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Photothermal Mucoadhesive Biomaterial Composites of Rambutan-like Gold Micro/Nanostructure and Chitosan

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Photothermal property is one of the many remarkable characteristics possessed by gold nanoparticles of which a near-infrared (NIR) laser can be converted into heat. This methodology can potentially be used to provide direct and local heating of sensitive organs. Such ability was incorporated along with the muco-adhesive property of chitosan and investigated in this work. The rambutan-like gold nanoparticles were bound onto chitosan and the resulting gold micro/nanostructure-chitosan composite was evaluated for its photothermal property. Upon irradiation with 808 nm laser, an increase of the surrounding heat of up to 11 °C was achieved with the biomaterial. This gold-chitosan composite was also investigated for its muco-adhesive binding onto mucosal membranes in the body and the interaction between mucin and the compo-site was found to be pH dependent. The mucoadhesion ability of the obtained composite, expressed by its adsorption of mucin, are 29.98 mg/g, 43.20 mg/g and 74.02 mg/g at pH 1.2, 4.0 and 6.4 respectively.

Keywords: Gold particles,Chitosan, NIR laser, Photothermal, Mucoadhesive.

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1. INTRODUCTION

Photothermal therapy of tumors is an attractive and minimally invasive method for treating tumors [1-4]. This technique, which typically involves the conversion of absorbed light into local heating through nonradiative mechanisms, is relatively simple for cancer cell ablation usage and may have several advantages, such as fast recovery, fewer complications, and shorter hospital stay [5]. In particular, the near-infrared (NIR) light used in this manner provides deep-tissue penetration with high spatial precision without damaging normal tissues due to the low-energy absorption of NIR light by normal tissues [6, 7].

Several nanostructures, including aggregated gold nanoparticles [8], gold nanoshells [9], gold nanocages [10], gold nanorods (GNRs) [11], carbon nanotubes [12], and copper sulfide (CuS) nanoparticles [13], have been investigated for NIR photoactivated cancer therapy. In all cases, light is converted into heat by surface plasmon resonance. Surface plasmon resonance (SPR) is a phenomenon in which free electrons in the nanostructures collectively oscillate and scatter or absorb the incident electromagnetic wave [14].

Another form of gold micro/nanostructure synthesized was the rambutan-like gold micro/nanostructure. This particular structure can be tuned to NIR light absorber in order to use in photothermal therapy.

Nano-biocomposites are obtained by adding nanostructure material to biopolymer in order to combine the surface plasmon resonance property of nanomaterial and the mucoadhesive property of biopolymer [15]. In this work, we are interested in using chitosan as a supported polymer for carrying the gold particles.

Chitosan is a linear chain polysaccharide comprising of glucosamine and N-acetyl glucosamine residues connected by ß-1,4-glycosidic bonds. A variety of fundamental properties such as good mucoadhesive, non-toxic, excellent biocompatibility and biodegradability make chitosan a very attractive material for biomedical applications including wound dressing, tissue engineering and drug delivery [16-18].

The aim of this work was to prepare a gold-chitosan composite in order to combine the photothermal property of the rambutan-like gold micro/nanostructure and mucoadhesive property of chitosan for the application in mucosal organs cancer therapy.

2. EXPERIMENTAL

2.1 Synthesis of the Rambutan-like Gold

The rambutan-like gold micro/nanostructure was prepared using $HAuCl_4$ and $AgNO_3$. First, $HAuCl_4$ and $AgNO_3$ were mixed in reducing agent and the solution was vortex for 10 sec. The mixture was subse-quently left at room temperature for 10 minutes. The color of the mixture was changed from yellow to colorless and the gold particles were settled. The mixture was centrifuged and washed with DI water for three times.

2.2 Preparation of the gold-chitosan composite

Two hundreds ppm of the rambutan-like gold micro/nanostructure solution and 8 mL of 1% chitosan solu-tion were mixed at room temperature for 2 days. The gold-chitosan composite were obtained by pouring the solution on 50 cm^2 polypropylene mold, followed by dry-ing at room temperature for 48 h.

