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Chitosan wound dressing with hexagonal silver nanoparticles for hyperthermia and enhanced delivery of small molecules

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ABSTRACT

Chitosan films were synthesized with hexagonal silver nanoparticles (Ag NP). The unique shape and size of the Ag NP shift the optical absorption into the infrared. Stimulation of the nanoparticles with infrared light was used to generate heat and facilitate intracellular delivery of fluorescently-labeled dextran molecules. Chitosan films prepared with hexagonal or spherical Ag NP were characterized by optical and thermal analyses, and X-ray diffraction. There were found to be slight differences between how the chitosan molecular chains interface with the Ag NP depending upon shape of the nanoparticle. Viability of cells associated with dermal wound healing was evaluated on chitosan films prepared with hexagonal or spherical Ag NP, with both keratinocytes and fibroblasts having normal or moderately enhanced growth on films containing hexagonally-shaped nanoparticles.

1. Introduction

Chitosan is a linear polysaccharide that is often explored as a wound healing material due to its low cost, ease of processing, biodegradability, and antimicrobial characteristics [1–8].Thin films of chitosan are especially suitable as wound dressings for dermal burns [9–12]. More recently, the inherent antimicrobial nature of chitosan has been supplemented by the incorporation of silver within the film [13–16]. Silver has been used as a broad-spectrum antibiotic agent for millennia, and it is frequently used in tissues where high bacterial burden is problematic, such as burns. Many silver containing compounds such as silver nitrate and silver sulphadazine need to be reapplied to the wound repeatedly. Within the past few decades, there has been much interest in the use of silver nanoparticles (Ag NP) in lieu of ionic silver, and there are a number of wound dressings with embedded nanosilver that are currently available on the market, specifically Acticoat™ manufactured by Smith and Nephew and PolyMem Silver® manufactured by Ferris Manufacturing Corp [17–19]. Ag NP with a truncated triangular shapes or hexagonal shapes displaying the {1 1 1} face plane have been shown to exhibit a stronger antimicrobial effect [20,21]. One of the benefits of using anisotropic Ag NP displaying the {1 1 1} face plane is that the particles display both increased oxygen reactivity due to the presence of extra vertices and there is a greater enhancement of incident electromagnetic fields, leading to enhancement of the surface plasmon resonance effect which imparts heating potential [22]. Other studies indicate that not only the size and chemistry, but also the shape of nanoparticles can have profound effects on cell viability, exhibiting both anti-inflammatory and anti-angiogenic responses [23–32].

A challenge with healing dermal wounds is that they tend to remain below basal body temperature due to compromised vasculature and evaporative water loss [33–37]. Wound dressings inherently help retard heat loss, and can be improved by offering mild hyperthermia to thermally stimulate tissue recovery [38–40]. A unique way to induce hyperthermia is to capitalize on the potential of anisotropic Ag NP to generate heat when illuminated by infrared light via the phenomenon of surface plasmon resonance (SPR) [41–43]. SPR from metal nanoparticles has been shown to be useful for photothermal therapy for ablation temperatures (above 45 °C) [25,44]. In addition photothermal therapy can also induce a mild hyperthermia (less than 44° C) for enhancing the delivery of drugs [45–47]. The goal of the present work was to develop and characterize a novel chitosan wound dressing material with Ag NP capable of generating hyperthermia and assisting in the intracellular delivery of small molecules.

