A STUDY ON DEVELOPING COPPER NANOPARTICLE AND PEPTIDE CONJUGATE – MICROBIOLOGICAL ANALYSIS OF CONJUGATE AGAINST DRUG RESISTANT ORGANISMS

Princy P.K[1] and *Rajeswari K[2]

[1]Research scholar, Department of Microbiology, Rathnavel subramaniam college of Arts and Science, Sulur, Coimbatore-641402, Tamil Nadu, India,
Mobile: +91 7907663107, email: princypk88@gmail.com

[2]Assistant Professor, Department of Microbiology, Rathnavel subramaniam college of Arts and Science, Sulur, Coimbatore-641402, Tamil Nadu, India,
Mobile: +91 8248971319, email: krmicrobiol@gmail.com

***Corresponding author:**
Dr. K.Rajeswari
Assistant Professor, Department of Microbiology, Rathnavel subramaniam college of Arts and Science, Sulur, Coimbatore-641402, Tamil Nadu, India,
Mobile: +91 8248971319
email: krmicrobiol@gmail.com

ABSTRACT

Marine peptides are antibacterial substances which are conjugated with copper nanoparticles to develop antibacterial agent with an aim of determining the microbiological analysis against drug resistant microorganisms. Synthesis of copper nanoparticles (CuNPs) using chemical reduction method was performed. Antimicrobial peptides were extracted from marine fish, *Carangoides malabaricus* (AMPs). Both agents were conjugated to develop the antibacterial agent (AMPs-CuNP conjugates). Antibacterial activity of AMPs-CuNP conjugates was evaluated against clinical pathogens. Using this conjugate, a novel dermal substitute was developed using fibroblast cell lines on wound healing spacer material. Scanning Electron Microscopy (SEM) analysis of cell adhesion assay (L_{929} epidermal cells) on the spacer materials was studied. Antibacterial activity and wound healing ability of the developed dermal substitute was investigated. The antibacterial activity of the developed conjugates exhibited inhibitory zones of 20.6 ± 1.25mm and 20.3 ± 1.04mm against *Escherichia coli* and *Staphylococcus aureus*. Morphological differences in the ways cells attach to the developed wound healing spacer, was investigated using Scanning Electron Microscopy. SEM images showed that the cells were firmly attached onto the developed mesh and the number of attached cells was found increasing with the increase in incubation period of time. Antibacterial activity of AMPs-CuNP conjugates coated wound healing spacer showed that *Escherichia coli* and *Staphylococcus aureus* exhibited inhibitory zones of 32.3 ± 0.57mm and 34.3 ± 1.25mm. *In vitro* wound healing assay revealed that conjugates used for the cell adhesion studies was determined for its ability to improve wound healing by acting directly on L_{929} mouse fibroblast cells. The findings of the research concluded that, this combination therapy two or more antimicrobials are blended at different combinations; so that the broader spectrum of activity is achieved at a lower concentration resulting in more effective therapy and decreased resistance.

Keywords: *Marine peptides, Copper nanoparticles, Dermal substitute, Fibroblast cells, Wound healing*

INTRODUCTION

The global emergence and spread of antimicrobial resistant strains of commonly encountered pathogens and their alarming increase has become a serious health issue threatening the achievements of modern medicine (Jasovsky *et al.,* 2016). Nosocomial infections by clinical pathogens are a major concern. The most serious, life-threatening infections by a group of drug-resistant bacteria are named by the IDSA as *ESKAPE* pathogens (*Enterococcus faecium*, S*taphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli*. Mechanisms involved in antimicrobial resistance in Gram negative *ESKAPE* pathogens are drug inactivation or alteration, modification of drug binding sites, reduced intracellular drug accumulation and biofilm formation. As the commercial antimicrobials showed resistance to pathogens, there is a need for the development of new antimicrobials from various sources (Santajit and Indrawattana, 2016).

