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Chitosan-Gold Nanoparticle Composites for Biomedical Application

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The aim of this work is to synthesize chitosan-gold nanoparticles films by direct chemical reduction of $HAuCl_4$ in a chitosan solution and to investigate the influence of gold nanoparticles concentration on the structure of films, conductivity and healing effect on mice skin after surgery. Results obtained have shown that new chitosan-gold nanoparticle-collagen bionananocomposites demonstrated better healing effect on the mice skin after surgery than control performed on commercial TheraForm™ material.

Keywords: Chitosan, Gold nanoparticles, Dielectric spectroscopy, Healing effect.

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1. INTRODUCTION

Metal nanoparticles such as gold (AuNP's) are excellent candidates for biomedical and tissue engineering applications. AuNP's have unique properties, such as size- and shape-dependent optical and electronic features, a high surface-to-volume ratio, and surfaces that can be readily modified with ligands containing functional groups such as thiols, phosphines, amines [1, 2]. Different methods for synthesis of gold nanoparticles have been proposed. The most common method for AuNP's synthesis is based on the reduction of $HAuCl_4$ by sodium citrate in solution at high temperature (Turkevitch method) [3]. The Brust-Schiffrin method usually involves use of organic solvents [4]. However, the use of organic solvents render AuNP's unsuitable for application in biomedicine. In ref. [1] to improve the stability and uptake of nanoparticles has been proposed a novel method for synthesis and stabilization of gold nanoparticles using a natural, biocompatible polymer, chitosan. Chitosan (CS) is well known for its hydrophilic, biocompatible, biodegradable and non-toxic properties. Furthermore, the NH_2 and/or OH groups of chitosan may act as reducing agents for the formation of gold nanoparticles [5]. Additional, chitosan promotes adsorption onto the surfaces of gold nanoparticles via electrostatic interactions between the amino groups and gold [6].

It is noteworthy that AuNP's embedded in the three-dimensional chitosan matrix according to the effective media theory [7] must sufficiently change conductivity mechanism of CS / AuNP's nanocomposites. But the majority of recent publications have been focusing on the fabrication, microstructural characterization and sensibility of CS / AunP's as biosensors, however, electrical properties of such CS / AuNP's films have not been reported which are important for optimization material for different applications. Additional, in spite of fact that many studies have been reported that chitosan is a substitute material in the skin tissue engineering [8, 9], but from our knowledge in the literature there are no any reports about application of CS / AuNP's nanocomposites for treatment of skin damaged.

Based upon the above discussion on the potential of AuNP's, the aim of this work is to synthesize CS / AuNP's films by direct reduction of $HAuCl_4$ in chitosan solution and to investigate the influence of gold nanoparticles concentration on the structure, conductivity and healing effect on the mice skin after surgery.

2. MATERIALS AND METHODS

The nanocomposites were synthesized by dissolving 2 wt. % of chitosan (with medium molecular weight 300,000 g/mol and 85 % of degree of deacetilation) in 1 wt. % acetic acid solution, afterward the $HAuCl_4$ solution ranging from 0.06 mM to 1.7 mM were added and the mixture. The solutions were mixed and heated at 75 °C under magnetic stirring until the solution changed its color from slight yellow to red. CS / AuNP's thin films (thickness ca. 30 nm) were prepared by the solvent cast method by pouring the final solution into a plastic Petri dish and allowing the solvent to evaporate in oven for 24 h at 60 °C.

CS / AuNP's films morphology was analyzed by JEOM JSM-7401F Field emission scanning electron microscope. Crystal structure analysis was performed using a Rigaku diffractometer ULTIMA IV, equipped with the CuKα radiation (λ = 1.5406 Å).

The amount of free water was determined by thermogravimetric analysis (TGA) (Metler Toledo 851e model). The measurements were made with a dry air flow from 25 to 300 °C with rate 10 °C/min.

Dielectric measurements in the frequency range from 40 Hz to 110 MHz were carried out Agilent Precision Impedance Analyzer 4249A. The amplitude of measuring signal was 100 mV.

3. RESULTS AND DISCUSSION

3.1 Structure and Properties of CS/AuNP's films

SEM micrographs show a homogeneous distribution

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of the nanoparticles with dimension between 8 and 11 nm embedded in the chitosan matrix (not shown).

XRD measurements (Fig. 1) show the peak of Au (111) appeared at around 38.1 ° of 2θ which is in agreement with previous reports [10]. It is clear that volume fraction of AuNP's increases with increasing with $HAuCl_4$ concentration (increasing of intensity of Au diffraction line on Fig. 1). The crystalline size of nanoparticles d was estimated from XRD measurements using the Scherrer formula [11]:

$$d = \frac{0.9\lambda}{FWHM \cdot \cos\theta},$$

where, λ is wavelength, FWHM is full-width at half-maximum and θ is the peak position of Au. The value of d was calculated about of 9 nm and is close to SEM measurements.

