alginate. The gold nanoparticles were obtained by reduction of tetrachloroauric acid with sodium citrate. To characterize the microcapsules, UV-Vis and FTIR spectroscopy, optical and confocal microscopy experiments were performed. In vitro cytotoxicity tests on HFL-1 cells were also performed. The capsules have spherical shape and 120 μm diameter. The presence of encapsulated gold nanoparticles is also shown by confocal microscopy. In vitro tests show that the microcapsules are not cytotoxic upon 24 h of cells exposure to microcapsules concentrations ranging from 2.5 to 25 capsules per cell. The obtained microcapsules of sodium alginate loaded with plasmonic gold nanoparticles could potentially be considered as release systems for biologically relevant molecules.

Keywords: gold nanoparticles, microcapsules, microencapsulation, sodium alginate

INTRODUCTION

From ancient times, metals have been used by man in various fields including medicine and curative sciences. One of the most precious metals discovered by man and used for his multiple properties for about 7000 years by now is gold who found his way through time by combining his aesthetic properties with a high value for trade market and with multiple curative properties discovered along the ages.

Modern research in fields like electronics and medicine had a dynamical evolution along with nanotechnology development which can be defined as a research for the design, synthesis and manipulation of particles with dimensions smaller than 100 nm (Ankamwar et al., 2005). Gold nanoparticles were used by man since ancient Roman times for decorative purposes but their synthesis in modern era begun over one and a half century ago along with Michael Faraday who observed for the first time the differences between the properties of colloid gold and the bulk gold (Giljohann et al., 2010). During the last half-century intense research regarding gold nanoparticles synthesis has been developed. Their unique properties such as their optical and electronic features which depend on the nanoparticles size and shape, make them promising candidates in various fields of nanoscience. A key element related gold nanoparticles is the high surface to volume ratio which can be readily modified with ligands containing functional groups such as amines, thiols, phosphines, which exhibit affinity for gold surfaces. Also the functional groups can be considered as anchors to other ligands, fact that allows gold nanoparticles to be used as...
carrier particles (Giljohann et al., 2010). Their high stability and exceptional properties allow gold nanoparticles to be used in applications such as targeted drug delivery, photothermal therapy, molecular imaging, in vivo diagnosis and cell labelling (Coman et al., 2015, Dreaden et al., 2012). The synthesis of gold nanoparticles was based on the reduction of gold atoms from salts where they are usually found in an oxidation state of +3 to the basic oxidation state of 0. The most commonly used salt is chlorauric acid (HAuCl₄) and as reduction agents a wide range of compound including sodium citrate, ascobate, polymers (polyethylene glycol), glucose, or natural extract from plants have been reported (Coman et al., 2013).

Plasmonic nanoparticles such as gold nanoparticles for example can be used as building blocks for the design of multifunctional systems based on polymeric capsules (Carregal-Romero et al., 2012, Song et al., 2009). The use of polymer encapsulated nanoparticles, as proposed here, is a subject of continuous interest and of great potential for biomedical applications, because of the great need towards developing new nanomaterials based on which one could build diagnosis and treatment systems. As opposed to regular nanoparticles, encapsulated nanoparticles are much more promising devices. For the final purpose of using them as effective therapeutic delivery systems, it is much more effective to encapsulate the cargo, because this would facilitate: (a) a controlled release of drugs and bioactive molecules; (b) homogeneous intracellular distribution of the loaded molecules; (c) in vitro and in vivo delivery of bioactive molecules; (d) protection of encapsulated molecules from enzymatic degradation (Carregal-Romero et al., 2015, del Mercato et al., 2014).

Here we have synthesized gold nanoparticles using sodium citrate as reducing agent for the gold salt and further we have encapsulated the nanoparticles in a polymeric matrix of sodium alginate. Alginate was chosen for encapsulation (Gombotz et al., 1998) because it is biocompatible, biodegradable and preffered system for applications related to controlled and targeted release of bioactive compounds. The obtained microcapsules were characterized by UV-Vis and FTIR spectroscopies, contrast phase and confocal microscopy. In vitro cellular viability tests on HFL-1 cells were also performed. The microcapsules had 120 µm in diameter, successful encapsulation of the gold nanoparticles was achieved and the obtained microstructures were found to be biocompatible with the HFL-1 cells.