In the development of new drugs, marine source possess an inexhaustible source of useful chemical substances. Marine Source are considered to be wide and largely unexplored environment which contains approximately half of the total global biodiversity. About 80% of all life forms on earth are present only in the oceans. Out of 33 known animal phyla, 32 are present in the aquatic environment and 21 are exclusively marine. Marine invertebrates, as a new and promising source of biologically active compounds, are currently of considerable interest (De Rosa *et al.,* 2002). Immunity among marine invertebrates is strictly related to the presence of antimicrobial agents. As exposed to a large number of pathogens some of which are also present in anthropic environments. Marine invertebrates have retained an ancestral, nonspecific innate immune defense system mainly composed of antimicrobial peptides (AMPs). The major bioactive compounds present in marine organisms are phenols, lactones, Sterols, Terpenoids, phthalates, fatty acids, terpenes, macrolides etc., (Anselmo *et al*., 2010)

Particles that are having at least one dimension ranging from 1nm to 100 nm are considered as nanoparticles (NPs). NPs provide potential solution to combat multidrug resistant pathogens. The large surface area to volume ratio of NPs provides a high loading of coated molecules. NPs by themselves (e.g. silver, other metal oxides such as titanium, copper, zinc and iron etc.) have been known to possess antimicrobial activities and work through numerous modes of action. NPs can disrupt the bacterial cell membrane, causing cell penetration, react with intracellular target and cause toxicity (Slavin *et al.,* 2017).

When peptides are conjugated to NPs, the resulting conjugate will have new critical properties like enhanced potency, site of action targeting capability, less toxicity, etc. that are directly acquired from the peptides but not previously possessed by the nanoparticles (Suhas *et al.,* 2016). Nanoparticles are excellent candidates for transporting drugs to their targets. The interaction between proteins and nanoparticles has been demonstrated to play a pivotal role in the nanomaterial's biocompatibility and ultimately, its antimicrobial performance. Proteins, peptides and free amino acids can be used to control the structure of NPs during synthesis and improve their stability under a variety of conditions. Nanoparticles are expected to have a favorable effect

on the strength and nature of nanoparticle-peptide interactions. Recent researches in which nanoparticles have been combined with AMPs are promising and shown an increasing trend toward a safer profile of the conjugates toward mammalian cells. Due to the antimicrobial potential of the AMPs-CuNPs conjugates, combination therapy was carried out in this research as reported by Saginur *et al* (2006).

To avoid the antibiotic resistance character the effects of introducing two different groups of synergistic antimicrobials (Anti-Microbial peptides and copper Nanoparticles) into the biomedical products gained lot of interest currently. This was well supported from the report of Saginur *et al* (2006); describing that the accepted clinical practice to treat biomedical-associated infections was the use of combination therapy in which two or more antimicrobials are blended at different combinations. So that broader spectrum of activity is achieved at a lower concentration resulting in more effective therapy and decreased resistance.

Objectives

Based on this concept, antimicrobial peptides extracted from marine sources, *Carangoides malabaricus* (Marine Trevally fish) and copper nanoparticles synthesized in the laboratory were made as conjugates and a biotechnological application study was conducted in the present research. As a preliminary attempt, a wound healing material impregnated with AMPs-AgNPs conjugates was developed and investigated for its tissue engineering properties by allowing fibroblast cells to grow on the material surface.

MATERIALS AND METHODS

Collection of *Carangoides malabaricus* fish

Carangoides malabaricus fishes were commercially purchased from fish market, Coimbatore, Tamil Nadu, India (Fig. 1). Fishes were collected in UV sterile container and brought to Microbiology Laboratory, RVS College of Arts and Science, Coimbatore, Tamil Nadu, India.

Extraction of antimicrobial peptides (AMPs) from *Carangoides malabaricus*

Carangoides malabaricus fish weighing about 1kg procured from a commercial fish market, at Coimbatore, Tamil Nadu, India. Procured fishes were washed once, packed in a transport polystyrene box covered with ice packs and brought to Microbiology Laboratory, RVS College of Arts and Science, Coimbatore, Tamil Nadu, India for further processing. The fishes were further processed for the extraction of AMPs using the procedure described by Nieto Lozano *et al*. (2012). The fishes were cut into pieces and the tissues were collected in a sterile container. About 100 g of tissues were mixed with 10% acetic acid and homogenized for 1 hour in a homogenizer (Fig. 2). The homogenized tissue mixtures were centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatants were collected and equal volumes of acetone was mixed and centrifuged at 5,000 rpm for 10 minutes at 4°C. Supernatant containing AMPs were partially

purified using standard ammonium sulphate precipitation and dialysis. AMPs as protein precipitates were collected finally and stored at 4°C.

