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Alginate-based composite sponge containing silver nanoparticles synthesized in situ

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ABSTRACT

Silver-based biomaterials have been developed in a variety of bactericidal applications, especially for wound dressings. In this study, silver nanoparticles (AgNPs) were synthesized in a sodium alginate solution and then the composite sponge containing AgNPs was prepared from the nanocolloid solution. The alginate-stabilized AgNPs had the mean negative zeta potential of −52.5 mV, suggesting that the surface charge prevents the nanoparticles from aggregating through electrostatic repulsion. The alginate–AgNPs composite sponge had a highly enhanced antimicrobial activity compared to the alginate sponge. In spite of excellent cytocompatibility of the alginate sponge, the viability of the cell treated with the alginate–AgNPs composite sponge extract decreased to 86% of the control. The amount of proinflammatory cytokines released from macrophages treated with the alginate–AgNPs composite sponge was reduced. For the preparation of AgNPs-embedded composites, alginate can be a potential candidate stabilizing AgNPs and providing synergistic antimicrobial and antiinflammatory activities with AgNPs.

1. Introduction

The development of materials containing substances with antimicrobial activity has been implicated in a variety of biomedical applications (Djoric & Burrell, 1998; Feng et al., 2000; Kraft, Hansis, Arens, Menger, & Vollmar, 2000). Silver or silver ions have long been known to exhibit powerful antimicrobial activity (Slawson, Van Dyke, Lee, & Trevors, 1992; Zhao & Stevens, 1998) and strong biocidal effects against as many as 16 species of bacteria including *Escherichia coli* (Spadaro, Berger, Barranco, Chapin, & Becker, 1974). For this reason, silver-based compounds have been used extensively in many bactericidal applications, including the formulation of dental resin composites (Yoshida, Tanagawa, & Atsuta, 1999; Yoshida, Tanagawa, Matsumoto, Yamada, & Atsuta, 1999), ion exchange fibers (Nonaka, Node, & Kurihara, 2000), and coatings of medical devices (Bosetti, Masse, Tobin, & Cannas, 2002; Hillyer & Albrecht, 2001; Schierholz, Lucas, Rump, & Pulverer, 1998; Schierholz, Beuth, & Pulverer, 1999).

The synthesis protocols for nanostructured materials have been developed (Andrew, Mirkin, & Letsinger, 2000; Duan, Cai, Luo, Li, & Lei, 2006). Recently, the preparation of ultrafine metal particles has attracted considerable attention because they can offer highly promising and novel options for a wide range of technical applications (Henglein, 1993; Hayward, Saville, & Aksay, 2000). The large specific surface area and high fraction of surface atoms on silver nanoparticles (AgNPs) leads to enhanced antibacterial activity compared to bulk silver metal. The preparation of a uniform and stable colloidal dispersion of AgNPs, free from agglomeration and precipitation, is a core technique. On the other hand, stable dispersions of AgNPs are generally short-lived in an aqueous media because they tend to agglomerate. For the preparation of metal particles, metal ions have often been reduced in protective colloids. Agglomeration is traditionally overcome through spontaneous adsorption on the particle surface of polymeric stabilizers. As stabilizers and protective media for colloids, water-soluble polymers have been used (Aslam, Fu, Su, Vijayamohanan, & Dravid, 2004; Brugger, Guendet, & Grätzel, 1981; Dunworth & Nord, 1954; Esumi, Sato, Torigoe, & Meguro, 1992; Esumi, Wakabayashi, & Torigoe, 1996; Hirai, 1979; Hirai, Nakao, & Toshima, 1979; Huang et al., 1996; Kattumuri et al., 2007; Kim, Park, Lee, Jeong, & Jon, 2007; Kiwi & Grätzel, 1979; Kuo, Chen, & Jao, 2005; Luo, Zhang, Zeng, Zeng, & Wang, 2005; Sun, Dong,

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& Wang, 2004; Sun, Dong, & Wang, 2005; Sun & Xia, 2002; Toshima, Harada, Yamazaki, & Asakura, 1992; Yamamoto & Nakamoto, 2003; Yonezawa & Toshima, 1995).

