J Mater Sci: Mater Med (2010) 21:807–813
DOI 10.1007/s10856-009-3877-z

Development of novel chitin/nanosilver composite scaffolds for wound dressing applications

K. Madhumathi · P. T. Sudheesh Kumar · S. Abhilash · V. Sreeja · H. Tamura · K. Manzoor · S. V. Nair · R. Jayakumar

Received: 20 May 2009 / Accepted: 17 September 2009 / Published online: 3 October 2009

Abstract Antibiotic resistance of microorganisms is one of the major problems faced in the field of wound care and management resulting in complications like infection and delayed wound healing. Currently a lot of research is focused on developing newer antimicrobials to treat wounds infected with antibiotic resistant microorganisms. Silver has been used as an antimicrobial agent for a long time in the form of metallic silver and silver sulfadiazine ointments. Recently silver nanoparticles have come up as a potent antimicrobial agent and are finding diverse medical applications ranging from silver based dressings to silver coated medical devices. Chitin is a natural biopolymer with properties like biocompatibility and biodegradability. It is widely used as a scaffold for tissue engineering applications. In this work, we developed and characterized novel chitin/nanosilver composite scaffolds for wound healing applications. The antibacterial, blood clotting and cytotoxicity of the prepared composite scaffolds were also studied. These chitin/nanosilver composite scaffolds were found to be bactericidal against *S. aureus* and *E. coli* and good blood clotting ability. These results suggested that these chitin/nanosilver composite scaffolds could be used for wound healing applications.

K. Madhumathi · P. T. Sudheesh Kumar · S. Abhilash · V. Sreeja · K. Manzoor · S. V. Nair · R. Jayakumar (✉)
Amrita Centre for Nanosciences, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham University, Kochi 682 026, India
e-mail: rjayakumar@aims.amrita.edu; jayakumar77@yahoo.com

H. Tamura
Faculty of Chemistry, Materials and Bioengineering and High Technology Research Centre, Kansai University, Osaka 564-8680, Japan

1 Introduction

Nanomedicine is an upcoming field, which integrates nanotechnology and medicine to develop newer drugs, drug delivery systems, biosensors and molecular diagnostic systems. Nanotechnology deals with nanoparticles with unique optical, chemical and mechanical properties. The particular importance in medical field are silver nanoparticles, which show excellent antimicrobial activity [1]. The use of silver as an antimicrobial agent is not new. Over centuries metallic silver and its salts like silver nitrate has been used to treat burns, wounds, abscesses and fistulas [2, 3]. The use of silver minimized as antibiotics came into prominence during last century. Silver was even combined with sulfonamide, an antibiotic to form silver sulfadiazine ointment and was used to treat burns [4, 5].

Silver returned to prominence recently due to the emergence of antibiotic resistant bacteria as a result of overuse of antibiotics [6]. These drug resistant microorganisms pose a grave danger to the health of patients, as they do not respond to any treatment. Silver is an effective antibacterial agent due to its polycationic nature. Silver containing materials are already used as prostheses, catheters, vascular grafts and as wound dressings [7]. Silver in the form of nanoparticles is very effective antimicrobial and is used in wound dressings [8, 9]. Silver nanoparticles exert their antibacterial activity by interacting with the sulfur containing proteins present in bacterial cell membrane as well as with phosphorus containing DNA [10–13]. They also attack the bacterial respiratory chain [10–13]. The nanoparticles release silver ions within the cells enhancing their bactericidal activity [14]. The surface area to volume ratio of silver nanoparticles is high providing better contact with bacteria due to which they exert

antibacterial activity even at low concentrations [10–13]. Silver has been incorporated into different natural and synthetic polymers like cellulose [15–17], chitosan [18, 19], polystyrene, acrylic acid [20], PCL [21] and gelatin [22] etc.