Extraction and Synthesis of copper nanoparticles (CuNPs) using chemical reduction method (Kooti and Matouri, 2010)

Copper oxide nanoparticles were prepared using two prepared solutions separately. Solution - 1: About 6.9g of copper sulphate pentahydarate was dissolved in 100ml of distilled water. To dissolve completely the mixture was stirred in a magnetic stirrer kept at constant conditions (37°C and 200rpm). Solution-2: About 34.6g of sodium potassium taratate and 12g of sodium hydroxide was dissolved in 100ml of distilled water. To dissolve completely similar stirring conditions were carried out using a magnetic stirrer kept at constant conditions (37°C and 200rpm). About 50ml of solution-1 and 50ml of solution-2 was mixed together with vigorous stirring and 5g of glucose was added and then the mixture was stirred vigorously was 10mins and then keep in boiling water bath at 60°C for 10mins. Then, the obtained mixture is centrifuged and washed with distilled water twice and with ethanol twice and it was air dried and the powdered substance was used for further analysis.

Conjugation of AMPs [*Carangoides malabaricus* and copper NPs]

Using *Carangoides malabaricus* and actinomycetes extracts, antimicrobial peptide conjugate [*Carangoides malabaricus* and copper NPs] was developed. Briefly, the *Carangoides malabaricus* peptide extracts was kept under stirring conditions using a magnetic stirrer (180rpm, 40ºC) in a beaker. Followed by actinomycetes peptide extracts was added drop wise onto the above extract at the rate of 1ml per minute. The magnetic stirring condition was kept constant for 2h till complete development of conjugate. Developed conjugates were stored in brown amber bottle at refrigeration temperature prior to antimicrobial activity and other tests.

Antibacterial activity of AMPs-CuNPs conjugates against clinical pathogens

The antibacterial activity of developed AMP conjugate - *Carangoides malabaricus* + copper NPs was evaluated separately against the test organisms. To determine the effective concentration of AMP conjugates as developed above, the antibacterial activity of four different concentrations (1X–10µg/ml, 2X–20µg/ml, 3X–30µg/ml) of each conjugates were tested against the test organisms (*Escherichia coli*, S*taphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* sp). The test organisms *Enterococcus faecalis* and *Candida albicans* were grown in 5ml of sterile Nutrient broth (Composition g/L: Peptone: 5g; Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g; Final pH - 7.0 ± 0.2) for 24hr at 37^{o}C. The growth of the organism was measured based on the turbidity in the test culture tubes. The turbidity of microbial suspension was compared and adjusted to 0.5 on the Mc Farland scale.

Selection of AMP conjugates based on the antibacterial activity was carried out using modified Kirby–Bauer test method – Agar well diffusion method. Briefly, sterile Muller– Hinton agar (MHA) plates (Composition g/L: Acid hydrolysate of Casein: 17.5g; Starch: 1.5g, Sodium chloride: 5.0g, Agar 17.0 g; Final pH - 7.0 ± 0.2) were prepared under sterile conditions. The

plates were allowed to solidify for 5 minutes and 0.1-ml inoculum suspension of test bacteria were swabbed uniformly on the agar surface separately. After swabbing the cultures, four 6-mm wells were punctured on the agar surface using a sterile well borer for the addition of four different concentrations. All the plates after inoculation and addition of AMP concentrations were incubated at 37°C for 24 hours. The antibacterial activity of each concentration against the test bacteria was evaluated based on the inhibitory zones around the well. Experiments were carried out in triplicates and antibacterial activity was expressed in standard deviation values using the Statistical Package for Social Sciences (SPSS 9 for Windows 7.0) software.

Developing fibroblast cells on conjugate coated wound healing materials under *in vitro* conditions (Houreld and Abrahamse, 2007)

Collection and procurement of wound healing spacer materials

Non adhering wound dressing material was used in the study. In Table-1 and Fig. 3, the sample material designation and the product description are mentioned.

Surface coatings of AMPs-CuNPs conjugates on the wound healing spacer materials (Gollwitzer *et al.*, 2003)

Surface coating of wound healing spacer material using the AMP conjugates was done by a standard two dip-coating technique. The coating protocol was carried out using the conjugate - *Carangoides malabaricus* and copper NPs. The technique started with the preparation of stable slurry with specific amount (5g) of AMPs in the molten polyethylene glycol (PEG). PEG (5g) with a predefined molecular weight was mixed with AMPs (10ml of purified fractions of AMPs) in a glass vial. The mixture was heated at the range of 60 to 70°C in a water bath to obtain homogeneous slurry. The resulting slurry was homogenized in a magnetic stirrer for 5 to 10min. The wound healing spacer disc was cut (10mm) using sterile devices and dip-coated twice with intermittent drying (suspension coating method). The dip-coating procedure was carried out in sterile glass beakers on a shaker (120rpm) for 30min, with a drying period of about 15min between the two coating procedures, followed by drying at room temperature. All coating steps were carried out under strict aseptic conditions. After coating procedure, the materials were stored at 4°C for upto 15min. In order to increase antimicrobial drug loading and prevent excessive increase in material thickness, the coating process were repeated for replicates of each sample. Subsequently, in order to slow down the release rate of antimicrobial drug from PEG coating and mitigate the friction effect between material surfaces, second coating layer was formed using polyvinyl alcohol (PVA). PVA was dissolved in DMSO to acquire a 10% (w/w) solution. PEG-coated materials were submerged into PVA solution three times for 1min each. The coated materials were left to dry on a clean bench for 24 to 48hours at room temperature to remove residual DMSO, followed by determining the antimicrobial activity (Fig. 4).