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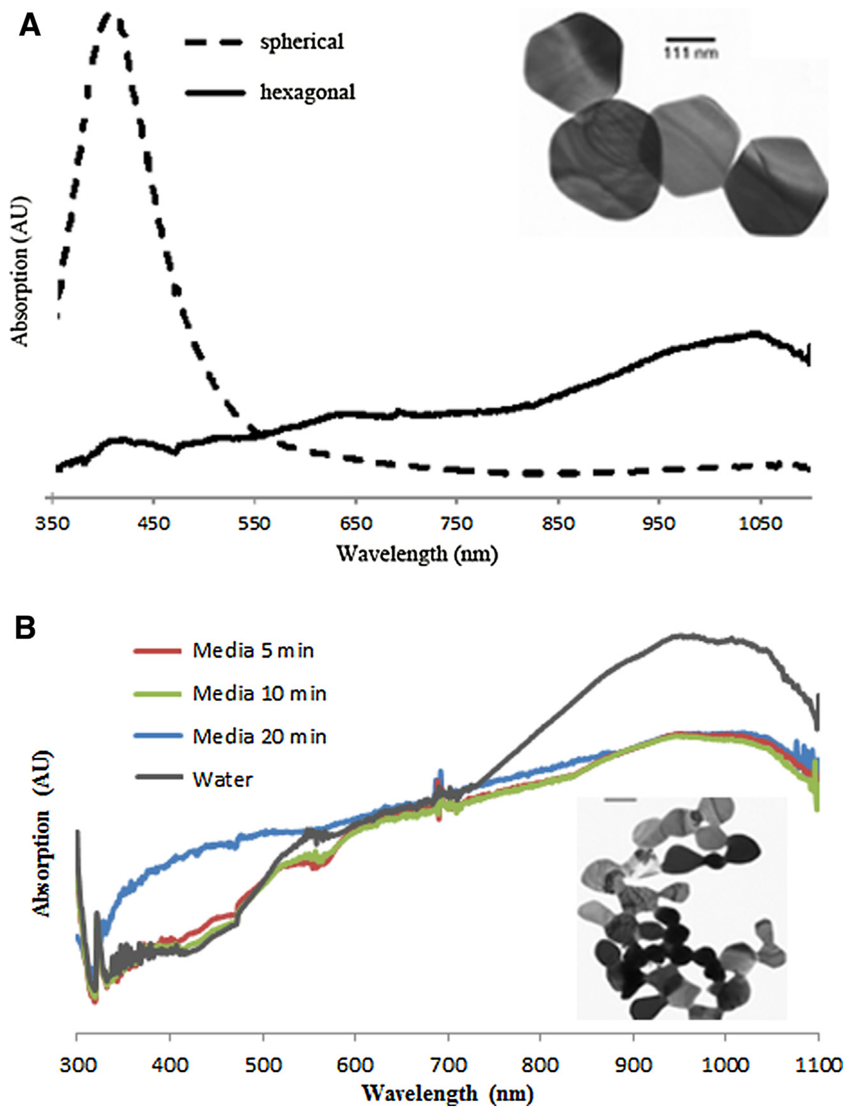

Fig. 1. (a) The optical absorption of spherical Ag NP in water shows an intense peak near 400 nm. Shape shifting of the nanoparticles into larger, hexagonal shapes red-shifts the absorption into the infrared. The insert shows a TEM image of the hexagonal Ag NP with an approximate edge length of about 100 nm (b) Stability of the hexagonal Ag NP in tissue culture media was evaluated over time by UV–vis. The insert TEM image shows that Ag NP tend to aggregate and exhibit a rounded corners when placed in cell culture media for twenty minutes.

2. Materials and methods

2.1. Materials

2.1.1. Cell lines

The CCD 1106 KERTr human keratinocyte cell line was purchased from American type culture collection (ATCC) and cultured in keratinocyte media (Gibco) supplemented with 0.05 mg/ml bovine pituitary extract, and 35 ng/ml human epidermal growth factor. Human epithelial palatal mesenchyme (HEPM) cells were purchased from ATCC and cultured in Eagle's minimum essential medium. HEPM cells were utilized to represent a fibroblast-like cell line. Hep G2 human hepatocellular carcinoma cell line was purchased from ATCC and cultured in Eagle's minimum essential medium. Peripheral blood mononuclear cells (PBMC) were isolated from donated heparinized porcine blood by centrifuging at 400 g for 30 min in conjunction with the density gradient medium, Histopaque 1119 (Sigma-Aldrich). They were suspended in Eagle's minimum essential medium during the experiments. All cell culture media were supplemented with 10% fetal bovine serum.

2.1.2. Reagents

Medium molecular weight chitosan (75–85% deacetylated), acetic acid, silver nitrate, sodium borohydride, ascorbic acid, trisodium citrate, fluorescein isothiocyanate (FITC)-labeled dextran (20,000 MW), and Rhodamine B isothiocyanate (Rhod)-labeled dextran (4000 MW) were all purchased from Sigma-Aldrich. CellTiter 96^{TM} (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay)) kit was purchased from Promega. Deionized water was obtained from a ThermoScientific Nanopure system.

2.2. Methods

2.2.1. Synthesis of silver

Synthesis of tri-sodium citrate (TSC) protected silver seed solution: 0.5 ml of 59 mM $AgNO_3$ and 1 ml of 34 mM TSC were added to 98 ml H_2O. 0.5 ml of a 0.02 M $NaBH_4$ solution (aged for 2 h) was added dropwise and then the solution was stirred for 1 h and aged for 24 h at room temperature, in the dark.

Synthesis of Ag NP: Ag NP were developed by a stepwise synthesis reaction to develop particles with optical absorption near