Polymeric materials like polyurethane [23], chitin and its derivatives are widely used as wound dressings due to their desired properties. In our study, we have incorporated silver nanoparticles into chitin scaffolds for wound dressing applications. Chitin has properties like wound healing ability, antimicrobial and anti-inflammatory activity [24, 25]. It is composed of poly (*N*-acetyl glucosamine) units. Chitin and its derivatives like chitosan are already being used as scaffolds in tissue engineering [26–29], drug delivery vehicles [30–32] and as wound dressings [33, 34]. The wound healing ability and antibacterial activity of chitin can be enhanced by the addition of silver nanoparticles [35]. Thus, the chitin/nanosilver composite scaffolds will function as ideal wound dressings.

2 Materials and methods

2.1 Materials

Chitin (Degree of Acetylation-72.4%) was purchased from KYOWA TECNOS Co. Ltd. $CaCl_2$ and Methanol were purchased from Qualigens. Silver Nitrate was obtained from S.d.FINE-CHEM and sodium citrate was purchased from MERCK. *Staphylococcus aureus* (ATCC 25923) and *E. coli* (ATCC 25922) strains were obtained from Microbiology lab of Amrita Institute of Medical Sciences, Kochi, India. The mouse fibroblast (L929) was provided by National Center for Cell Sciences, Pune, India.

2.2 Preparation of chitin/nanosilver composite scaffolds

2.2.1 Preparation of chitin hydrogel

Chitin hydrogel was prepared by the following method. 5 g of chitin was added to a mixture of saturated $CaCl_2$/methanol to prepare 0.5 w/v chitin hydrogel. This mixture was stirred for 2 days after which the undissolved chitin was removed by filtration. This was followed by addition of water (to break the bond between chitin and $CaCl_2$) and stirring for 2 h. The hydrogel was kept aside undisturbed for a day following which the water (containing $CaCl_2$) was removed by suction filtration. The hydrogel was then dialyzed for 2 days to remove impurities, methanol and remaining $CaCl_2$.

2.2.2 Preparation of nanosilver solution

Nanosilver solution was prepared by the well-known Turkevich method as described in the literature [36]. This method is based on the reduction of silver nitrate to metallic silver nanoparticles using sodium citrate as the reducing agent. 125 ml solution of 1 mM silver nitrate in water was made and was heated until it begins to boil. 5 ml of 1% sodium citrate solution was added as soon as boiling commences. This reduction occurs at high temperature and nanosilver formation is observable by a visible color change of the solution to pale yellow. The solution was then stirred until it has cooled to room temperature. By this method, 125 ml of 1 mM nanosilver solution was obtained.

2.2.3 Preparation of composite scaffolds

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles are in the range of 1.56–6.25 and 12.5 μg/ml, respectively [37]. Chitin/nanosilver composite scaffolds were prepared by adding different amounts of nanosilver solution to chitin hydrogel. 10 (sample A), 15 (sample B) and 20 (sample C) ml of 1 mM nanosilver solution was added to 40 g of chitin gel, stirred well for 15 min followed by lyophilization to obtain chitin/nanosilver composite scaffolds having 54, 80 and 107 μg silver nanoparticles per cm^2. However, half of the silver nanoparticles only come to contact with the bacteria in the plate containing broth. So, we suggest that this much concentration will be needed per cm^2 of the wound. Figure 1 shows the image of composite scaffolds while Table 1 shows the concentration (%) of nanosilver in these composite scaffolds.

Fig. 1 Picture showing chitin scaffold (*left*) and chitin/nanosilver composite scaffold (*right*)

Table 1 Concentration of nanosilver in chitin scaffolds

Scaffolds	Amount of chitin gel (in grams)	Volume of 1 mM nanosilver solution (in ml)	Amount of nanosilver present (in mg)	Concentration of nanosilver (%) in each scaffold
A	40	10	1.07	0.003
B	40	15	1.6	0.004
C	40	20	2.14	0.005

2.3 Antibacterial activity studies

The chitin/nanosilver composite scaffolds were tested for their antibacterial activity using disc diffusion method. *E. coli* and *S. aureus* were taken as model gram-negative and gram-positive bacteria. The test specimens were chitin and chitin/nanosilver composite scaffolds, which were compressed into pellets of 1 cm diameter, and UV sterilized for 24 h while Gentamycin (10 mg) was taken as control. The specimens along with control were placed in MHA medium inoculated with *E. coli* and *S. aureus.* The agar plates were then incubated for 24 h at 37°C after which the 'Zone of Inhibition' was measured for each specimen.