Antibacterial activity of AMPs-CuNPs conjugates coated wound healing spacer against clinical pathogens

The antibacterial activity of wound dressing spacer coated with conjugate - *Carangoides malabaricus* + copper NPs was evaluated separately against the test organisms. AMPs coated wound dressing materials were subjected to evaluate the qualitative antibacterial activity against the test organisms individually. Standard Disc diffusion method was used in the research to evaluate the antibacterial activity. Briefly, Mueller-Hinton agar (MHA) plates were prepared by pouring 15 ml of media into sterile Petri dishes. The plates were allowed to solidify for 5 min and 0.1 ml inoculum was swabbed uniformly and allowed to dry for 60 sec. Premeasured disc shaped (20 mm in diameter) AMPs coated wound dressing materials were placed on the MHA plates (seeded with bacterial inoculum). The disc was gently pressed to attach on the agar surface using sterile conditions. A plain spacer without AMPs was also kept in the plate as control. All the plates were incubated at 37 ºC for 24 to 48 h. At the end of incubation, the zone of inhibition formed around each material was measured in millimeter. Experiments were carried out in triplicates and antibacterial activity was expressed in Standard deviation values using Statistical Package for Social Sciences (SPSS - 9 for Windows 7.0) software.

Cultivation of L_{929}dermal fibroblasts

L_{929} dermal fibroblasts were cultured according to the standard culture method described by Houreld and Abrahamse, (2007). Briefly, cells were cultured in Dulbecco´s modified Eagle's Medium (DMEM) supplemented with and 10% fetal calf serum, 1mM sodium pyruvate, 2mM L-glutamine, 0.1mM non-essential amino acids, 1% Penicillin-Streptomycin and 0.2% Amphotericin-B. The cells were cultured for 7 days in 75cm^2 cell culture flasks at 37°C and in humidified atmosphere containing 5% CO_2 atmosphere.

Culture of fibroblasts on wound dressing material – A Dermal Substitute

Wound dressing samples coated with conjugate [*Carangoides malabaricus* + copper NPs] were cut aseptically (size - 1.5 cm × 1.5 cm) and stabilized with a stainless steel ring. Cultured fibroblasts were harvested through trypsin-EDTA (Gibco) treatment and seeded at 2×10^4 cells/cm^2 on to the wound dressing samples by placing the material in a cell culture dish. Fibroblasts were incubated directly with the test specimen in complete DMEM (containing 1% antibiotic - antimycotic solution and 10% fetal calf serum) for 24h and the culturing process was carried out using rotary cell culture system. During the incubation period, cell morphology and cell proliferation on the wound dressing samples were recorded.

Proliferation of fibroblasts and construction of Dermal Substitute

Rotary cell culture system was used for the proliferation of fibroblast at a revolution rate 15-20 rotation per min. The proliferation was carried out at 37°C and in humidified atmosphere containing 5% CO_2 atmosphere. The increase in the cell number and attachment on the surface and interstices of the fibres were recorded at 0^{th}hr, 6^{th}hr, 12^{th}hr and 24^{th}hr. These developed living dermal substitute shall be used to promote diabetic wound healing and neovascularization. This method may be used to treat diabetic ulcers and other chronic wounds in the clinic.

SEM Analysis of cell adhesion assay (L_{929} epidermal cells) on the spacer materials

Developed epidermal layer on the material with attached fibroblasts was immediately visualized after thawing. The thawed dermal substitute samples were fixed, dehydrated, and observed under a field emission scanning electron microscope (FESEM).

