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Research Article

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Preparations of Chitosan silver nanoparticles for burns treatment: wound-healing effects

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Abstract

Chitin, β-(1-4)-poly-*N*-acetyl-D-glucosamine) is widely distributed in nature and is the second most abundant polysaccharide after cellulose. Chitin occurs in nature as ordered macrofibrils and is the major structural component in the exoskeletons of crab, shrimp shells and the cell walls of fungi and yeast. In this study silver nanoparticles were successfully synthesized by using marine shells such as prawn and crab shells,formation of synthesized silver nanoparticles wasdetermined by UV-Vis spectrophotometer were Silver nano composites plasmon peak was observed at nearly 450 nm,morphology was characterized by scanning electron microscope were particle size was found to be minimu\m of 96.3nm. Chitin and chitosan are biocompatible, biodegradable, and non-toxic. are also used in various types of biomedical applications such as drug and gene delivery, wound healing,burn healing, tissue engineering, and stem cell technology,the main kind of nanoparticles which have been studied for the use in food packaging systems are overviewed as well as their applications.

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Keywords: SEM, chitosan, nanocomposite.

Introduction

Nanotechnology has been established recently as a new interdisciplinary science. Nanotechnology enters into all the field of sciences.Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Kumar et al., 2004).Silver has a long history as an antimicrobial agent, especially in the treatment of wounds. Silver nanoparticles (AgNPs) show strong inhibitory and antibacterial effects and limited toxicity to mammalian cells (Di et al. 2012).Chitin, a long-chain polymeric polysaccharide, renewable organic resource and the supporting material of some plants and many sea creatures and is produced by a number of living organisms.The matrix is proteinaceous, where the protein is hardened by a tanning process (Yogeshkumar et al. 2013). Chitosan is a linear polysaccharide consisting of β-(1→4)-linked 2-amino-2- deoxy-D-glucose residues, originating from deacetylated derivative of chitin, which is the second most abundant polysaccharide in nature after cellulose. It is non-toxic, biodegradable, biofunctional, and biocompatible.(Dutta et al.,2004) studied on chitin and chitosan properties. They are considered as versatile and promising biomaterial. Deacetylated chitin derivative and chitosan is more useful and interacting bioactive polymer. Chitosan and chitosan nanomaterials have a wide array of applications in biomedical, pharmaceutical, nutraceutical, foods and personal care areas. In health care industry, chitosan and chitosan materials are used as antimicrobial, antitumor, antiulcer, antidiabetic and cholesterol lowering agent. One of the most effective and promising materials are nanoparticles based on silver nanoparticles (AgNPs) and chitosan (Kumar et al., 2004).

The present study was carried out to give an example of an effective burn wound healing agent based on synergistic effect from using silver nitrate. We synthesized chitosan silver nanopartlicles using chitosan of crustacean shells by an environment friendly method. Furthermore, chitosan silver nanopartlicles ointment was prepared to evaluate the healing effect for burn wound using rat model with the expectation of applications in pharmaceutical and biomedical fields.

Materials and Methods

Collection of sample

Crab and prawn shells were collected from Kundapura beach, Udupi District, Karnataka during August 2015.The prawn and crab shells cleaned and washed it with water until proteins are removed and dried at 60°C. Chemicals such as Sodium hydroxide, Hydrochloric acid and Silver nitrate were purchased from Durga Research Laboratories, Mangalore.

Synthesis of chitosan from Crab and prawns shells (Sivakumar et al., 2014)

The dried Crab and prawns shells were grinded using mortar and crusher. Powdered shells were treated with 1% NaOH at 60ºC for 8 hour to remove chitin. Higher solid to alkali solution ratio was preferred. The powder was filtered and demineralized using 5% Hydrochloric acid at 45ºC. It was then washed and decolourized in direct sunlight for about 10 hour. Chitin observed was deacetylated by treating it with 50% NaOH at 85ºC for 2 hour. Finally chitosan was synthesized from crustacean shells.