2.4 Blood clotting studies

Blood clotting studies were done according to the reported literature [38]. Blood was drawn from human ulnar vein using 10 ml BD discardit^{TM} II sterile syringe and mixed with anticoagulant agent Acid citrate dextrose at a ratio of 9:1. The blood clotting efficiency of chitin/nanosilver composite scaffolds were compared with chitin scaffold and a commercial collagen scaffold (Kollagen^{TM}). Triplicate samples were used for this study and blank 50 ml tubes without scaffold were used as control. 4.5 ml of whole blood was taken and 0.5 ml of Acid Citrate Dextrose (ACD) was added to it. 200 µl of this blood sample was added to each scaffold placed in a 50 ml tube, which was followed by the addition of 20 µl of 0.2 M $CaCl_2$ solutions to begin blood clotting. Then these scaffolds were incubated at 37°C for 10 min. 25 ml distilled water was then added drop wise without disturbing the clot. Subsequently, 15 ml of solution was taken from the tubes and was centrifuged at 1000 rpm for 1 min. The supernatant was collected for each sample and kept in 37°C for 1 h. 200 µl of this solution was added to each well of a 96-well plate. The optical density was measured at 540 nm using a plate reader (BioTek PowerWave XS).

2.5 Cytotoxicity studies

Cytotoxicity of the chitin/nanosilver composite scaffolds was evaluated by indirect cytotoxicity test using MTT assay. L929 Mouse fibroblasts were seeded into each well of a 96 well plate at a density of 1×10^4 cells/well. Triplicates of each sample were taken and UV sterilized for a day following which they were incubated in serum containing media for 24 h at 37°C. 100 µl of the media from each sample was taken and transferred into each well. MTT [3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium] assay was used to quantify the live cells. 5 mg of MTT (Sigma) was dissolved in 1 ml of PBS and filter sterilized. 10 µl of the MTT solution was further diluted to 100 µl with 90 µl of serum–free phenol red free minimum essential medium. 100 µl of the above solution was added to each well and incubated to form formazan crystals by mitochondrial dehydrogenases. After 4 h of incubation at 37°C, 100 µl of the solubilization solution (10% Triton X-100, 0.1 N HCl and isopropanol) was added in each well plate to dissolve the formazan crystals. The optical density of the solution was measured at a wavelength of 570 nm using an Elisa plate reader [Beckmann Coulter DTX 880].

2.6 Characterization

The nanosilver solution was characterized using TEM (JEOL Ltd., JEOL-3010). The chitin/nanosilver composite scaffolds were characterized using SEM (JEOL Ltd., JEOLJSM-6490LA), XRD (PANalytical X'Pert PRO) and FT-IR (PerkinElmer Co., SPECTRUM RX1, FT-IR) instruments.

3 Results and discussion

3.1 Characterization

3.1.1 TEM studies

The nanosilver solution was characterized using TEM for analyzing the particle size and morphology. Figure 2 shows the TEM image of the spherical silver nanoparticles. The silver nanoparticles showed spherical morphology and well dispersed. The size of the silver nanoparticles in nanosilver solution was found to be 5 nm in diameter from the TEM analysis.

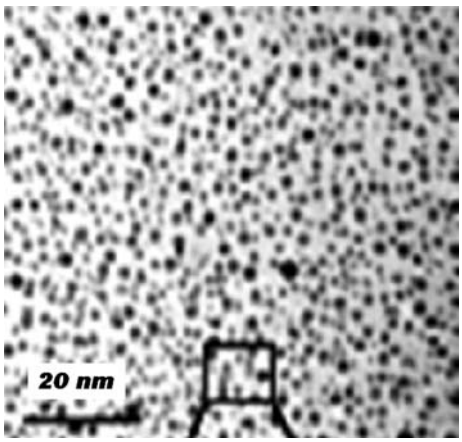

Fig. 2 TEM image of nanosilver solution prepared by Turkevich method showing well-dispersed spherical particles of 5 nm diameter

3.1.2 SEM studies

Figure 3 shows the surface morphology of chitin/nanosilver composite scaffold. Since silver nanoparticles are very small (5 nm) and well dispersed they are not visible in SEM. The scaffold appears as a very porous structure with smooth surface morphology.