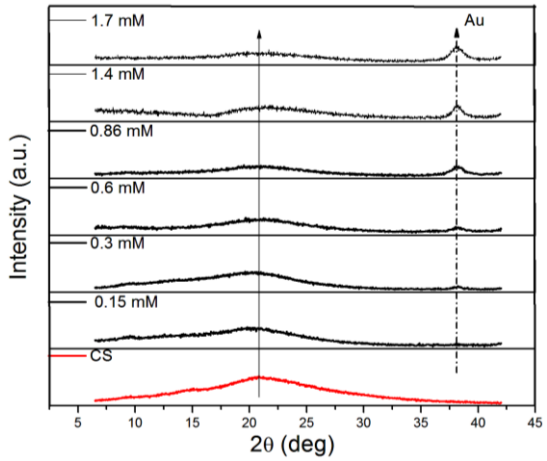

Fig. 1 – XRD pattern of films obtained from solution with different $HAuCl_4$ concentration

Free water content was determined by thermogravimetric analysis (TGA). The amount of free water may be evaluated by the decrease of sample weight during the heating scan. The loss in weight at the temperature 120 °C was taken to be the result of water evaporation. Fig. 2 shows the dependence of moisture content on the $HAuCl_4$ concentration: increasing of $HAuCl_4$ leads to the lower moisture content in CS / AunP's composites.

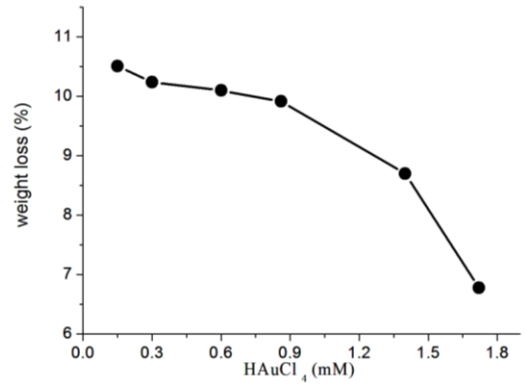

Fig. 2 – Dependence of water content on $HAuCl_4$ concentration

This effect can be related to the interaction of AuNP's with the reactive NH_2 and OH groups and the formation of a polymeric composite structure with nanoparticles which impeded the formation of H-bonding with water and decrease the water absorption ability with increasing of AuNP's concentration in composites. This lower weight loss by increasing of the nanoparticles concentration has been previously observed in chitosan-Ag nanocomposites [12].

Fig. 3 shows the plots of the DC electrical conductivity as a function of the $HAuCl_4$ concentration obtained from impedance measurements at room temperature.

Insert in Fig. 3 shows the typical complex impedance spectra measured in all films (open circles). This plot exhibits characteristic depressed semicircles at high frequencies and a quasi-linear response at low frequencies. According to previously reported studies [13], a depressed semicircle describes properties of bulk material and a quasi-linear response at low frequencies is associated with interfacial polarization and/or surface and contact effects.

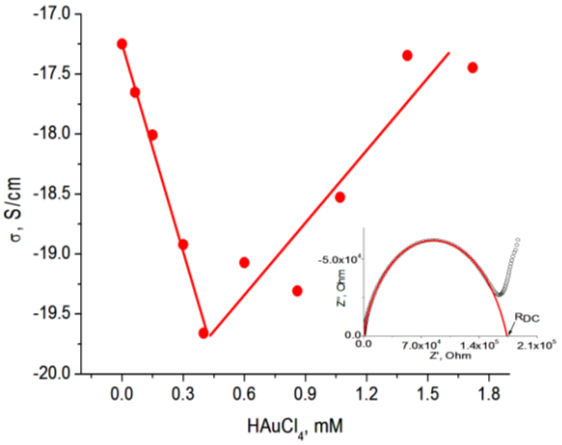

Fig. 3 – DC conductivity of CS / AuNP's films as a function of the of the $HAuCl_4$ concentration obtained from impedance measurements at room temperature. Insert shows complex impedance spectra

The values of DC resistance R_{dc}, and the corresponding DC conductivity σ_{dc} ($\sigma_{dc} = d/(R_{dc} \times S)$, where d = film thickness and S = contact area) have been obtained from the intersection of the high frequency semicircle and the real-part axis on the impedance plane as it is shown in insert of Fig. 3 (red line).