Analyzing the wound healing ability: *in vitro* wound scratch assay for selected concentrations of AMP conjugates (Katyakyini Muniandy et al., 2018)

The migration rates of fibroblast cells were assessed by the scratch assay method. The cell density of 2×10^5 cells was seeded into each well of a 24-well plate and incubated with complete medium at 37°C and 5% CO_2. After 24h of incubation, the monolayer confluent cells were scrapped horizontally with a sterile P_{200} pipette tips. The debris was removed by washing with PBS. The cells were treated with AMP conjugates with various concentrations (5, 10, 15, 20, 25 µg/ml) by diluting with serum-free Dulbeco modified eagle medium (DMEM). The cells treated with allantoin (Sigma Aldrich, Germany) were used as the positive control. The scratch induced as represented wound, was photographed at 0h using phase contrast microscopy at ×40 magnification. After 4h, 18h and 24h of incubation, the second set of images was photographed. To determine the migration rate, the images were analyzed using "image-J" software, and percentage of the closed area was measured and compared with the value obtained at 0h. An increase in the percentage of the closed area indicated the migration of cells.

Wound closure (%) = (Measurement at 0h - Measurement at 24 h)/ Measurement at 0h X 100

RESULTS AND DISCUSSION

Antibacterial activity of AMP-CuNPs against clinical pathogens

The antibacterial activity of the developed conjugates were evaluated for three different concentration against the test organisms. In Table-2, the zone of inhibition measured for each concentration were presented. The results showed that the antibacterial activity was concentration dependent. All test organisms showed good inhibitory zones for all three concentrations. When compared to standard streptomycin, the concentration (30µg/ml) revealed almost similar inhibitory zones against all the test organisms. *Escherichia coli* and *Staphylococcus aureus* exhibited inhibitory zones of 20.6 ± 1.25mm and 20.3 ± 1.04mm; *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* exhibited the inhibitory zones of 22.3 ± 0.75mm and 19.3 ± 0.57mm; and *Enterobacter* sp showed 19.9 ± 0.57mm of inhibitory zone (Fig. 5).

Antibacterial activity of AMP-CuNPs conjugates coated wound healing spacer

After developing the fibroblast cell lines on the AMP-CuNPs coated wound healing spacer, the antibacterial activity was investigated against the test bacteria. This test was performed to determine the persistence of conjugates on the cell developed mesh materials. Interestingly, the antibacterial activity was found retained. *Escherichia coli* and *Staphylococcus aureus* exhibited inhibitory zones of 32.3 ± 0.57mm and 34.3 ± 1.25mm for the coated mesh samples. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* exhibited the inhibitory zones of 32.6 ± 1.04mm and 33.9 ± 0.75mm against their respective coated samples. *Enterobacter* sp showed significantly 33.3 ± 1.25mm of inhibitory zones for the test samples. No inhibitory zones were recorded for plain mesh samples during the study (Table-3; Fig. 6). The obtained results revealed that the antimicrobial conjugates increased the therapeutic effect against the test organisms. The mode of action of AMP-CuNP conjugates was reported to penetrate the pyramidal wall of bacteria followed by disrupting the cytoplasmic contents; and hence adhesion of pathogen at the wound site was prevented.

Development of epidermal layer on the wound healing mesh materials

A. L_{929} fibroblast cell proliferation and adhesion

Tissue engineered wound dressing material was developed as final end use product using L_{929} fibroblast cells. The cells were allowed to attach and its stage wise development on wound healing spacer materials were microscopically observed. Inverted microscopic images showing the extent of cell attachment and proliferation of L_{929} cells on the fibres and interstices were noted from 0^{th}hr to 24^{th} hr. The increase in the cell number and attachment on the surface and interstices of the fibres were observed at 0^{th}hr, 6^{th}hr, 12^{th}hr and 24^{th}hr. No growth was observed on 0^{th}hr. Partial cell growth was observed on 6^{th} hr. Cell attachment was found to be evident on 12^{th}hr and proliferated cell covering the entire surface and interstices of the fibres was observed at 24^{th}hr (Fig. 7A, 7B, 7C and 7D).

B. Investigating the cell adhesion of developed epidermal layer using SEM analysis

To investigate if there are morphological differences in the ways cells attach to the developed wound healing spacer, the nature of the cell-cell and cell-material interactions was investigated and presented using Scanning Electron Microscopy. SEM images showed that the cells were firmly attached onto the developed mesh and the number of attached cells was found increasing with the increase in incubation period of time. It is remarkable that the morphology of attached L_{929}fibroblast cells was found to be different on different time period. Cells were almost flat on first 6^{th} hour of incubation time. Followed, the attached cells displayed typical elongated and spindle-like morphology after 12^{th} hour of incubation on the mesh. After 12hours of incubation, the cells covered the surface of the material. As expected after 24hours, the total surface of the wound healing spacer was completely covered by L_{929} cells. With increasing cultivation period of time, fibroblasts showed a preference for aligning in parallel to one another. The present findings indicate multiple layers of cells on mesh were resulted from intense matrix production by fibroblasts in the developed conditions.