3.1.3 FT-IR studies

Figure 4 shows the FT-IR spectrum of chitin (control) and chitin/nanosilver composite scaffolds. There are no observable peak shifts in chitin/nanosilver composite scaffold compared to chitin scaffold. This may be because there is no chemical interaction between chitin and nanosilver but only a physical adsorption of silver nanoparticles on chitin.

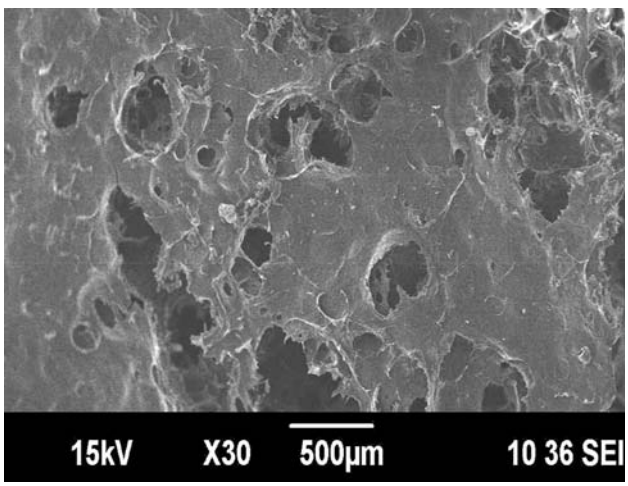

Fig. 3 SEM image of chitin/nanosilver composite scaffold

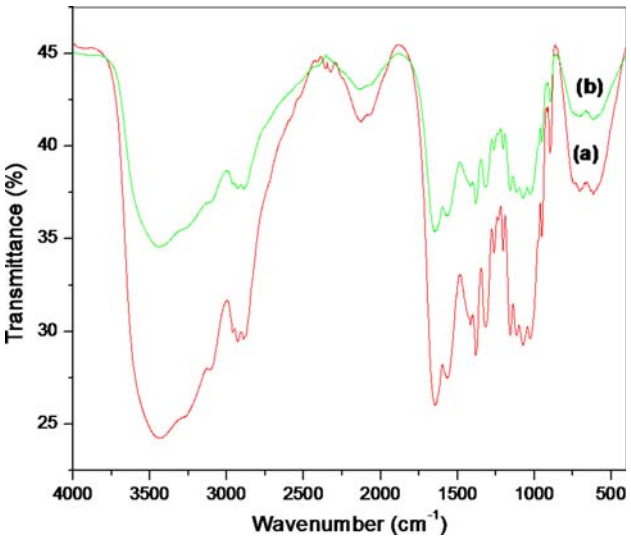

Fig. 4 FT-IR spectra: **a** chitin scaffold **b** chitin/nanosilver composite scaffold

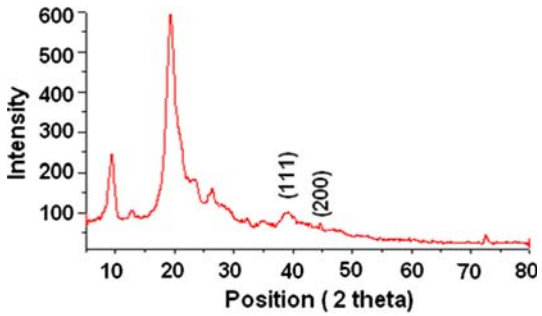

Fig. 5 XRD spectra of chitin/nanosilver composite scaffold

3.1.4 XRD studies

Figure 5 shows the XRD image of chitin/nanosilver composite scaffolds. The peak at $2\theta = 20°$ can be attributed to chitin [39]. Peaks at 39.8° and 44.5° can be attributed to (111) and (200) crystalline planes of silver [40].