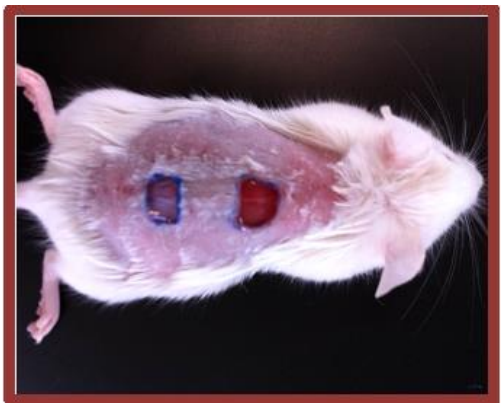

Fig. 4 – Surgery process. The lesion nearby to head was implanted with material synthetized, the lesion ubicated in the inferior part of animal was used like control

According to effective media theory DC conductivity of system with conductivity inclusion in polymer dielectric matrix must increasing [7]. But, in the case of CS / AuNP's systems, DC conductivity at the low nanoparticles concentration decreases. This conductivity decreasing can be explained by interaction of AuNP's with citosan matrix. Such interaction is specific for chitosan composites and related with decreasing of moisture content (Fig. 2) and number of free H^+ and OH^- ions which responsible for conductivity of pure chitosan [14]. At the higher AuNP's concentration conductivity of CS / AunP's nanocomposites increases due to the formation of conductivity paths in the composite formed by nanoparticles. That is why DC conductivity demonstrate minimum. This minimum appear due to competition of two mechanisms: decreasing number of free H^+ and OH^- ions and the formation of conductivity paths in the composite formed by nanoparticles.

As a rule, interaction of AuNP's with chitosan matrix can be confirm by FTIR measurements by changing the bands shapes and frequencies. But these changes can be observed at relative high AuNP's concentration [15]. In our case the formation of a polymeric composite structure with nanoparticles has been confirmed by TGA and conductivity measurements as have been discussed above.

3.2 Healing effect of CS/AuNP's nanocomposites

To check the possibility application of CS / AuNP's nanocomposites in the tissue engineering healing effect on the mice skin have been investigated. For application in tissue engineering, the material should meet the following requirements: biocompatibility with the tissues; biodegradability; nontoxicity and has good mechanical property. CS / AuNP's nanocomposite respondents to all these requirements but has relative bad mechanical strength. To improve mechanical properties 20 % of Glydidyl methacrylate (GMA) has been add to CS / AunP's solution. GMA is a synthetic monomer which belongs to epoxy group. GMA can be functionalized on aqueous medium and can react with amines, carboxylic acids and hydroxides groups. This monomer has been used in dentistry like diluents of epoxy resins and is biodegradable. This new hybrid natural-synthetic hydrogel (CTS-g-GMA) change the mechanical properties, viscosity and swelling index of CS and can be used like cellular support for biomedical applications [16].

Additional to GMA 0.3 % of collagen (Col) dissolved in the acetic acid solution has been added to CS / AuNP's solution. Collagen is a major protein component of bone, cartilage, skin, and connective tissue and also an important part of extracellular matrixes in animals [17]. More, chitosan-collagen membranes have great potential in skin tissue engineering [18]. CS-g-GMA-AuNP´s-Col thin films were prepared by the solvent cast method by pouring the final solution into a plastic Petri dish and allowing the solvent to evaporate in oven for 24 h at environment temperature.

Four CS-g-GMA-AuNP´s-Col films obtained from solution with different $HAuCl_4$ concentration (0.15, 0.3, 0.6 and 1,07 mM) have been evaluated for investigation healing effect. Additional, commercial material TheraForm™ and CS-g-GMA film were used like control. TheraForm™ is a commercial absorbable biomaterial based on collagen fabricated by Regenerative Medical System industry. It is biocompatible implant and using in the tissue regeneration [19]. The materials were cut into squares of 8×8 mm; afterwards cut materials were washed with buffer phosphate solution (PBS) 600 μL by 5 minutes two times and 600 μL of Dulbecco's Modified Eagle Medium (DMEM) by 5 minutes three times. Finally the materials were sterilized by ultraviolet light during 10 minutes.

In vivo tests were carried out on the female mice stain BALB / C at 6 weeks old, with an average weight of 21 g. Each mouse was anesthetized with ketamine hydrochloride 100 mg/kg and xylazine 5 mg/kg. Subsequently the back of animal was shaved carefully and the surgery consisted on removed two skins square of 5×5 mm (dermis and epidermis) on the back. The square nearby to head of mice was implanted with the synthesized materials and the other square was used like a control, without any materials (Fig. 4).