Sodium alginate, a linear copolymer of 1,4-linked β-D-mannuronate (M) and α-D-guluronate (G) residues, is isolated from marine algae and well dissolved in water due to negatively charged carbonyl group. Alginate is widely used in industry and medicine for many applications such as scaffolds and wound dressings due to low toxicity, favorable mechanical properties, and capacity for bioresorption of the constituent materials (Boontheekul, Kong, & Mooney, 2005; Kim et al., 2005). Alginate dressings are widely used in the treatment of exuding wounds. The ion exchange occurring between the calcium ions of the dressing and the sodium ions in the exudates results in the formation of a gel on the surface of the wound. This gel maintains an appropriate moist environment (Winter, 1962). Alginate non-woven fabrics are clinically applied for moisture wound healing.

Wound infection contributes to the formation of a nonhealing wound (Warrier & Burrell, 2005). Bacterial load on the surface of a wound amplifies and/or perpetuates a pro-inflammatory environment. Therefore, antimicrobial agents, such as silver-based formulations, are often used for wound healing. Silver-coated dressings are commonly used because they are effective in killing a broad range of bacteria. In this study, AgNPs were synthesized in a viscous solution of sodium alginate so that alginate–silver nanocolloid solution was prepared. Alginate–AgNPs composite sponge was then produced from the nanocolloid solution. The characteristics of alginate–AgNPs sponge were examined by transmission electron microscopy (TEM) and X-ray diffraction (XRD). Antimicrobial activity, cytotoxicity, and pro-inflammatory cytokine level were also checked to examine its potential for biomedical application as a wound dressing material.

2. Materials and methods

2.1. Synthesis of the alginate-stabilized AgNPs and preparation of the alginate–AgNPs composite sponge

1.0 g of sodium alginate (M_w ~500,000, FMC Biopolymer, Norway) was dissolved completely in 100 ml of deionized water for an hour. Subsequently, 2 ml of an aqueous silver nitrate ($AgNO_3$) (Junsei Chemical, Japan) solution (5.0 wt%) was dropped into the sodium alginate solution with magnetic stirring. After 1 h, a freshly prepared sodium borohydride ($NaBH_4$) (Duksan Pure Chemical, Korea) solution (2 ml, 1.0 wt%) was added to the mixture. A rapid color change to dark brown indicated the formation of AgNPs. The mixture was stirred for 3 h at room temperature to ensure that complete reduction had occurred. The solution was dialyzed using a dialysis tube with a molecular weight cut-off of 1000 Da (Membrane Filtration Products Inc., USA) with repeated water changes for more than 2 days to eliminate any unreacted chemical remains of $AgNO_3$, $NaBH_4$, and any salts formed during synthesis. The sodium alginate–silver nanocolloid was freeze-dried for 3 days. The freeze-dried matrix was cross-linked by dipping in 0.2 M $CaCl_2$ solution, washed thoroughly with deionized water, and freeze-dried again to prepare an insoluble the alginate–AgNPs composite sponge.

2.2. Characterization of AgNPs and alginate–AgNPs composite sponge

The morphology of AgNPs in the alginate-stabilized nanocolloid was examined by TEM (H-7600 TEM Hitachi, Japan). For TEM, one drop of the nanocolloid was placed onto a carbon-coated copper grid and dried at room temperature for 1 day. The mean hydrodynamic size of the silver nanocluster was measured by dynamic light scattering using a ZEN 3600 (Malvern Instruments, UK). Electrophoretic light scattering was used to detect the surface charge of the nanoparticles. This technique allowed a determination of the zeta potential of the nanoparticles. The measurements were conducted using a ZEN 3600 (Malvern Instruments, UK) at a fixed angle of 90°. The XRD pattern of the alginate–AgNPs sponge was acquired using an X'pert PRO MRD X-ray diffractometer (Philips, Netherland) with Cu-Kα radiation. The alginate–AgNPs composite sponge was coated with gold and its morphology was observed with an S-4200 Field Emission Scanning Electron Microscope (FE-SEM) (Hitachi, Japan).