Wound healing ability test: *In vitro* wound scratch assay of AMP-CuNP conjugates

In vitro wound healing assays have commonly been applied to measure cell migration, cell proliferation and wound closure in response to stimulation with specific agents. In this study, the AMP-CuNP conjugates used for the cell adhesion studies was determined for its ability to improve wound healing by acting directly on L_{929} mouse fibroblast cells. After creating a scratch on L_{929} mouse fibroblast cell lines, the cell migration, cell proliferation and wound closure was measured for a known concentration (100µg) of AMP conjugates at three different time periods (0^{th} hour, 12^{th} hour and 24^{th} hour). The self-wound healing ability of the developed AMP-CuNP conjugates showed that, at 0^{th} hour, no cell migration and proliferation was observed for the known concentrate (100µg) including control. At 12^{th} hour, positive cell migration and cell proliferation was observed when compared to the control sample (Fig. 8B). After 24hours, more cell proliferation was evident indicating the wound closure. The control sample showed a little more cell proliferation than the treatment group after 24^{th} hour (Fig. 8A).

REFERENCES

Anselmo Jesus Otero-Gonzalez, Beatriz Simas Magalhaes, Monica Garcia-Villarino, Carlos Lopez-Abarrategui, Daniel Amaro Sousa, Simoni Campos Dias, and Octavio Luiz Franco. Antimicrobial peptides from marine invertebrates as a new frontier for microbial infection control. The FASEB Journal. 2010; 1320-1334.

De Rosa S, Mitova M, De Caro S. New peptide from a bacterium associated with marine sponge Irciniamuscarum. In: Sener B, editor. Biodiversity: biomolecular aspects of biodiversity and innovative utilization. Springer Science & Business Media. 2002; 335-340.

Gollwitzer, H., Ibrahim, K., Meyer, H., Mittelmeier, W.R., Busch, R and Stemberger, A. (2003) Antibacterial poly (D, L-lactic acid) coating of medical implants using a biodegradable drug delivery technology, Journal of Antimicrobial Chemotherapy, 51: 585–591

Houreld N and Abrahamse H. In vitro exposure of wounded diabetic fibroblast cells to a helium-neon laser at 5 and 16 J/cm2. Photomed Laser Surg. 2007; 25:78–84.

Jasovský D, Littmann J, Zorzet A, Cars O. Antimicrobial resistance-a threat to the world's sustainable development. Ups J Med Sci. 2016; 121(3):159–164

Katyakyini Muniandy, Sivapragasam Gothai, Woan Sean Tan. In Vitro Wound Healing Potential of Stem Extract of Alternanthera sessilis. Evidence-Based Complementary and Alternative Medicine. 2018; 1:125-133.

Saginur R, Denis MS, Ferris W, Aaron SD, Chan F, Lee C, Ramotar K. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. Antimicrob Agents Chemother. 2006; 50-61.

Santajit S, Indrawattana N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. Biomed Res Int. 2016; 247-267.

Slavin YN, Asnis J, Hafeli UO, Bach H. Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. J Nanobiotechnology. 2017; 5(1): 65-70.

Suhas Raspacer, Marcelo Grijalva, Alexis Debut, Beatriz G. de la Torre, Fernando Albericio and Luis H. Cumbal. Peptides conjugated to silver nanoparticles in biomedicine – A Value added phenomenon. Biomaterials science. 2016; 12-21.

Table-1: Details of Wound dressing Material

Product Designation	Product Description
Polyester + TLC	Polyester Non-adhering wound dressing spacer with thin lipido-colloid (TLC) matrix

Table-2: Antibacterial activity of conjugate - AMP-CuNPs

S. No	Test organism	Zone of inhibition (mm)			
		10µg/ml	20µg/ml	30µg/ml	STD (Streptomycin)
1	*Escherichia coli*	09	18.3 ± 1.04	20.6 ± 1.25	23.6 ± 0.75
2	*Staphylococcus aureus*	08	16.6 ± 0.57	20.3 ± 1.04	22.9 ± 1.25
3	*Klebsiella pneumoniae*	08	17.9 ± 1.04	22.3 ± 0.75	23.9 ± 1.04
4	*Pseudomonas aeruginosa*	09	15.6 ± 0.75	19.3 ± 0.57	22.3 ± 1.04
5	*Enterobacter* sp	09	16.3 ± 1.25	19.9 ± 0.57	21.9 ± 0.57