**MATERIALS AND METHODS**

Hydrogen tetrachloroaurate (III) dihydrate (HAuCl₄·2 H₂O) was purchased from Alfa Aesar. The rhodamine 6G (R6G) and sodium citrate were purchased from Sigma. Fetal bovine serum (FBS)-Lonza, Ham’s F12 Nutrient Mixture (Ham’s F12), and Dulbecco’s Modified Eagle Medium (DMEM) were purchased from Lonza Group Ltd. (Basel, Switzerland). Glutamine, penicillin, streptomycin, and amphotericin were purchased from Sigma Chemical Co. (St. Louis, MO). The HFL-1 human lung embryonic cell line was obtained from American Type Culture Collection (Rockville, MD, USA).

The gold nanoparticles were obtained by reduction of the tetrachlororocuric acid (HAuCl₄) with sodium citrate. 100 ml of 0.01% solution of HAuCl₄ were boiled under constant magnetic stirring. At boiling, 2.5 ml of 1% sodium citrate solution was added. The formation of the gold nanoparticles is indicated by the colour change of the solution. Within seconds the solution changes colour from almost colourless to wine red. Next, the nanoparticles were embedded in the polymeric matrix of sodium alginate using the Multinozzle Biotech Encapsulator (EncapBioSistems Inc.). This is a successful tool for encapsulation (Heinzen et al., 2004), the apparatus being able to produce different sizes of capsules (diameter given by nozzle) using the method of uniform bead production, based on the proven principle that a laminar jet of liquid is broken in equal beads when vibration at the appropriate frequency is applied. The gold nanoparticles (3 ml, prior washed by repeated centrifugation cycles and concentrated 8X) were mixed with 100 ml of 1% sodium alginate solution. The polymer-nanoparticle mixture was then passed through a nozzle and the obtained microspheres were hardened in a sterile 2% CaCl₂ bath. A 120 µm nozzle was used for obtaining the microcapsules and the encapsulator was operated under the following parameters: 1020 Hz frequency, 1610 V electrode potential and 640 bars pressure.

The UV-VIS measurements were carried out with a PerkinElmer Lambda 25 UV-Vis spectrometer. The FTIR Attenuated Total Reflectance spectra were recorded on a Schimadzu IR-Prestige FTIR Spectrometer equipped with a diamond PIKE.
MIRacle single reflection plate unit. The spectra were taken on the dried samples by co-adding 64 interferograms with 4 cm\(^{-1}\) resolution. Contrast phase microscopy images of the samples were obtained using a Zeiss Observer A1 microscope (Carls Zeiss). The confocal microscopy was performed with a Zeiss LSM 710 confocal microscope, equipped with Zen software for image processing. For the confocal microscopy experiments, the encapsulated gold nanoparticles were also labelled with rhodamine 6G (R6G). The nanoparticles (5 ml) were mixed with 150 µl of R6G 10\(^{-4}\) M and kept under vigorous magnetic stirring for 3 hours prior to encapsulation.

For testing the microcapsules effect on the cellular viability of human fetal lung fibroblasts (HFL-1), the MTT viability assay was performed. The HFL-1 cells were grown in a mixture of 1:1 v/v Ham's F12 and DMEM containing 4.5 g/L glucose, supplemented with 10% fetal bovine serum, 2 mM glutamine, 1% penicillin and streptomycin, and 0.1% amphotericin. The cells were kept in 5% CO\(_2\) atmosphere, 37 °C, and 95% relative humidity. After seeding 8×10\(^3\) cells in 96-well plates and allowing cells to attach for 24 h, the cells were incubated for another 24 h with three different concentrations of encapsulated gold nanoparticles. Then, the cell culture media was removed and MTT reagent in HBSS buffer (0.5 mg/ml) was added to each well. After 2 h of incubation, the MTT was removed and the resulted formazan crystals were solved in DMSO. The solubilized formazan formed in viable cells was measured at 550 nm and 630 nm (for sample and background, respectively). The absorbance was measured using the HT BioTek Synergy microplate reader (BioTek Instruments, USA). The results were expressed as percent survival relative to an untreated control.

**RESULTS AND DISCUSSION**

Gold nanoparticles show characteristic surface Plasmon resonance peaks in the range 520-540 nm (Daniel et al., 2004). The absorption maxima for the nanoparticles obtained in our study is at 518 nm. A comparison between the UV-Vis spectrum of the citrate reduced gold nanoparticles (Fig. 1.) before and after encapsulation shows the dissappearance of the UV-Vis absorption band at 518 nm upon encapsulation. This confirms the formation of the microcapsules and the successful encapsulation of gold nanoparticles in the sodium alginate matrix.