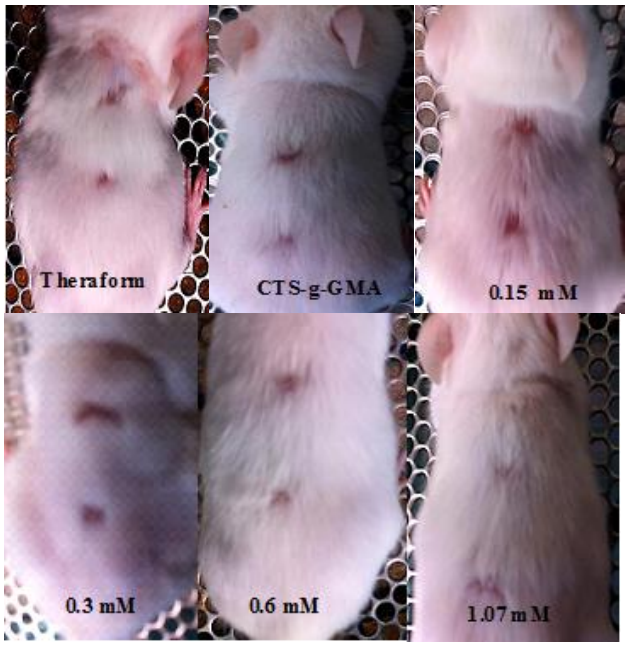

Fig. 5 – Cicatrization process of mices after 18 days. Numbers on Figure show $HAuCl_4$ concentration in solution from which films have been prepared

Fig. 5 shows cicatrization process of mices after 18 days after surgery. All materials have shown that new skin was growing without infection and inflammatory sings during healing process.

Fig. 6 shows percent of recuperation of skin (estimated from dimension of cicatrize) using different materials: TheraForm™, CTS-g-GMA and CS-g-GMA-AuNP´s-Col films obtained from solution with different $HAuCl_4$ concentration (0.15, 0.3, 0.6 and 1,07 mM). TheraForm™, CTS-g-GMA and CS-g-GMA-AuNP´s-Col with low AuNP´s (below 0.6 mM) demonstrated approximately the same recuperation ability. But films with high higher AuNP´s concentration have been demonstrated better recuperation ability than control commercial TheraForm™ and CTS-g-GMA materials.

The healing of a skin wound is complicated courses,

including a wide range of cellular, molecular, physiological, and biological processes. Many studies and different explanations have been reported on the use of chitosan as a skin substitute material in skin tissue engineering due to its many advantages such as hemostasis, accelerating the tissue regeneration and stimulating the fibroblast synthesis of collagen [8]. One of explanation proposed that the cationic nature of chitosan allows the electrostatic interactions with anionic glycosaminoglycans and proteoglycans distributed widely throughout the body and other negatively charged species [8].

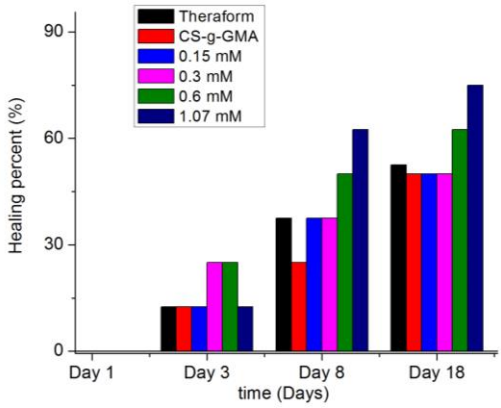

Fig. 6 – Estimation of healing effect on mices. Numbers on Figure show $HAuCl_4$ concentration in solution from which films have been prepared

From another point of view AuNP´s wide used in fundamental cell biology applications [1]. As is the case with all nanomaterials, little is known about the interactions of nanoparticle with cells at the molecular level [1]. But it was been reported that AuNP´s can be modified with chitosan reactive groups [20, 21]. So, we can propose that high surface area of AuNP´s and the cationic nature of chitosan increase the electrostatic interactions with anionic group and other negatively charged species that accelerate the tissue regeneration.

4. CONCLUSION

Chitosan-AuNP´s (with nanoparticle dimension about of 9 nm) films have been synthesized by direct reduction of $HAuCl_4$ in chitosan solution. It was shown that DC conductivity dependent on AuNP's concentration and demonstrate minimum. This minimum appear due to competition of two mechanisms: decreasing number of free H^+ and OH^- ions and the formation of conductivity paths in the composite formed by nanoparticles.

Possible application of CS / AunP's nanocomposites in the tissue engineering has been demonstrated on the healing effect on the mice skin.

New CS / AuNP´s-Glydidyl methacrylate-collagen nanocomposites have been synthesized. It has been shown that new nanocomposite demonstrated better recuperation ability of skin than control commercial TheraForm™ material. This study indicated that a small amount of AuNP´s could induce significant changes in the physicochemical properties of chitosan, which may increase its biocompatibility and can be used in the tissue engineering.

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