*Mean ± Standard deviation

Table-3: Antibacterial activity of AMP-CuNPs coated wound healing spacer

S. No	Test organism	Zone of inhibition (mm)	
		Conjugate (ABA) coated materials*	Uncoated materials*
1	*Escherichia coli*	32.3 ± 0.57	0
2	*Staphylococcus aureus*	34.3 ± 1.25	0
3	*Klebsiella pneumoniae*	32.6 ± 1.04	0
4	*Pseudomonas aeruginosa*	33.9 ± 0.75	0
5	*Enterobacter* sp	33.3 ± 1.25	0

*Mean ± Standard deviation

Fig. 1: *Carangoides malabaricus* fish and collected tissue

Fig. 2: Collected tissue from *Carangoides malabaricus*

Fig. 3: Non-woven wound dressing spacer used in the study

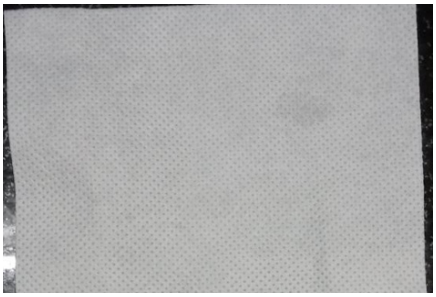
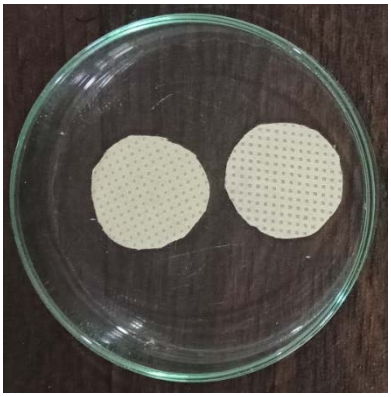


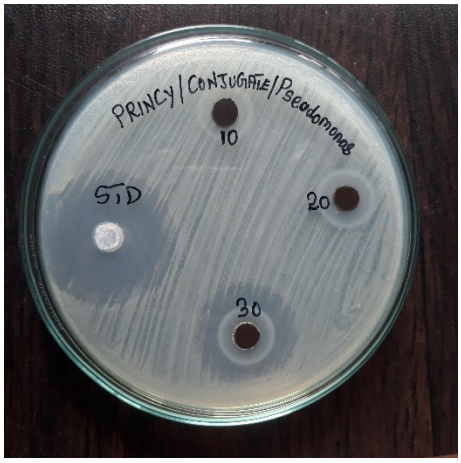
Fig. 4: Conjugate coated and uncoated wound dressing spacer samples