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2.3 Characterization of Gold and gold-chitosan composite

The rambutan-like gold micro/nanostructure was characterized using a scanning electron microscope (SEM, JEOL). Gold-chitosan composite was characterized by FTIR (Nicolet 6700, Thermo) in ATR mode.

2.4 Photothermal Test

The rambutan-like gold micro/nanostructure 500 ppm dispersed 5 mL of water was irradiated by NIR laser and measured the temperature from room temperature to constant temperature. The gold-chitosan composite film was irradiated by NIR laser with a continuous wave at the wavelength of 808 nm with diode laser (Dragon laser, China) and measured the temperature using a thermocouple probe (Microtherma 2T thermometer and T needle penetration probe, Thermoworks, UK). The irradiation of the composite film was a cycle irradiation of opened laser for 5 minutes and closed for 5 minutes for three times.

2.5 Mucus glycoprotein assay

The mucus glycoprotein assay was studied using the periodic acid schiff (PAS) method [19]. The PAS colorimetric assay for the detection of glycoprotein was used for the determination of the free mucin concentration, in order to evaluate the amount of mucin adsorbed onto the chitosan.

A 0.5% (w/v) mucin solution in each of three broadly isosmotic solutions that differ in pH media, pH 1.2, pH 4.0 and pH 6.4 were prepared. Gold-chitosan composite was dispersed (at 5 mg/1.5 mL final) in the above mucin solutions and shaken at 37 °C for 2 h. Then the dispersions were centrifuged at 12,000 rpm for 2 min to pellet the gold-chitosan-mucin complex and the supernatant was harvested and used for the measurement of the free mucin content. The mucin concentration was calculated by referenced to the calibration curve, and the amount of mucin adsorbed to the composite was calculated as the difference between the total amount of mucin added and the free mucin content in the supernatant.

3. RESULTS AND DISCUSSION

3.1 Morphology of Gold particles

Figure 1 shows the SEM image of the rambutan-like gold micro/nanostructure particles. The size of the rambutan-like gold micro/nanostructure particles are about 3-5 μm (see Fig. 1a) and the length of the hairs are 300-400 nm (see Fig. 1b). The appearance seen here was the hairs of these particles grew on the gold microsphere by the induction of Ag ion [20].

The temperature of the rambutan-like gold micro/nanostructure particles in water immediately increased about 1 °C within the first minute of irradiated with NIR laser and gradually increased in temperature to a constant level of about 3 °C higher than the original temperature in 15 minutes (see Fig. 2). The gold nanorod was heated when irradiated NIR laser because of surface plasmon resonance. However, the gold microsphere (more than 1 μm) has no photothermal

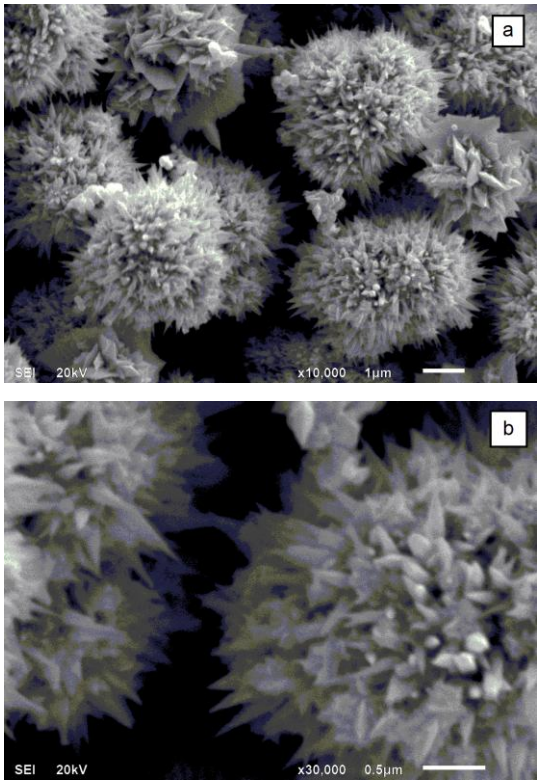

Fig. 1 – SEM micrographs of the rambutan-like gold micro/nanostructure magnification 10,000x (a) and magnification 30,000x (b)

