Production of antimicrobial adhesives

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Abstract

Aim: To produce a bio mimetic consortium embedded with chitosan, haemolymph and calcium nanoparticles to enhance its activity in wound healing.

Methodology: Haemolymph was collected with a syringe from the abdominal region of the snails. Nanoparticles were prepared from the powdered samples of shells and operculum by addition of HCl and NaOH. Later, it was calcinated in the muffle furnace. SEM analysis was performed to check for the formation of nanoparticles. To extract chitosan, the cockroaches were dried in hot air oven, then crushed and powdered. By the process of de-protenization and de-mineralization, chitin was obtained by de-acetylation process to produce chitosan. Haemolymph was checked for anti-bacterial property which was determined by agar dilution method.

Results: The results obtained indicated that the consortium prepared from the combination of calcium nanoparticles, chitosan and haemolymph proved to have an impact on the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus licheniformis*. These synthesized compounds has individual potency too.

Interpretation: All the samples, haemolymph, calcium nanoparticles and chitosan showed anti-microbial property and were embedded in the bandages. Hence, the wound healing property of bandages were increased and its activity was verified.

Key words: Antibacterial activity, Bandages, Calcium nanoparticles, Chitosan, Haemolymph.

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Introduction

Injury of any kind caused to the epidermis, local vasculature and the underlying tissues creates a potential threat to the integrity of the organism as it can allow the entry of pathogens, as well as blood loss (Daunton *et al.*, 2012). Wound healing concludes with up to 80% original tensile strength being restored to the site. It involves four tightly overlapping phases: Hemostasis (formation of fibrin clot); Inflammation (body's immune response); Proliferation (cell migration and wound closing) and Remodeling (reorganization of the disorganized matrix to uninjured tissue).

The use of gauze and bandage to dress wound plays an essential role in health care sectors. It ranges from simple dressing to superficial wounds to holding together fractured bones or body parts from rehabilitation and recovery. They are mainly used in wound healing mechanism.

The material are of two types: Woven gauze – cotton cloth and Synthetic gauze – rayon or synthetic materials (Daunton *et al.*, 2012). Current wound dressings have disadvantages like poor flexibility, poor mechanical strength, lack of porosity, and a tendency for dressings to adhere onto the wound surface (Jayakumar *et al.*, 2011). It is well known that bacterial adhesion is the first step in colonization of wounded skin and in formation of a bio-film. Therefore, it is very important to have the antibacterial surface directly on to the bandage to avoid the formation of these bacteria films, preventing bacterial multiplication inside the bandage (Juknius *et al.*, 2016). Bio-materials can assist in proper physiological reconstruction of skin and reduce or prevent scar tissue formation. Some of them are chitin, chitosan, silver, iodine, calcium, etc. (Azad *et al.*, 2004). In large open wounds, the process of new tissue formation is recognized as granulation, which should form before wound epithelialization begins. Therefore, chitosan has an adaptable property for the wound (Ueno *et al.*, 2001). Materials for wound dressing and tissue engineering are important but still under development. New adhesives have been proposed. Chitosan derivative is non-toxic, cyto-compatible and mechanically suitable for peripheral surgeries. The poor solubility of chitin is the major limiting factor in its utilization and investigation of its properties and structure. Despite these limitations, various applications of chitin and modified chitins have been reported as raw materials for absorbable sutures and wound dressing (Dutta *et al.*, 2004).

Mollusca innate immunity relies on both cellular and humoral component. Cellular components comprehends, among the most important protagonists, circulating cells able to engulf, encapsulate or simply kill the invaders and potential pathogens. These cells are usually called hemocytes, and despite the considerable efforts spent for their characterization, their origin and classification still remain to be firmly established in mollusks (Accorsi *et al.*, 2013). Three circulating hemocyte types were

distinguished such as hyalinocytes, agranulocytes and granulocytes (Cueto *et al.*, 2011). Cockroaches are used in Traditional Chinese Medicine (TCM) against bone tuberculosis, cirrhosis, liver cancer, breast cancer and as facial beauty mask because of their purported anti-aging effects. Chitin is especially important in the extracellular structures and is available biopolymer which is used in various industrial and pharmaceutical applications. Chemical composition of chitin is β - (1-4) 2 acetamido-2-deoxy- β -D-glucose (N-acetyl glucosamine) and the deacetylated derivatives of chitin are chitosan which is a linear polymer of α (1-4 linked-2- amino-2-deoxy- β -D-glucopyranose). These have specific applications in drug delivery, tissue engineering, food sectors, waste water treatments, imprinting and metal nano-composites (Kaewboonruang *et al.*, 2016).

Snails are preferably available and does not cause any harmful effects to humans. In countries like China, Japan and Europe cockroaches are consumed. Insects can play a vital roles in the transmission of pathogenic bacteria and fungi with antibiotic resistance in households and in hospital environments (Salehzadeh *et al.*, 2007).

The present study aimed to produce bandages embedded with chitosan, haemolymph and calcium nanoparticles to enhance its activity in wound healing.

Materials and Methods

The consortium was developed from a mixture using the operculum and shell of *Pomacea canaliculata*: Common name - Golden apple snail. Family- Ampullariidae and the haemolymph of *Periplaneta americana*: Common name - Cockroaches Family - Blattidae.