2.3. Antimicrobial activity of the alginate–AgNPs composite sponge

The broth dilution method was used to determine the antimicrobial activity of the AgNPs synthesized in a sodium alginate solution. The sodium alginate–silver nanocolloid was centrifuged using a VS-30000i ultracentrifuge (Vision Scientific, Korea) at 24,000 rpm for 1 h to remove the excess of sodium alginate molecules and the precipitates were collected to obtain AgNPs. *Staphylococus aureus* (KCTC 3881) and *Klebsiella pneumoniae* (KCTC 2690) were incubated in a nutrient broth (8.0 g/l) at 37 °C for 24 h. Duplicate two-fold serial dilutions of each sample were added to the nutrient broth at fixed final concentrations and autoclaved at 120 °C for 15 min. Subsequently, 0.1 ml of each suspension was inoculated in a nutrient medium. After inoculation, the test tubes were incubated at 37 °C using a shaking incubator for 24 h. The antimicrobial activity was confirmed by checking the transparency of the samples.

The antimicrobial activity of the alginate–AgNPs composite sponges was determined according to the internationally recognized standard (Test Method 100-2004, 149, AATCC Technical Manual (2006)). Circular swatches (4.8 ± 0.1 cm in diameter) were cut from the alginate–AgNPs composite sponge (test) and the alginate sponge (control) and sterilized by ethylene oxide gas. The swatches prepared were inoculated in sterile petri dishes with *S. aureus* (KCTC 3881) and *K. pneumoniae* (KCTC 2690) and incubated in jars at 37 ± 2 °C for 24 h. Then, 100 ml of sterile distilled water was added in the jars. After vigorously shaking for 1 min, the serial dilutions were plated on nutrient agar and all plates were incubated for 48 h at 37 ± 2 °C. Antimicrobial activity was evaluated using the following formula.

$$\%\text{reduction} = \frac{B - A}{B} \times 100$$

where, A is the number of colonies recovered from the inoculated test swatches in the jar incubated over the desired contact period, and B is the number of colonies recovered from the inoculated control swatches in the jar incubated over the desired contact period.

2.4. Cytotoxicity of the alginate–AgNPs composite sponge

The cytotoxicity of sponge was estimated based on the international standard used for biological evaluation of medical device (ISO 10993-12 and ISO 10993-5) (Wiegand, Heinze, & Hipler, 2009). Briefly, 0.5 g of the sponge sterilized by ethylene oxide gas was incubated in 50 ml of Dulbecco's modified Eagle's medium (DMEM) (HyClone, USA) at 37 °C for 24 h under shaking. Afterwards, the sponge extracts were filtered to remove insoluble material residues and sterilized by passage through a 0.2 mm filter. Human fibroblasts were cultured in DMEM supplemented with 1% penicillin and 10% fetal bovine serum. The cells were cultured at 37 °C in 5% CO_2 atmosphere for 3–5 days and 1.0×10^6 cells were seeded into each well of 96-well culture plates. After 24 h, the culture medium was replaced by either fresh DMEM or sponge extracts. Cells were then further incubated for 24 h.

After replacing the old medium, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, USA) solution (5.0 mg/ml) was added to each well, and the cells were incubated for 4 h. The cell viability was obtained from the degree of mitochondrial reduction of MTT to formazan by succinic dehydrogenase. The absorbance at 570 nm was measured using a microplate reader (Molecular devices, USA). Cell viability (%) was expressed as the relative absorbance of the sample to that of the control (means ± SD). Differences were considered statistically significant at a level of $p < 0.05$.

2.5. Evaluation of proinflammatory cytokine levels

RAW 264.7 cells (murine macrophage cell line, Korean Cell Line Bank) were cultured in the growth medium at 37 °C. When the cells reached 80% confluence, they were trypsinized with 0.25% trypsin containing 1 μM ethylenediaminetetraacetic acid (Gibco, USA) and counted by a hemacytometer (Hausser Scientific, USA) prior to further use. Then the cells seeded in the sponges and stimulated by 1 μg/ml of lipopolysaccharide for 24 h. The pro-inflammatory cytokine level of each culture supernatant was quantified using an enzyme-linked immunosorbent assay kit (R&D system, USA) according to the manufacturer's protocol. Cytokine (%) was expressed as the relative absorbance of the sample to that of the control (means ± SD). Differences were considered statistically significant at a level of $p < 0.05$.