3.2 Photothermal Property of gold solution

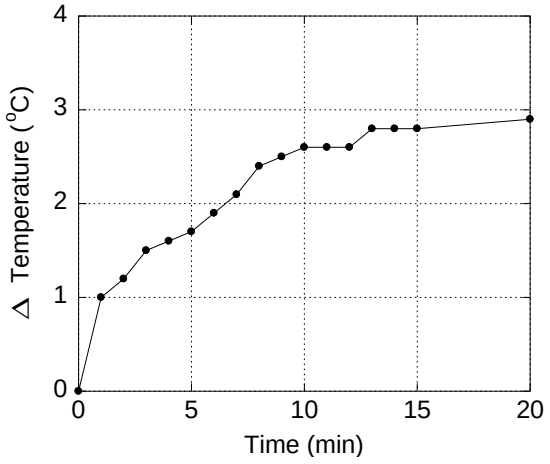

Fig. 2 – The Temperature profile of the rambutan-like gold micro/nanostructure in water

property due to the fact that the surface plasmon resonance is a property of nanoparticles exclusively [21]. The rambutan-like gold micro/nanostructure was heated when irradiated with NIR laser for the reason that they have plasmon resonance property at the wavelength of 808 nm. This surface plasmon resonance effect of the rambutan-like gold micro/nanostructure occurred at the hair structure of these particles. Thus, these particles behaved in the analogous way as the aggregation of gold nanorods on the spherical gold particles (see Fig. 3).

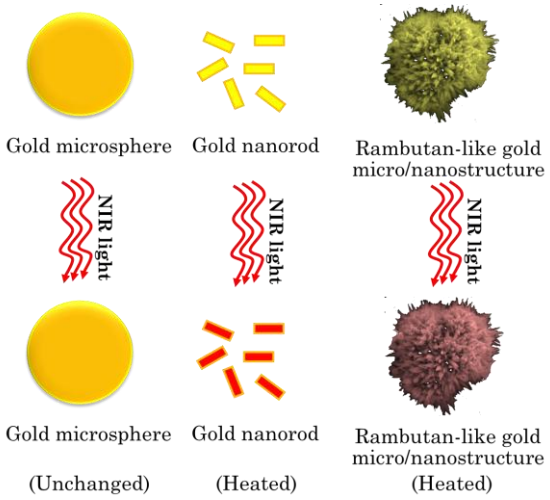

Fig. 3 – Schematic drawing of gold microsphere, gold nanorods and the rambutan-like gold micro/nanostructure before and after irradiated by NIR laser