Snails and cockroaches were collected from different localities in Chennai. The snails were allowed to grow with proper aeration and regular feeding. The haemolymph was collected the abdominal region. The collected samples was centrifuged at 3000 rpm for about 3 times and EDTA was added to the supernatant and stored in refrigerator for further use. It is well known that haemolymph has anti-microbial activity and it was tested by using agar dilution method in subsequent steps. The snail shell and operculum was removed from its body after euthanizing the animals. The haemolymph was collected from the cockroaches by a syringe near the base of their legs. Both the samples were dried in the hot air oven at 65°C for about 3 days. The samples were crushed separately with a mortar and pestle to make a fine powder.

Anti-bacterial assay was confirmed with the collected haemolymph by using four different bacterial samples by agar dilution method. Agar was selected as a medium for growth of microorganisms and the culture was streaked with L-rod. Haemolymph was poured in the centre of Petriplate by gel

Fig. 2 : Graph of FTIR analysis of the snail shell

Fig. 3 : Graph of FTIR analysis of the operculum in the snail

Fig. 4 (a) : SEM analysis of calcium nanoparticles

Fig. 4 (b) : A result of SEM analysis

punching method. It was incubated for about 48 hours and the plates were checked for the microbial growth.

Calcium nanoparticles were prepared from the shells and the operculum of the snail. Nanoparticles was prepared by

precipitation method (Abdul *et al.*, 2013, Ferraz and Monteiro, 2004, Teerawat, 2014, Himanshu and Satya, 2016) 2-5. 1M calcium nitrate and 0.6M Di-ammonium hydrogen phosphates was dissolved in distilled water and adjust the pH 10-11 by adding ammonia with cationic surfactant CTAB. The precipitate was

obtained after washing with distilled water and ethanol. Finally Nano particles were rinsed with distilled water and dried in an oven at 80° C for 12 hrs and then powder was crushed and kept at 800°C in a muffle furnace for 2 hrs. The confirmation of nanoparticles were done using FTIR and SEM analysis.

Results and Discussion

The collected haemolymph showed resistance against all the bacterial samples namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Bacillus licheniformis* cultured in our laboratory. Pavlina et al., (2011) states that the snails have a complex mixture of biochemically and pharmacologically active components. It can be used in the medicinal field and also as an antibiotic.

The nanoparticles synthesized from the operculum and shell of golden apple snail was subjected to FTIR analysis to confirm the function groups (Fig. 2) and SEM analysis to confirm their size (Fig. 4 a, b).

The characteristic peaks of C-O stretching and bending modes of CaCO₃ are observed at 2522cm⁻¹, 1477cm⁻¹, 861cm⁻¹ and 467cm⁻¹. The observed peaks are compared with the standard graph to confirm its contents. The peak of the operculum is shown in (Fig.3).

By comparing the two results obtained from the operculum and the shell, calcium peaks are confirmed in both the shell and the operculum.

Ongluki et al. (2012) had discussed his FTIR results of the Golden apple snail and the inference of the peaks. This was used as a standard graph for the sample. Hence, the comparative study was useful in adding the operculum powder to prepare nanoparticles. Vanitha et al, 2017, obtained strong bands at 640 and 3702 cm⁻¹ indicate the presence of hydroxyl groups in hydroxy apatite. The band at 1976 cm⁻¹ is the presence CO₃²⁻ groups.

The SEM studies indicated that the calcium particles obtained slightly exceeded the size of nanoparticles but were able to mix along with the other ingredients during the preparation of the adhesives (Fig. 4 a, b)

Nanoparticles are known to have anti-bacterial activity; hence the obtained particles were tested for their antibacterial efficacy. These nanoparticles have efficiency against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Bacillus licheniformis*. The nanoparticles were deposited at the centre by gel punching. After 48 hrs of incubation, the plates were checked for its zone of inhibition. As they showed effective inhibition against all the four bacterial species, it was evident that the calcium nanoparticles are efficient in wound healing activity against microbes. (Ataee et al., 2011) reported that the calcium

nanoparticles have anti-bacterial effect on Gram positive and Gram negative bacteria. This was performed by inoculating both Gram positive and Gram negative bacteria and incubation was carried out for 48 hr and the result obtained was positive.

Chitosan extracted by crushing whole cockroach was tested for confirmation. There was a change in colour of the solution during the addition of iodine followed by a colour change to dark purple when HCl was added. Wanule et al., (2017) suggested the confirmatory test for the chitosan. It states that the colour change is the best confirmatory test for the chitosan solution. The change of colour from yellow to dark purple indicates the presence of chitosan. The synthesized chitosan can be embedded in hydrogel to be used for wound healing (Wang et al., 2017). Chitosan is supposed to act as drug carriers as well (Jose et al., 2016).

Haemolymph, calcium nanoparticles and chitosan have anti-bacterial activity and it enhances the wound healing property. All the prepared particles were mixed together and the moisture content was removed by drying. Then it was lyophilized and stored. Later, it was embedded in the cotton area of the bandages and sealed. This adhesive would help both in the absorption and also wound healing activity.

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