3.2 Antibacterial activity studies

Figure 6a and b show the results of antibacterial activity studies. Figure 6a shows bactericidal efficiency of chitin/nanosilver composite scaffolds against gram-positive *S. aureus* while Fig. 6b shows activity against gram-negative *E. coli*. It can be seen that the inhibition zone is higher in *E. coli* than *S. aureus* indicating higher susceptibility of gram-negative bacteria to nanosilver [41, 42]. This may be because gram-positive bacteria are protected by a thick peptidoglycan wall, which limits the penetration of silver nanoparticles [10]. It can also be seen that as the nanosilver concentration increased, the zone of inhibition

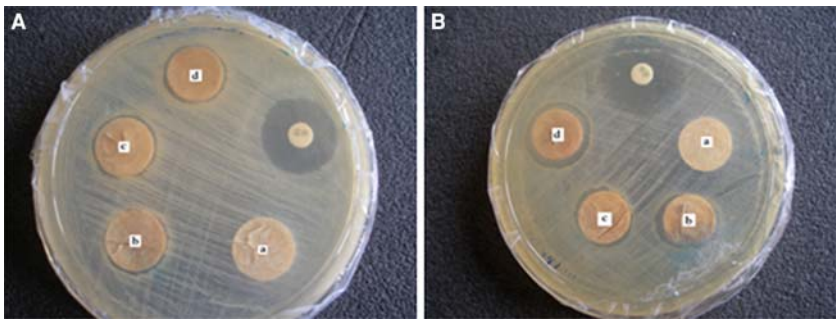

Fig. 6 **A** and **B** Antibacterial activity of chitin/nanosilver composite scaffolds against **A** *S. aureus* and **B** *E. coli*. The specimens used were (*a*) Chitin (*b*) Sample **A** (*c*) Sample **B** and (*d*) Sample **C** along with Gentamycin (10 mg) control

Table 2 Diameter of 'zone of inhibition' of chitin/nanosilver composite scaffolds against *S. aureus* and *E. coli*

Samples	Diameter of zone of inhibition (mm)	
	S. aureus	*E. coli*
Gentamycin control (10 mg)	21 ± 1.2*	23 ± 1.4*
Chitin	–	–
Sample A	16 ± 1.4*	19 ± 2*
Sample B	17 ± 2.2*	20 ± 1.8*
Sample C	18 ± 1.8*	21 ± 1.3*

The data are expressed as mean ± (standard deviation (SD)) of three independent experiments. * Denotes a statistically significant difference compared to chitin control ($P < 0.05$)

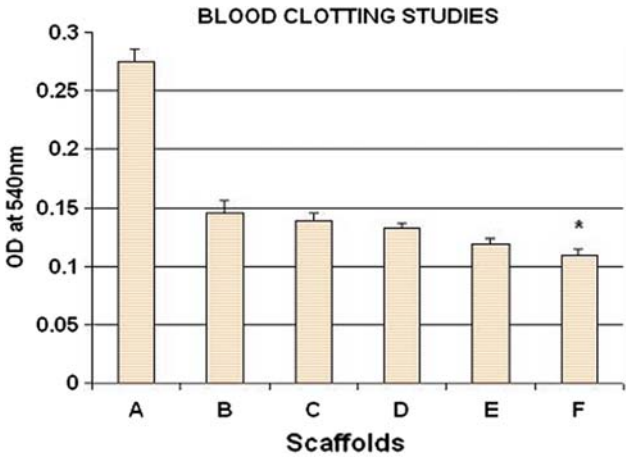

Fig. 7 Blood-clotting efficiency of chitin/nanosilver composite scaffolds **a** control (Without scaffold), **b** collagen, **c** chitin control, **d** sample A, **e** sample B and **f** sample C. *Asterisk* (*) denotes a statistically significant difference compared to positive control (collagen) ($P < 0.05$)

also increased. These results show that the antibacterial activity is due to the presence of nanosilver in chitin scaffolds. The interaction between chitin and nanosilver will be physical adsorption of silver nanoparticles on chitin and the release of silver will be controlled. Ong et al. [18] reported that silver release per cm^2 dressing was 4.4 ± 0.6 μg at 2 h, increasing to 19.2 ± 2.5 μg at 24 h, to a maximum of 23.9 ± 2.1 μg of dissolved silver by 48 h. Table 2 gives the values of 'zone of inhibition'. ($n = 3$ at each time point).