Synthesis of silver nanocomposites in various concentrations

0.05g of silver nitrate and 0.25% of glucose were added with 10ml of distilled water, autoclave at 121°C for 20 minutes and cooled at room temperature again centrifuged at 3000 rpm for 20 minutes. Supernatant was stored at 4°C for overnight. Next day chitosan was added in various concentration(10mg, 20mg, 30mg and 40mg) in 1ml of supernatant, centrifuged at 6000 rpm for 10 minutes, dried at 70°C hot air oven for 2 hours, nanocomposites was obtained.

Characterization of silver nanopartlicles

The reduction of silver ions was monitored by measuring double beam UV-Vis spectra of the reaction medium at different wave length from 300 to 500nm at different functional time. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 7000 rpm for 15 min and dried at 100°C. Crystalline nature of the nanoparticles was analyzed by XRD at 2θ ranges from 20 to 80°C. The morphology and size of the silver nanoparticles were found by Scanning Electron Microscope.

Preparation of wax

The wax was prepared by taking 50% of emulsifying bee wax and 49.5% of liquid paraffin were heated to 70-75°C and after it cools down to ointment form, 0.5% of chitosan silver nanoparticles was added.To experimentally evaluate the efficacy of chitosan silver nanoparticles by the method of Molten wax burn method in rats.

Drugs used

- Control
- Standard test (silver nitrate ointment)
- Test drug(chitosan silver nanoparticles wax)

Method

The albino ratsselected for emulsifying wax burn method was divided into 3 groups and named as control, standard and trial group.The animals were anaesthetized under ether. Once the rats are sufficiently anaesthetized, they were secured to the dissection plate in prone position. The hairs were shaved from the part to be operated. Using a coin, mark the measurement for burn wound on the shaven back of the rats.This was poured with molten wax of 80°C and the wax was allowed to solidify onto the shaven back of the rats. Eight minutes after this, the solidified wax adhering to the layers of skin was gently removed to inflict a distinctly demarked burn wound.

Application of Drugs and its activity

External application of the trial drugs and standard drug were started to the respective groups from 1st day of wounding. Every post wounding day, Silver nanoparticles wax was applied to trial group and silver nitrate ointment was applied to the standard group. The control group rats received normal diet and water without any drug application for natural healing. To monitor the change in wound shapes and sizes, the wound margins were traced on a trace paper from the day of wounding and continued till the complete healing of the wound. This was again retraced on a millimeter scale graph paper. The rats were inspected daily and the health was assessed based on physical parameters such as wound contraction

Results

Characterization of silver nanocomposites

Ultraviolet (UV) - visible studies

UV-Visible spectroscopy is one of the most widely used techniques for structural characterization of silver

nanoparticles. Silver nanocomposites Plasmon peak was observed at nearly 450nm in size from the below given peak. It was clearly indicating the presence of silver nanocomposites. Figure:1 shows the absorbance spectrum of synthesized silver nanocomposites from chitosan.

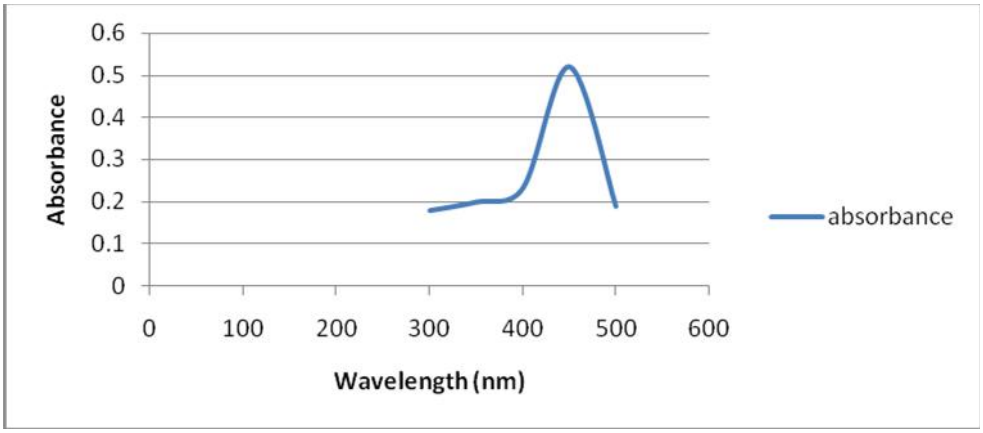

Figure 1: UV-Visible spectroscopy, absorption of Plasmon peak of Ag nanocomposites from chitosan

SEM

Size of chitosan Ag nanocomposites was determined by scanning electron microscope. The size of silver nanocomposites was found to be minimum of 96.3nm shown in figure 2.

