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Development of novel wound care systems based on nanosilver nanohydrogels of polymethacrylic acid with *Aloe vera* and curcumin

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ABSTRACT

This study is aimed at the development of a composite material for wound dressing containing nanosilver nanohydrogels (nSnH) along with *Aloe vera* and curcumin that promote antimicrobial nature, wound healing and infection control. Nanosliver nanohydrogels were synthesized by nanoemulsion polymerization of methacrylic acid (MAA) followed by subsequent crosslinking and silver reduction under irradiation. Both the polymerization and irradiation time had significant influence on the nanoparticle shape, size and its formation. Polyvinyl alcohol/polyethylene oxide/carboxymethyl cellulose matrix was used as gel system to blend with nSnH, *A. vera*, curcumin and coat it on the hydrolysed PET fabric to develop antimicrobial dressings. The cumulative release of silver from the dressing was found to be ~42% of the total loading after 48 h. The antimicrobial activity of the dressings was studied against both *Staphylococcus aureus* and *Escherichia coli*. *In vivo* wound healing studies were carried out over a period of 16 d on full-thickness skin wounds created on Swiss albino mice. Fast healing was observed in Gel/nSnH/Aloe treated wounds with minimum scarring, as compared to other groups. The histological studies showed *A. vera* based dressings to be the most optimum one. These results suggest that nSnH along with *A. vera* based dressing material could be promising candidates for wound dressings.

1. Introduction

Infection is one of the main problems of today's wound care management systems. Bacterial infection in a wound can impede the healing process and may lead to the life threatening complications. One of the approaches for treating wound infection is the use of wound dressings with antibacterial agents having broad-spectrum of activity and high kill rate [1]. Although, a wide variety of antimicrobial agents are available, antibiotic resistance of microorganisms poses a major threat, resulting in the complications such as delayed wound healing. From a huge class of antimicrobial agents, the medicinal properties of silver have been extensively investigated [2]. A large number of silver coated dressings are used extensively for wound management, particularly in burn wounds, chronic leg ulcers, diabetic wounds and traumatic injuries [3–5].

In recent years, extensive research has been dedicated to the development of nanosilver based systems due to its high effectiveness against microbes [6]. Nanosilver is effective against more than 200 species of microorganisms and can accurately be termed as a "wonder drug" [7]. The polymer nanocomposites containing silver nanoparticles can be prepared by several methods. One of the common methods is the *in situ* polymerization of a monomer in the presence of metal nanoparticles, or *in situ* reduction of metal salts or complexes in a polymer. The *in situ* synthesis of silver nanoparticles on oxidized cotton fabrics has been an interesting approach [8]. Radiation induced synthesis of silver nanoparticles within a hydrogel has been recently reported by our group, where gamma radiation was used for the simultaneous polymerization of methacrylic acid and the reduction of silver ions [9]. Tummalapalli et al. has investigated *in situ* synthesis of silver nanoparticles by oxidized pectin for further blending with gelatin for wound dressings [10]. In another study, we investigated the chemical reduction of nanosilver loaded polyvinyl alcohol (PVA) nanogels for wound dressings [11]. In this study, fructose was used as reducing agent and PVA was taken as stabilizing as well as gel matrix for the nanosystem.

To accelerate the healing process, several bioactive products such as *Aloe vera* and curcumin have been traditionally used. *A. vera* is a plant belonging to the Liliaceae family, with two major components i.e., yellow latex and leaf pulp. The raw pulp contains about 98.5% water while the remaining contains a range of compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids [12]. *A. vera* has been used

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in wound healing since ancient times and number of recent studies has also available for this purpose [12–17]. These studies have shown that *A. vera* is widely used in wound dressings for excellent healing along with removal of pain and scar from the wound site. On the other hand, curcumin, a yellow pigment obtained from the rhizomes of *Curcuma longa* Linn (Zingiberaceae) has been widely used for centuries as indigenous medicine for the treatment of a variety of inflammatory conditions [18]. Topical application of curcumin with antioxidant activities for the treatment of wound damage has been extensively studied in past two decades [19,20]. In earlier studies, the medicinal extracts of both *A. vera* and curcumin incorporated membranes in varying ratios have been found to exhibit excellent antimicrobial activity against both gram-positive and gram-negative bacteria [21].

The present new approach is dedicated to the development of nSnH by two-step process comprising of the chemical polymerization of a nanoemulsion of methacrylic acid followed by the reduction of silver ions and crosslinking of polymer chains by using gamma radiation. The nSnH was blended with carboxymethyl cellulose, polyvinyl alcohol and polyethylene oxide based gel along with *A. vera* and curcumin. This developed gel was coated on the functionalized polyester fabric and the resultant dressings were evaluated using *in vitro* antimicrobial and *in vivo* animal studies. The incorporation of nSnH with *A. vera* and curcumin into gel makes these systems antimicrobial in nature which prevents infection around the wound. Especially presence of *A. vera* promotes the wound healing and minimizes the scar on wound site.