3.3 Blood clotting studies

Figure 7 shows the result of blood clotting experiment. Blood clotting ability of the chitin/nanosilver composite scaffolds were significantly higher than chitin scaffold and a commercial collagen dressing. It can be seen that the OD values at 540 nm was highest for control (without scaffold) followed by commercial collagen scaffold, chitin scaffold, chitin/nanosilver composite scaffolds A, B and C in that order. A decrease in OD values indicates the higher blood clotting efficiency. Thus from the figure, it can be seen that with increase in the amount of nanosilver the blood clotting efficiency was increased. Chitin as such is hemostatic since being a cationic polymer it tends to aggregate negatively charged red blood cells. This activity is further enhanced by the addition of silver nanoparticles. Silver denatures the anticoagulant proteins and affects the intrinsic pathway of blood coagulation by producing a shortened clotting time [43]. ($n = 3$ at each time point).

3.4 Cytotoxicity studies

Indirect cytotoxicity test done using MTT assay with L929 mouse fibroblasts showed that while chitin scaffold was biocompatible, all the chitin/nanosilver composite scaffolds were found to be cytotoxic (Fig. 8). This toxicity is due to the silver nanoparticles present in these scaffolds. Whether this may have an effect on wound healing is doubtful as previous studies have shown that nanosilver containing wound dressings are cytotoxic in vitro, while in vivo they perform satisfactorily [18, 44]. In an article by Ong et al. [18] reported chitosan wound dressing containing nanosilver and polyphosphate showed severe cytotoxixity in vitro while in vivo the wound healing was satisfactory and the wounds stained with H&E showed good fibroblast proliferation and keratinocyte maturation in epidermis when tested on murine models. This apparent

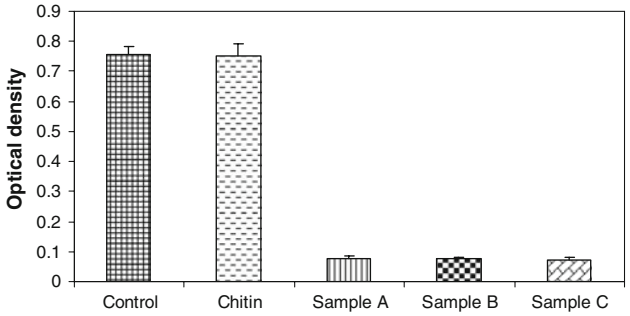

Fig. 8 Biocompatibility of chitin and chitin/nanosilver composite scaffolds (samples A, B and C)

discrepancy between in vitro and in vivo results may be explained by the rapid inactivation of silver ions in presence of physiologic concentrations of chloride and protein in the wound [45]. The antimicrobial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical–induced bacterial membrane damage [46]. ($n = 3$ at each time point).

4 Conclusion

In this work, novel chitin/nanosilver composite scaffolds were synthesized using chitin hydrogel with silver nanoparticles and characterized using SEM, FT-IR and XRD. These scaffolds were found to be bactericidal against both *S. aureus* and *E. coli* and were also found to have superior blood clotting ability, which will be useful for wound healing applications. However, these scaffolds were cytotoxic to mouse fibroblasts under in vitro conditions. It is uncertain whether this in vitro cytotoxicity will affect in vivo wound healing response.

Acknowledgments One of the authors R. Jayakumar is grateful to SERC Division, Department of Science and Technology (DST), India, for providing the fund under the scheme of "Fast Track Scheme for Young Investigators" (Ref. No. SR/FT/CS-005/2008). Dr. S. V. Nair also grateful to DST, India, which partially supported this work, under a center grant of the Nanoscience and Nanotechnology Initiative program monitored by Dr. C. N. R. Rao. The authors are also thankful to Mr. Sajin. P. Ravi for his help in SEM studies.