3.3 Photothermal Property of gold-chitosan composite

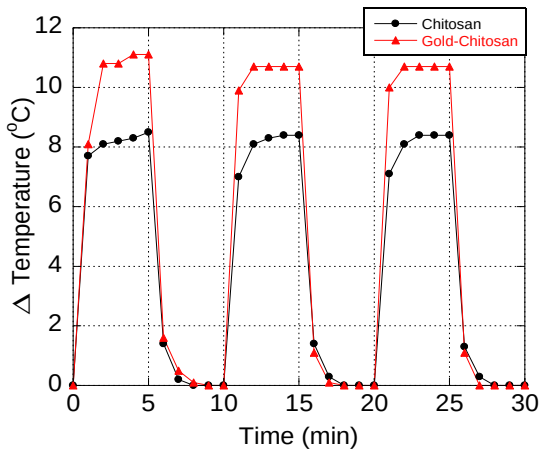

Fig. 4 – The temperature profile of chitosan and gold-chitosan composite

The photothermal property of gold-chitosan composite was measured to confirm the ability of the gold micro/nanostructure in converting light into heat during the binding process on the polymer. The gold-chitosan composite was irradiated by NIR laser and measured the surrounding temperature (2 mm. from laser spot). The chitosan film and gold-chitosan composite increased the surrounding temperature up to 8.4 °C and 11.1 °C respectively, during the irradiation with NIR laser for 5 minutes. The surrounding temperature decreased back to room temperature in 3 minutes after the NIR laser was turned off. The chitosan film can increase the temperature, because chitosan has the absorption spectrum at 700-850 nm (data not shown). Hence, chitosan can absorb NIR laser and transfers the heat to the surrounding surface. The gold-chitosan composite increased the temperature 3 °C higher than chitosan since the gold particles have the photothermal property. Therefore, this result can confirm that the rambutan-like gold micro/nanostructure-chitosan composite has the photothermal property.

3.4 Fourier transformed infrared spectroscopy (FTIR)

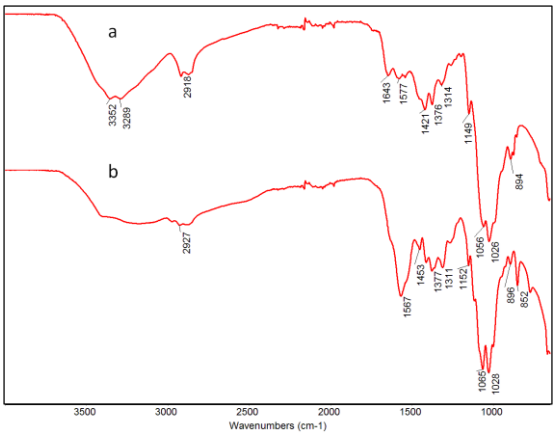

Fig. 5 – FTIR spectra of chitosan (a) and gold-chitosan composite (b)

The FTIR spectra of chitosan and gold-chitosan composite were shown in Figure 5. The broad band found at 3352 cm-1 is due to the overlapped of –OH and –NH group in chitosan. The band observed at 2918 cm-1 attributed to C-H bands. The band at 1643 cm-1 is due to amide band C-O stretching, along with N-H deformation. The 1577 cm-1 band is due to the characteristic peak of the NH2 group (see Fig. 5a). The peak of N-H bending at 1567 cm-1 was shifted with the increasing of intensity. The additional N-H bending peak at 852 cm-1 (see Fig. 5b) is due to the amino group in chitosan interaction with gold particle [22].

3.5 Mucoadhesive Property

The adsorption of mucin onto the rambutan-like gold micro/nanostructure chitosan composite was measured to evaluate the mucoadhesive property. The adsorptions of mucin were measured in three pH values, namely SGF (pH 1.2), 0.1 M sodium acetate buffer (pH 4.0) and SIF (pH 6.4). The adsorptions of mucin on the chitosan are 28.65 mg/g, 42.60 mg/g and 74.25 mg/g at pH 1.2, 4.0 and 6.4 respectively and the adsorptions of mucin on the gold-chitosan composite are 29.98 mg/g, 43.20 mg/g and 74.02 mg/g at pH 1.2, 4.0 and 6.4 respectively. The amount of mucin that was adsorbed onto the polymer decreased at lower pH values, being maximal at pH 6.4 and minimal at pH 1.2, because the degree of ionization of sialic acid and the different forms of the glycoprotein were influenced by the pH value of the environment [23]. The adsorptions of mucin values of gold-chitosan composite and chitosan were not significantly different. Hence, the addition of gold particles in chitosan did not decrease the mucoadhesive property of chitosan.

4. CONCLUSION

The rambutan-like gold micro/nanostructure chitosan composite increased the surrounding temperature up to 11.1 °C. The mucoadhesion ability of the obtained composite, expressed by its adsorption of mucin, were 29.98 mg/g, 43.20 mg/g and 74.02 mg/g at pH 1.2, 4.0 and 6.4 respectively.

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