3. Results and discussion

3.1. Synthesis and preparation of AgNPs and alginate–AgNPs composite sponge

In comparison with other water-soluble polymers, polyelectrolytes can stabilize nanocolloids better due to electrostatic repulsion (Kim et al., 2011; Lee et al., 2011). Alginate is an anionic polymer with a high charge density. Alginate fragments containing carboxylic groups can provide nanoparticles with a negative charge and stability against agglomeration. Fig. 1 shows TEM images of the AgNPs synthesized in aqueous solution of sodium alginate. The AgNPs exhibited a spherical shape with a particle size of 3–15 nm. The particle size is one of the factors affecting the hydrodynamic size of the nanocluster. In addition, it is also important to consider how many particles are involved in a nanocluster. Fig. 2a shows the hydrodynamic size of the AgNPs clusters in sodium alginate solution. The mean hydrodynamic size of the silver nanoclusters was 79.7 nm. The alginate-stabilized AgNPs had the mean negative zeta potential of −52.5 mV (Fig. 2b), suggesting that the surface charge of the nanoparticles was dominated by the adsorption layer of alginate, preventing the nanoparticles from aggregating through electrostatic repulsion. The particle size of polyethyleneimine (PEI)-stabilized AgNPs had some dependence on the polymer concentration (Lee et al., 2011). At relatively high PEI concentrations (>0.04 wt%), several large particles were produced. The role of polymer molecules as a stabilizer was weakened at high concentrations, such that nucleation and growth proceeded rapidly, resulting in the formation of large particles. In addition, the mean hydrodynamic size of the nanoclusters also increased at high PEI concentrations. Although the AgNPs were produced in the viscous solution of alginate (1.0 wt%), which was inevitable for the preparation of a sponge, a stable nanocolloid were formed in this study. This suggests that alginate is an excellent stabilizer for AgNPs which can be synthesized in situ when producing fibers, films, and sponges from concentrated solutions of alginate. Generally, AgNPs-containing polymer composites are fabricated from physical mixtures of polymer solutions and silver nanocolloids which were previously synthesized or purchased.

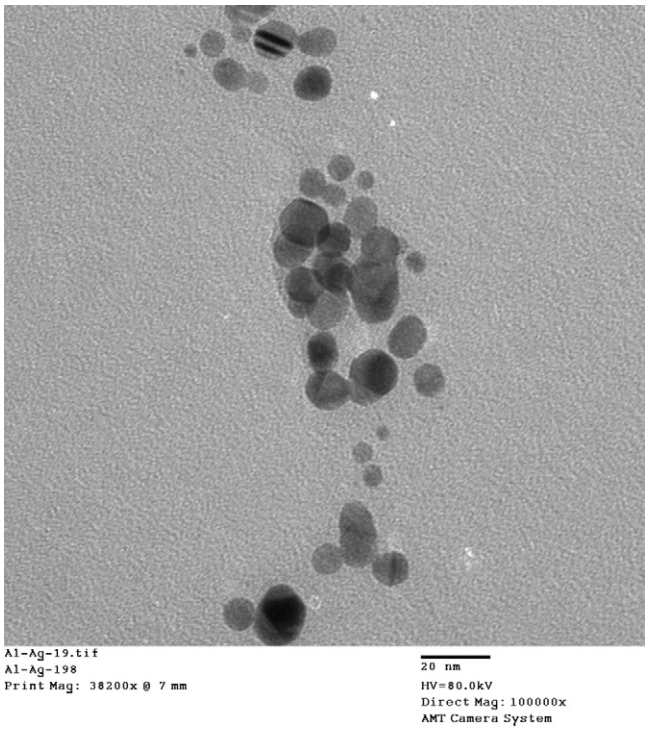

Fig. 1. TEM image of the AgNPs synthesized in 1.0 wt% sodium alginate solution.

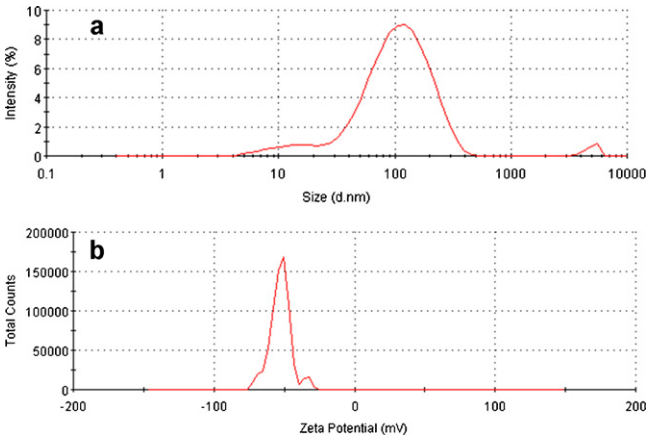

Fig. 2. Hydrodynamic cluster size (a) and zeta potential (b) of the AuNPs.