References

1. Gong P, Li H, He X, Wang K, Hu J, Tan W. Preparation and antibacterial activity of Fe3O4@Ag nanoparticles. Nanotechnol. 2007;18:604–11.
2. Klasen HJ. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns. 2000;26:131–8.
3. Castellano JJ, Shafii SM, Ko Donate FG, Wright TE, Mannari RJ. Comparative evaluation of silver-containing antimicrobial dressings and drugs. Int Wound J. 2007;4:114–22.
4. Fox CL, Modak SM. Mechanism of silver sulphadiazine action on burn wound infections. Antimicrob Agents Chemother. 1974;5:582–8.
5. Fox CL. Silver sulfadiazine: a new topical therapy for Pseudomonas in burns. Arch Surg. 1968;96:184–8.
6. Gemmell CG, Edwards DI, Frainse APJ. Guidelines for the prophylaxis and treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in the UK. J Antimicrob Chemother. 2006;57:589–608.
7. Furno F, Morley KS, Wong B, Sharp BL, Arnold PL, Howdle SM. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? J Antimicrob Chemother. 2004;54:1019–24.
8. Ip M, Lui SL, Poon VKM, Lung I, Burd A. Antimicrobial activities of silver dressings: an in vitro comparison. J Med Microbiob. 2006;55:59–63.
9. Leaper DL. Silver dressings: their role in wound management. Int Wound J. 2006;3:282–94.
10. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater. 2000;52:662–8.
11. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface. 2007;275:177–82.
12. Morones JR, Elechiguerra JL, Camacho A, Ramirez JT. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16:2346–53.
13. Song HY, Ko KK, Oh LH, Lee BT. Fabrication of silver nanoparticles and their antimicrobial mechanisms. Eur Cells Mater. 2006;11:58.
14. Jain P, Pradeep T. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng. 2005;90:59–63.
15. Hu W, Chen C, Li X, Shi S, Shen W, Zhang Z, et al. In situ synthesis of silver chloride nanoparticles into bacterial cellulose membranes. Mater Sci Eng C. 2009;28:1216–9.
16. Maneerung T, Tokura S, Rujiravanit R. Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. Carbohydr Polym. 2008;72:43–51.
17. Barud HS, Barrios C, Regiani T, Marques RFC, Verelst M, Dexpert-Ghys J, et al. Self-supported silver nanoparticles containing bacterial cellulose membranes. Mater Sci Eng C. 2008;28:515–8.
18. Ong SY, Wu J, Shabbir MM, Mui-Hong T, Jia Lu. Devolopment of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. Biomaterials. 2008;29:4323–32.
19. Lu S, Gao W, Gu HY. Construction, application and biosafety of silver nanocrystalline chitosan wound dressing. Burns. 2008;34:623–8.
20. Paula MM, Vitorio Franco C, et al. Synthesis, characterization and antibacterial activity studies of poly-{styrene-acrylic acid} with silver nanoparticles. Mater Sci Eng C. 2009;29:647–50.
21. Jeon HJ, Kim S, Kim TG. Preparation of poly(-caprolactone)-based polyurethane nanofibers containing silver nanoparticles. Appl Surf Sci. 2008;254:5886–90.
22. Rujitanaroj P, Pimpha N, Supaphol P. Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles. Polymer. 2008;49:4723–32.
23. Guncem G, Cigdem AO, Sibel E, Fikret S, Djursun K, Birgul S, et al. Fatty acid-based polyurethane films for wound dressing applications. J Mater Sci: Mater Med. 2009;20:421–31.
24. Lee SB, Kim YH, Chong MS, Lee YM. Preparation and characteristics of hybrid scaffolds composed of β-chitin and collagen. Biomaterials. 2004;25:2309–17.