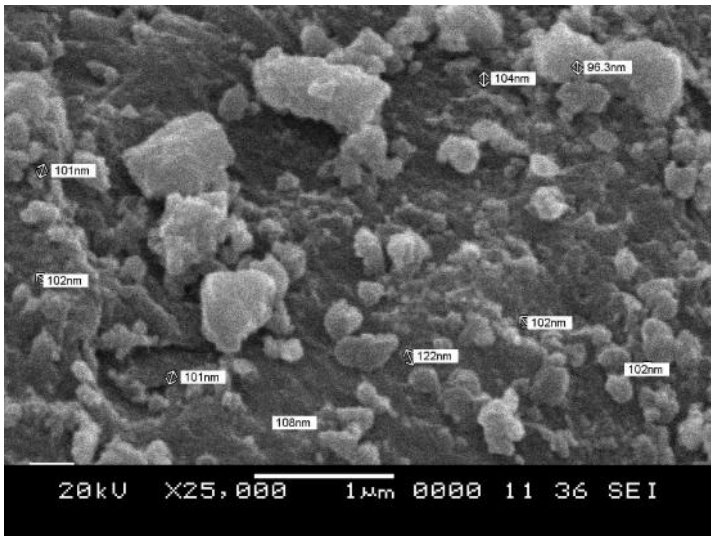

Figure 2. SEM analysis of chitosanAg nanocomposites

Burn healing activity

The percentage wound closure was observed on 4th, 8th, 12th and 16th post wounding day. To monitor the change in wound shapes and sizes, the wound margins were traced on a trace paper from the day of wounding and continued till the complete healing of the wound(Figure 3).

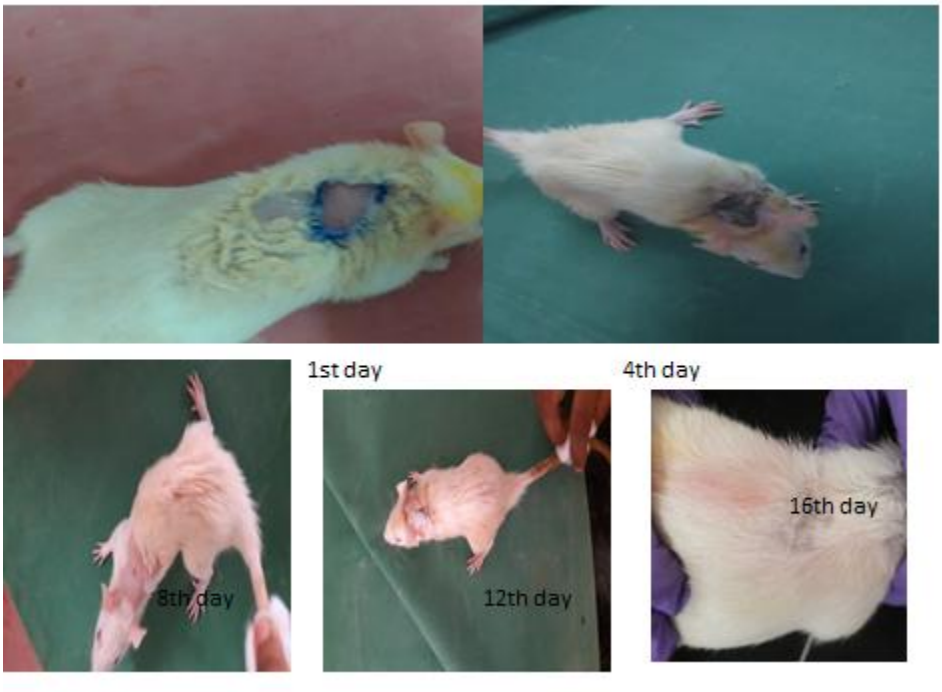

Figure 3 Healing activity of wound

Statistical inference

Test drug (chitosan silver nanoparticle)

As for the group received the test drug (chitosan nanopaticle) for burn treatment, the 4th day wound mean diameter was 210.5±5.4, 8th day mean diameter was 161.6±4.6, the 12th day wound mean diameter was 87.6±5.1, 16th day wound mean diameter was 25.3±3.3. Hence there was marked decrease wound diameter with every point of time the observation made.