From the stable alginate–silver nanocolloid solution, the alginate–AgNPs composite sponge was prepared. Fig. 3 shows XRD patterns of bulk silver powder and the alginate–AgNPs composite sponge. Several distinct diffraction peaks at approximately 38.1°, 44.3°, 64.4° and 77.5° were assigned to reflections from the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of the silver crystal, respectively, which confirmed the presence of silver (Brugger et al., 1981). The FE-SEM image of the alginate–AgNPs composite sponge is shown in Fig. 4. The open structure which was found in the alginate sponge mostly disappeared in the alginate–AgNPs composite sponge. However, the hydrophilic nature of alginate would enable the application of alginate–AgNPs sponges as moisture wound dressings. In fact, water absorption of the alginate–AgNPs composite sponge was comparable to that of the alginate sponge.

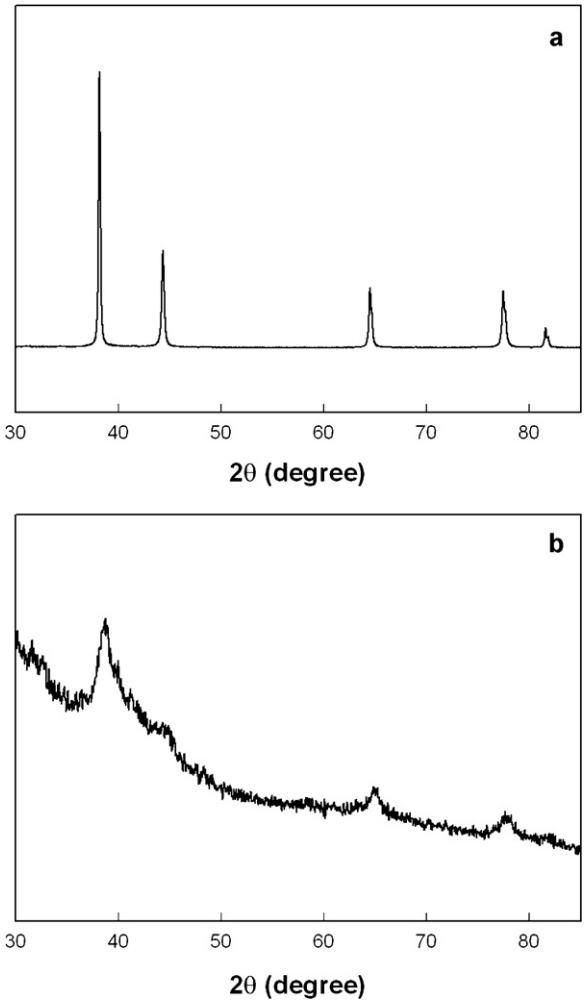

Fig. 3. X-ray diffraction patterns of the bulk silver powder (a) and the alginate–silver composite sponge (b).

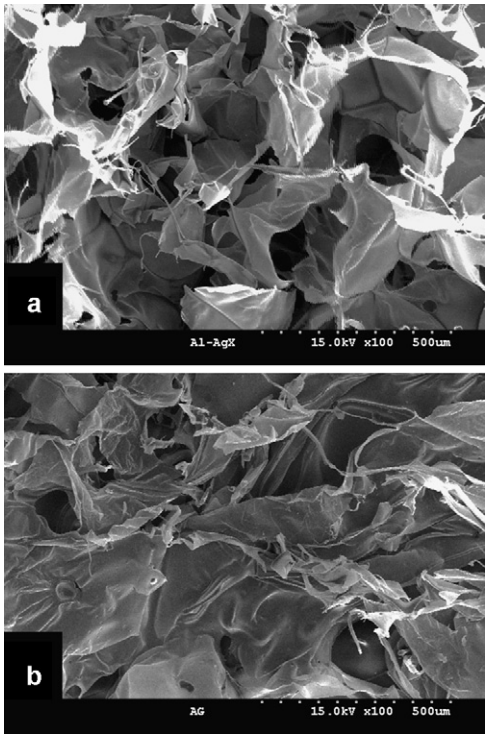

Fig. 4. FE-SEM images of the alginate sponge (a) and the alginate–silver composite sponge (b).