![Fig. 1. UV-Vis absorption spectra of the gold nanoparticles obtained by the citrate reduction method (upper spectrum) and of the solution obtained after encapsulating the gold nanoparticles in the sodium alginate matrix (bottom spectrum).](image)

Fig. 2(a) illustrates the gold nanoparticles solution before encapsulation (left-wine red) and after encapsulation in the micrometric matrix of

![Fig. 2. (a) The gold nanoparticle solution before (left) and after (right) encapsulation; (b) contrast phase microscopy image of the microcapsules; (c) confocal microscopy image of the microcapsules.](image)
sodium alginate (right side of the picture). The morphology of the microcapsules was studied by contrast phase microscopy and confocal scanning microscopy. Contrast phase images show that the capsules have spherical shape and mean diameter of 120 µm (Fig. 2(b)) The presence of the nanoparticles inside the polymeric microcapsules can was visualised by confocal microscopy. R6G was used as fluorophore for the fluorescence confocal microscopy. R6G was attached to the nanoparticles surface prior to encapsulation. Fluorescence confocal microscopy images of the microcapsules with R6G-labelled gold nanoparticles inside are shown in Fig. 3(c). The R6G-labelled gold nanoparticles within the microcapsules can be visualized din red.

The structural analysis of the microcapsules was carried out by FTIR spectroscopy. Fig. 3 illustrates the characteristic FTIR spectra for a solution of sodium alginate and also the FTIR spectrum characteristic for the gold nanoparticles-alginate microcapsules. For the measurements, a drop of solution was placed onto the ATR diamond plate and allowed to dry. The FTIR spectrum of the capsules was obtained by centrifuging 2 ml of solution containing the capsules, collecting the sediment and drying in order to eliminate the intense peaks characteristic to O-H vibrations from the water molecules. The two spectra show similar vibrational bands, characteristic to sodium alginate. The vibrational bands at 3280 represent characteristic stretching vibrations of –OH and the 2929 cm⁻¹ band is assigned to C–H stretching vibrations and C–O stretching vibrations of the carboxyl groups. The FTIR spectra also show a region rich in vibrational features below 1700 cm⁻¹.

![FTIR spectra](image)

**Fig. 3.** FTIR spectra of the encapsulated nanoparticles and of the sodium alginate solution.

![Viability graph](image)

**Fig. 4.** Viability of HFL-1 cells after 24 h exposure to 2.5, 10, 25 gold nanoparticle loaded microcapsules per cell. Values are expressed as a percentage of control (untreated) cells (mean ± SEM).
The intense peaks at 1595 and 1404 cm\(^{-1}\) correspond to symmetric and asymmetric stretching vibrations of the carboxyl COO\(^{-}\) groups (Dai et al., 2008). The peaks at 1296, 1125 and 1082 cm\(^{-1}\) are characteristic to oligosaccharides and the peaks at 1024 and 945 cm\(^{-1}\) are specific to the vibrations of the C-O bonds from the saccharide chain and they thus indicate the presence of gluconic and mannuronic acids (Darget et al., 2000, Puttipipatkhachorn et al., 2006) being known that sodium alginate contains two uronic acids residues, α-L-guluronic and β-d-mannuronic, in varying amounts and alternating sequences (Pongjanyakul et al., 2006).

**In vitro** cellular viability tests were also conducted on HFL-1 cells. 24 h after seeding, the HFL-1 cells were exposed to three different concentrations of microcapsules (2.5, 10, and 25 capsules per cell were used) and cellular proliferation tests by the MTT assay were carried out 24 h after exposure to microcapsules. An untreated control was also used. Contrast phase microscopy images taken 24 h after cell exposure to microparticles show that the cell morphology does not change compared to control (not shown here). A maximum of 30% decrease in cellular viability is observed 24 h from the treatment. Exposure to 2.5 capsules per cell leads to 22% decrease in cellular viability compared to control, while the 10 and 25 capsules per cell exposure leads to 25 and 30% decrease in the cellular viability. It can thus be concluded that the microcapsules are not cytotoxic to the HFL-1 cells. This makes the obtained microparticles suitable for further tests related to biological applications such as controlled release of bioactive molecules.

**CONCLUSION**

Spherically shaped microcapsules of sodium alginate loaded with gold nanoparticles were successfully obtained. The microcapsules could potentially be considered as release systems for biologically relevant molecules.

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**REFERENCES**