Standard drug (silver nitrate)

As for the group received the standard drug for burn treatment, the 4th day wound mean diameter was 231.3±7.0, the 8th day wound mean diameter was 183.5±4.2, the 12th day wound diameter was 134.8±5.2, and the 16th day wound diameter was 46.8±6.3. Hence there was marked decrease wound diameter with every point of time the observation made.

Control drug

As for the group not received any group the 4th day wound diameter was 238.4±4.9, the 8th day wound mean diameter was 206.8±4.8. , the 12th day wound diameter was 167.8±4.5, the16th day wound diameter was 69.5±5.4. (Table 1)

DEVIATION

Table 1

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Chitosan silver nanoparticles	4thday	6	210.5000	5.46809	2.23234	204.7616	216.2384	202.00	217.00
	8thday	6	161.6667	4.63321	1.89150	156.8044	166.5289	155.00	168.00
	12thday	6	87.6667	5.16398	2.10819	82.2474	93.0859	81.00	94.00
	16thday	6	25.3333	3.32666	1.35810	21.8422	28.8244	20.00	30.00
	Total	24	121.2917	72.23361	14.74462	90.7901	151.7932	20.00	217.00
Standard Drug	4thday	6	231.3333	7.08990	2.89444	223.8929	238.7737	222.00	240.00
	8thday	6	183.5000	4.23084	1.72723	179.0600	187.9400	178.00	189.00
	12thday	6	134.8333	5.23132	2.13568	129.3434	140.3233	128.00	142.00
	16thday	6	46.8333	6.36920	2.60021	40.1493	53.5174	38.00	55.00
	Total	24	149.1250	69.88395	14.26500	119.6156	178.6344	38.00	240.00
Control	4thday	6	238.5000	4.96991	2.02896	233.2844	243.7156	230.00	244.00
	8thday	6	206.6667	4.84424	1.97765	201.5829	211.7504	200.00	213.00
	12thday	6	167.8333	4.53505	1.85143	163.0741	172.5926	163.00	174.00
	16thday	6	69.5000	5.43139	2.21736	63.8001	75.1999	62.00	76.00
	Total	24	170.6250	65.05236	13.27876	143.1558	198.0942	62.00	244.00

Discussion

Formation of silver nanoparticles by reduction of silver nitrate during exposure to chitosan nanoparticle from crab and prawn shells can be easily monitored by change in color in the reaction mixture. Silver nanoparticle bears characteristic dark brown color due to the excitation of Surface Plasmon vibrations. Similarly chitosan nanoparticle from crab and prawn shells Kumar et al. (2004) revealed the synthesis of silver nanoparticles by change in color of the reaction mixture from white and dark brown.In the present study, the synthesized nanoparticles were detected by UV-Vis Spectroscopy at various nanometers and the particle has increasingly sharp absorbance peak maximum at 450nm. Kumar et al. (2004) reported a narrow absorption peak at nearly 430nm in sizeand Nigam et al. (2009) reported that the absorption peak of silver nanoparticles was at 425nm..Size of chitosan Ag nanoparticles was determined using scanning electron microscope. The size of silver nanoparticles was found to be maximum at 96.3-101 nm. Kumar et al. (2004) reported that the size of silver nanoparticles was found to be maximum of 110nm.In the present study burn healing treatment of injured tissue in less time was observed. The group which received the test drug for burn treatment the 4^{th} day, 8^{th} day, 12^{th} day and 16^{th} day shows that the mean diameter was decreased with every point of observation made, the standard drug on 4^{th} day , 8th day, 12^{th} day and 16^{th} day marked decreased in wound diameter but less than that of the test drug used.

The stastical data proves that the test drug is more effective on 4th day, 8th day, 12th day and 16th day than the standard drug used and there was significance of difference in wound diameter.The chitosan silver nanoparticles explore the potential application in the field of burn healing. Chitosan nanoparticle can be used as a promising candidate for burn treatment which forms tough, water absorbent, biocompatible films.

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