2. Materials and methods

2.1. Materials

Methacrylic acid, heptane, silver nitrate, methanol, acetone, hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium persulfate (KOH), *o*-cresol, potassium hydroxide, dichloromethane and bromophenol blue were purchased from Merck, India. PVA (degree of polymerization 1700–1800 and molecular weight 115,000 g/mol) and carboxymethyl cellulose (CMC) sodium salt of high viscosity were received from LobaChem Pvt. Ltd., Mumbai, India. Poly(ethyleneoxide) (PEO) of molecular weight 300,000, dioctylsulfosuccinate sodium salt (AOT) and curcumin (Cur) were supplied by Sigma Aldrich, India. Freeze dried *A. vera* (Aloe) gel powder was purchased from Biomax lifesciences, Hyderabad, India. Luria broth and agar-agar, were obtained from Hi Media Laboratories, India. Bacterial strains of *Escherichia coli* and *Staphylococcus aureus* were provided by Department of Microbiology, AIIMS, New Delhi. Polyester (PET) fabric (40 GSM) was supplied by Thermoband, India. Ultra-pure water, resistivity 18 MΩ cm, produced by Millipore Milli-Q system was used throughout the experimental work. All the chemicals were used without further purification.

2.2. Preparation of nanosliver nanohydrogel

The nanosliver nanohydrogel (nSnH) was prepared by the coupled process of chemical polymerization of methacrylic acid as water/oil nanoemulsion and subsequent irradiation to reduce silver ions into nanosilver. AOT was used as the surfactant for the stabilization of the nanoemulsion. In oil phase, 8 g AOT was added to 100 mL of heptane. Aqueous solution of silver nitrate (6 mmol/L), methacrylic acid (0.12 mol/L) and 0.2% potassium persulfate was added as the initiator. 2 mL water phase (comprising of monomer and silver nitrate) was added to 98 mL oil phase (comprising of AOT and heptane) and the whole mixture was then placed on constant stirring for 10 min. The solution was transferred to a reaction tube and deaerated by bubbling nitrogen for 15 min and was sealed. Subsequently, the tube was placed in a thermostated water bath at 50 °C for polymerization at different intervals of time.

After the polymerization, above solution was subjected to gamma irradiation (60Co gamma radiation source (dose rate of 0.10 kGy/h), supplied by Bhabha Atomic Research Centre, India) for the reduction of silver nitrate and crosslinking of the hydrogel at ambient conditions. After irradiation, the nanoemulsion was destabilized by the addition of methanol so that nanoparticles settled down. The precipitate was washed repeatedly with acetone to remove AOT and the nanoparticles were separated. The nSnH was obtained as precipitate and was repeatedly washed with acetone and dried under vacuum oven at 60 °C for 24 h. The molecular weight measurements were carried out according to the procedure followed by Gupta et al. using an Ubbelohde viscometer at a temperature of 25 ± 5 °C using Julabo 31A constant temperature water bath. [22]. PMAA solutions of varying concentrations were prepared using water as the solvent. The molecular weight (M) was calculated according to the following equation.

$$[\eta] = KM^{\alpha}$$

The intrinsic viscosities [η] were calculated by extrapolating the η_{sp}/C *vs.* C plot to infinite dilution. M is the molecular weight, (K and α) are Mark-Houwink constants (α:K = 0.5:0.0006 in dL/g) [23].

2.3. Energy dispersive X-ray microanalysis (EDX)

The presence of silver in nSnH was ascertained by RONTEC's EDX Model QuanTax 200 (SDD technology, USA). The sample was placed on a sample stub and coated with carbon with Auto-Fine Coater JFC-1600 (Joel, USA Inc., USA).

2.4. Fourier transforms infrared spectroscopy (FTIR)

The IR spectra of samples were recorded on a Perkin–Elmer FTIR System Spectrum GX. The spectra of PMAA and nSnH samples were recorded by using the potassium bromide disk technique, in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and averaged over 25 scans.

2.5. Dynamic light scattering (DLS)

Dynamic light scattering based on the concept of laser light scattering by particles in constant Brownian motion was employed to analyze the particle size. A high-performance DLS instrument (Beckmann Coulter Delsa™ Nano) was used for the size determination of nSnH in the wet stage.

2.6. High resolution transmission electron microscopy (HRTEM)

The morphology of nSnH particles was observed under a TECNAI TEM (Fei, Electron Optics) HRTEM operated at 200 kV equipped with Olympus Soft Imaging Solutions GmbH (software: iTEM; TEM Camera: Morada 4008 × 2672 pixel max) recording system. For HRTEM analysis, the samples were prepared by placing one drop of the aqueous nSnH on a carbon-coated copper TEM grid and dried at room temperature.

2.7. Fabrication of bioactive drug loaded composite dressings

PVA/PEO/CMC blend in the ratio of (72:8:20) was prepared by using water as solvent under continuous stirring for 8 h at 70 °C to get the gel, as reported earlier [24]. The gel content was varied from 1 to 6% by weight. Further, nSnH compositions of 200–1000 ppm were added to the blend solution and stirred for homogenization. *A. vera* and curcumin were also added into the optimized gel/nSnH blend system to monitor the synergistic effect of combination of synthetic and natural bioactive drugs. Both *A. vera* (Aloe) and curcumin (Cur) of (10% each) were added into gel/nSnH (1000 ppm). The composition of *A. vera* and curcumin were optimized from earlier studies by our group [25,26]. After complete dissolution, blends were coated on the hydrolyzed PET fabric and dried at ambient temperature. Initially, PET fabric was functionalized by alkaline hydrolysis of ester groups on the fabric so that the fabric acquires hydrophilic nature. The carboxyl group estimation