25. Jayakumar R, Nwe N, Tokura S, Tamura H. Sulfated chitin and chitosan as novel biomaterials. Int J Biol Macromol. 2007;40:175–81.
26. Maeda Y, Jayakumar R, Nagahama H, Furuike T, Tamura H. Synthesis, characterization and bioactivity studies of novel β-chitin scaffolds for tissue-engineering applications. Int J Biol Macromol. 2008;42:463–7.
27. Madhumathi K, Binulal NS, Nagahama H, Tamura H, Shalumon KT, Selvamurugan N, et al. Preparation and characterization of novel β-chitin–hydroxyapatite composite membranes for tissue engineering applications. Int J Biol Macromol. 2009;44:1–5.
28. Madhumathi K, Shalumon KT, Divya Rani VV, Tamura H, Furuike T, Selvamurugan N, et al. Wet chemical synthesis of chitosan hydrogel–hydroxyapatite composite membranes for tissue engineering applications. Int J Biol Macromol. 2009;45:12–5.
29. Jayakumar R, Prabaharan M, Reis RL, Mano JF. Graft copolymerized chitosan—present status and applications. Carbohydr Polym. 2005;62:142–58.
30. Janes KA, Fresneau MP, Marazuela A, Fabra A, Alonso MJ. Chitosan nanoparticles as delivery systems for doxorubicin. J Control Rel. 2001;73:255–67.
31. Jayakumar R, Reis RL, Mano JF. Synthesis and characterization of pH-sensitive thiol-containing chitosan beads for controlled drug delivery applications. Drug Deliv. 2007;14:9–17.
32. Jayakumar R, Reis RL, Mano JF. Phosphorous containing chitosan beads for controlled oral drug delivery. J Bioact Compat Polym. 2006;21:327–40.
33. Lee YM, Kim SS, Park MH, Song KW, Sung YK, Kang IK. β-Chitin-based wound dressing containing silver sulfurdiazine. J Mater Sci: Mater Med. 2000;11:817–23.
34. Campos MGN, Henry RR, Lucia H, Innocentini M, Neera S. In vitro gentamicin sustained and controlled release from Chitosan cross-linked films. J Mater Sci: Mater Med. 2009;20:537–42.
35. Demling RH, DeSanti L. Effects of silver on wound management. Wounds. 2001;13:4–9.
36. Katrin K, Yang W, Harald K, Perelman LT, Itzkan I, Dasari RR. Single molecule detection using surface enhanced Raman scattering. Phys Rev Lett. 1997;78:1667–70.
37. Jaya J, Sumit A, Jyutika M, Rajwade, Pratibha O, Sanjeev K, and Kishore MP. Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. Mol pharm A. doi:10.1021/mp900056g.
38. Shih MF, Shau MD, Chang MY, Chiou SK, Chang JK, Cherng JY. Platelet adsorption and hemolytic properties of liquid crystal/composite polymers. Int J Pharm. 2006;327:117–25.
39. Jayakumar R, Egawa T, Furuike T, Nair SV, Tamura H. Synthesis, characterization, and thermal properties of phosphorylated chitin for biomedical applications. Polym Eng Sci. 2009;49:844–9.
40. Chen Q, Zhou M, Fu Y, Weng J, Zhang Y, Yue L, et al. Magnetron sputtering synthesis silver and organic PEO nanocomposite. Surf Coat Technol. 2008;202:5576–8.
41. Siddhartha S, Tanmay B, Arnab R, Gajendra S, Ramachandrarao P, Debabrata D. Characterization of enhanced antibacterial effects of novel silver Nanoparticles. Nanotechnology. 2007;18:1–9.
42. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nanomed: Nanotechnol Biol Med. 2007;3:168–71.
43. Kapadia NP, Kristol D, Spillert CR. Effect of endotoxin and silver ion on the clotting time of blood. Proceedings of the IEEE 31st annual northeast bioengineering conference. 2005. p. 161–162.
44. Lam PK, Chan ES, Ho WS, Liew CT. In vitro cytotoxicity testing of nanocrystalline silver dressing (Acticoat) on cultured keratinocytes. Br J Biomed Sci. 2004;61:125–7.
45. Poon VKM, Burd A. In vitro cytotoxicity of silver: implications for clinical care. Burns. 2004;39:140–7.
46. Kim JS, et al. Experimental antimicrobial effects of silver nanoparticles. Nanomed: Nanotechnol Biol Med. 2007;3:95–101.