3.2. Antimicrobial activity of alginate–AgNPs composite sponge

Silver in an aqueous solution releases silver ions, which are biologically active and have bactericidal effects (Chaloupka, Malam, & Seifalian, 2010; Cho, Park, Osaka, & Park, 2005; Lok et al., 2007; Morones et al., 2005; Sondi & Salopek-Sondi, 2004). In the case of AgNPs, they interact extensively with the bacteria cell walls and cause lysis. AgNPs release silver ions, which make an additional contribution to the bactericidal effect. Poly(*N*-vinylpyrrolidone) (Cho et al., 2005) and PEI-stabilized AgNPs were reported to show complete inhibition against *S. aureus* and *E. coli*. They can interact with the cell walls of the bacteria due to the positively charged molecules bound to the surfaces of the nanoparticles. On the other hand, the AgNPs stabilized with sodium dodecylsulfate (SDS) have no antibacterial activity because the negatively charged SDS interferes with the absorption of microbes to the surface of the AgNPs or silver ions (Cho et al., 2005). In this study, *S. aureus* and *K. pneumonia* was inhibited completely by the AgNPs synthesized in a sodium alginate solution over the concentration of 200 ppm, showing the antibacterial effect of the original AgNPs. When AgNPs are incorporated in polymer-based matrices such as fibers, films, gels, and sponges, more things should be taken into consideration. *S. aureus* and *K. pneumonia* growth was inhibited significantly by incorporation of AgNPs in the alginate sponge (Table 1) although the alginate-stabilized AgNPs had a negative zeta potential. Alginate dressings are widely used in the treatment of exuding wounds enhancing the healing process (Warrier & Burrell, 2005). The alginate fibers absorb water and swell, the spaces between the fibers are closed, and any bacteria that are carried in the wound exudates are trapped in the wound dressing (Qin, 2005). In comparison with polyester fabric (control) which is known not to inhibit microbial growth, the commercialized alginate non-woven dressing sufficiently inhibited the growth of Gram-positive and Gram-negative bacteria by immobilizing them within its fibrous matrix (Wiegand et al., 2009). In the case of alginate–AgNPs composite sponge, such "swelling" and "trapping" of alginate-based matrix helps microbes and AgNPs contact each other to provide a synergistic bactericidal effect. For this reason, the alginate–AgNPs composite sponge had a highly enhanced antimicrobial activity as compared with the alginate sponge in this study.

3.3. Cytotoxicity of the alginate–AgNPs composite sponge

It is well known that alginate is a biocompatible polymer and the treatment of cells with the extracts of alginate dressings

Table 1
Antimicrobial activity of alginate–AgNPs sponge.

	S. aureus		*K. pneumoniae*	
	Number of colonies	% reduction	Number of colonies	% reduction
Alginate sponge (control)	1425	–	1175	–
Alginate–AgNPs sponge (test)	7	99.51	28	97.62

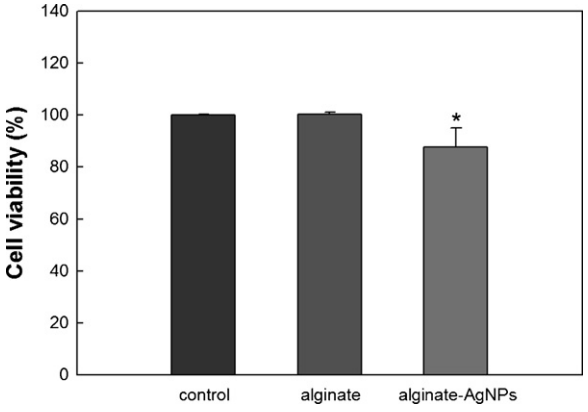

Fig. 5. Relative viabilities of human fibroblasts treated with the alginate sponge extract and the alginate–silver composite sponge extract ($n = 3$, $^{*}p < 0.05$).

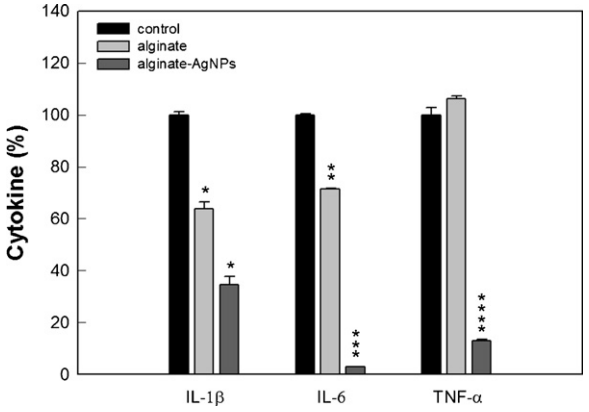

Fig. 6. Relative amounts of proinflmmatory cytokines released by RAW 264.7 cells treated with the alginate sponge and the alginate–silver composite sponge ($n = 3$, $^{*}p < 0.005$; $^{**}p < 0.0005$; $^{***}p < 0.00001$; $^{****}p < 0.001$).

had little negative influence on cell viability and cell proliferation (Wiegand et al., 2009). Extract of alginate sponge showed the comparable cell viability to control as shown in Fig. 5. On the other hand, AgNPs are cytotoxic to several cells (Braydich-Stolle, Hussain, Schlager, & Hofmann, 2006; Carlson et al., 2008; Foldbjerg et al., 2009; Hsin et al., 2008; Hussain, Hess, Gearhart, Geiss, & Schlager, 2005). The viability of C18-4 spermatogonial stem cells cultured for 48 h was approximately 20% with 10 ppm AgNPs synthesized using a gas phase process. In the case of PVP-coated AgNPs, the viability of THP-1 monocytes treated with the nanoparticles for 24 h was only 6% at 5 ppm. The cytotoxic effects of PEI and PEI-stabilized AgNPs were also confirmed depending on concentration (Lee et al., 2011). Considerable evidence suggests that AgNPs are cytotoxic through their interaction with the mitochondria and induction of the apoptosis pathway via the production of reactive oxygen species (ROS). A drastic increase in ROS levels suggests that oxidative stress is an important mediator of the cytotoxicity caused by AgNPs. AgNPs-containing wound dressings also had cytotoxicity. Chitin–AgNPs (Madhumathi et al., 2010) and chitosan–polyphosphate–AgNPs (Ong, Wu, Moochhala, Tan, & Lu, 2008) composite wound dressings exerted severe cytotoxicity against fibroblasts. The non-woven dressings of alginate–AgNPs and alginate–ionic silver revealed cytotoxic effects depending on concentration of the extract (Wiegand et al., 2009). In spite of excellent cytocompatibility of the alginate sponge, the incorporation of AgNPs in the alginate sponge had a cytotoxic effect on the human fibroblast. The viability of the cell treated with the alginate–AgNPs composite sponge extract decreased to 86% of the control.

3.4. Proinflammatory cytokine levels

The wound healing process is separated into three overlapping phases: (1) inflammation, (2) re-epithelialization and granulation tissue formation, and (3) matrix formation and remodeling (Clark, 1995; Martin, 1997). It has long been speculated that proinflammatory cytokines play an important role in wound repair. The invasion of monocytes into the wound tissue and their differentiation into macrophages are essential for normal repair (Leibovich & Ross, 1975). Activated macrophages can produce several proinflammatory cytokines, including interleukins l alpha and beta (IL-1α and IL-1β) (March et al., 1985), interleukin 6 (IL-6) (Delgado, McManus, & Chambers, 2003), and tumor necrosis factor alpha (TNF-α) (Beutler & Cerami, 1986; Leibovich et al., 1987; Nathan, 1987; Wong & Wahl, 1989). These cytokines exert a series of biological activities which might be important for wound healing. IL-1 is a pleiotropic proinflammatory cytokine responsible for fundamental functions in wound healing, inflammation, and host antitumor responses (Dinarello, 1996; Meghji, Qureshi, Henderson, & Harris, 1996; Wustrow, 1994). A small amount of IL-1 is necessary for host defense and wound healing, whereas overproduction of IL-1 can hinder the early phase of wound healing (Angele et al., 1999; Trengove, Bielefeldt-Ohmann, & Stacey, 2000). TNF is another central mediator of inflammatory responses, playing important roles in antimicrobial defense, wound healing, and defense against malignant disorders (Kohno et al., 1990). TNF-α can stimulate apoptosis-related events (Declercq, Denecker, Fiers, & Vandenabeele, 1998). Although small amounts of TNF are necessary for host defense against infection, overproduction of TNF can be detrimental. TNF-α, associated with chronic inflammation, is secreted by macrophages and mast cells. The levels of these cytokines are profoundly elevated in chronic wounds (Barrick, Campbell, & Owen, 1999; Mast & Schultz, 1996; Trengove et al., 1999).

It was reported that alginate is able to bind proinflammatory cytokines such as TNF-α in a time-dependent manner (Wiegand et al., 2009). For this reason, the amounts of IL-1β and IL-6 released by macrophages treated by the alginate sponge were lowered as shown in Fig. 6. However, the level of TNF-α was increased in this case. The cytokine-binding effect of alginate was not sufficient enough to reduce the amount of TNF-α. On the other hands, the levels of proinflammatory cytokines including TNF-α were lowered remarkably in the case of the alginate–AgNPs composite sponge. The TNF-α binding capacity of nanosilver-containing alginate non-woven dressing was slightly lower than that of alginate non-woven dressing without nanosilver (Wiegand et al., 2009). In other words, nanosilver had little effect on the binding capacity of proinflammatory cytokines. This suggests that the proinflammatory cytokine levels were decreased by incorporation of AgNPs in alginate sponge due to inhibition of release from macrophages. Unfortunately, for AgNPs alone, inflammatory responses were observed. Significant levels of proinflammatory cytokines released by macrophages were detected for AgNPs (Carlson et al., 2008). Cytokines including IL-1 and IL-6 were increased in mice by repeated oral administration of AgNPs (Park et al., 2010). However, reverse trends were found when AgNPs were embedded in polymer matrices. Nanocrystalline silver-coated dressings have been reported to modulate the inflammatory response at or above the level of TNF-α expression, thus generating an anti-inflammatory effect. (Warrier & Burrell, 2005; Nadworny, Wang, Tredget, & Burrell, 2008). AgNPs are well known to induce apoptosis (Carlson et al., 2008; Foldbjerg et al., 2009). Nevertheless, with only AgNPs, the proinflammatory cytokine levels cannot be decreased because there is no way to eliminate cytokines released although macrophages may die by apoptosis. With only Alginate,

the amount of cytokines cannot be reduced significantly because macrophages continue to release cytokines though alginate binds the released cytokines to some degrees. Hence, it is suggested that the synergistic effect of AgNPs and alginate is required to reduce the proinflammatory cytokine levels. The amounts of proinflammatory cytokines released by macrophages treated with the alginate–AgNPs composite sponge could be lowered significantly due to both the elimination of inflammatory cells by apoptotic induction of AgNPs and the cytokine-binding capacity of alginate.

4. Conclusions

AgNPs were synthesized in a sodium alginate solution and then the composite sponge containing AgNPs was prepared from the stable nanocolloid solution. The alginate-stabilized AgNPs had the mean negative zeta potential, suggesting that the surface charge of the nanoparticles was dominated through the adsorption layer of alginate, preventing the nanoparticles from aggregating by electrostatic repulsion. Alginate is considered an excellent stabilizer for AgNPs which can be synthesized in situ when producing fibers, films, and sponges from concentrated solutions of alginate. Hence, synthesis of AgNPs and preparation of AgNPs-embedded alginate matrices can be conducted through a batch-process. *S. aureus* and *K. pneumonia* growth was inhibited significantly by incorporation of AgNPs in the alginate sponge. "Swelling" and "trapping" of alginate-based sponge may help microbes and AgNPs contact each other to provide a synergistic bactericidal effect. In spite of excellent cytocompatibility of the alginate sponge, the incorporation of AgNPs in the alginate sponge had a cytotoxic effect on the human fibroblast. The amount of proinflammatory cytokines released from macrophages was reduced remarkably for the alginate–AgNPs composite sponge. It is suggested that the proinflammatory cytokine levels are lowered due to both the elimination of inflammatory cells by apoptotic induction of AgNPs and the cytokine-binding capacity of alginate. In nanosilver-based wound dressings, synergistic effects of AgNPs and polymer matrix accommodating antimicrobial and antiinflammatory activities are very important. Alginate is a potential candidate for the preparation of AgNPs-embedded composite, playing roles of stabilizing AgNPs, trapping bacteria, and binding proinflammatory cytokines.

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