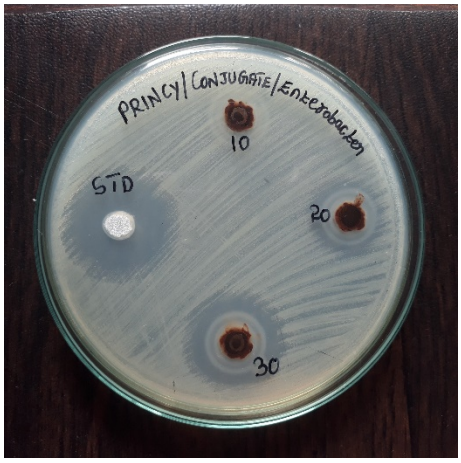
A B

A - Conjugate coated spacer, B - Uncoated spacer

Fig. 5: Antibacterial activity of AMP-CuNPs against clinical pathogens

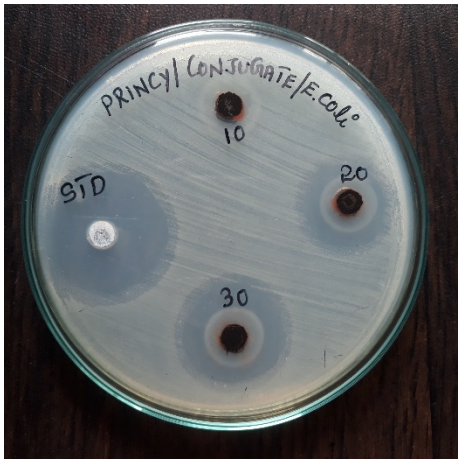


Pseudomonas aeruginosa



Enterobacter sp

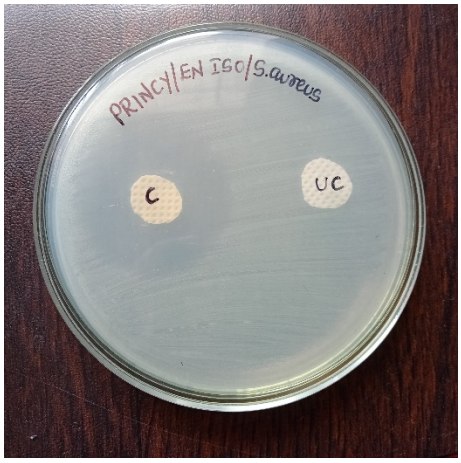
Staphylococcus aureus



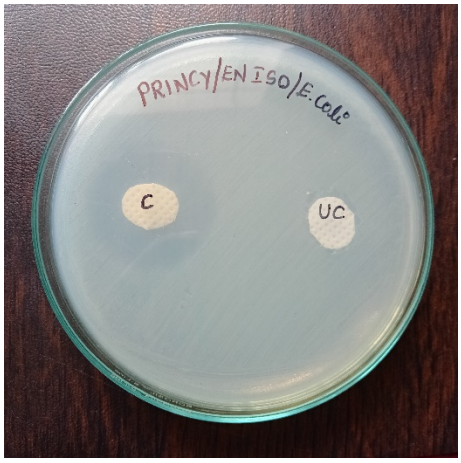
Escherichia coli

Klebsiella pneumoniae

Fig. 6: Antibacterial activity of AMP-CuNPs coated wound healing spacer



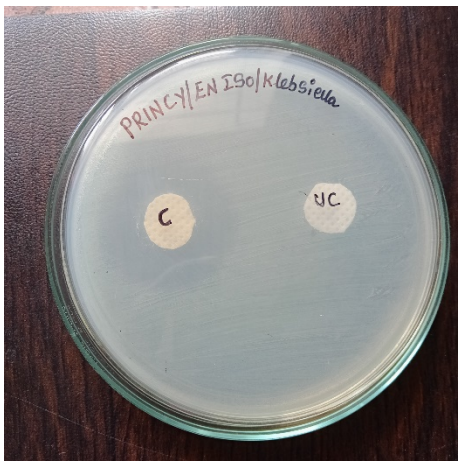
Staphylococcus aureus



Escherichia coli

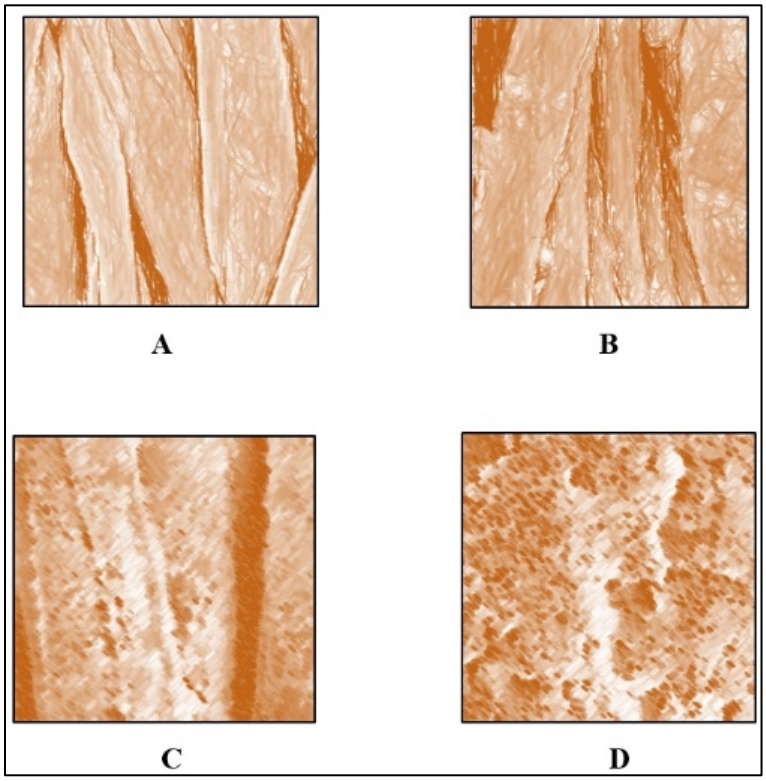
Enterobacter sp

Pseudomonas aeruginosa



Klebsiella pneumoniae

Fig. 7: L_{929} fibroblast cell proliferation and adhesion – SEM analysis



A: 0th hour, B: 6th hour, C: 12th hour, D: 24th hour

Fig. 8: *In vitro* wound scratch assay of AMP-CuNP conjugates

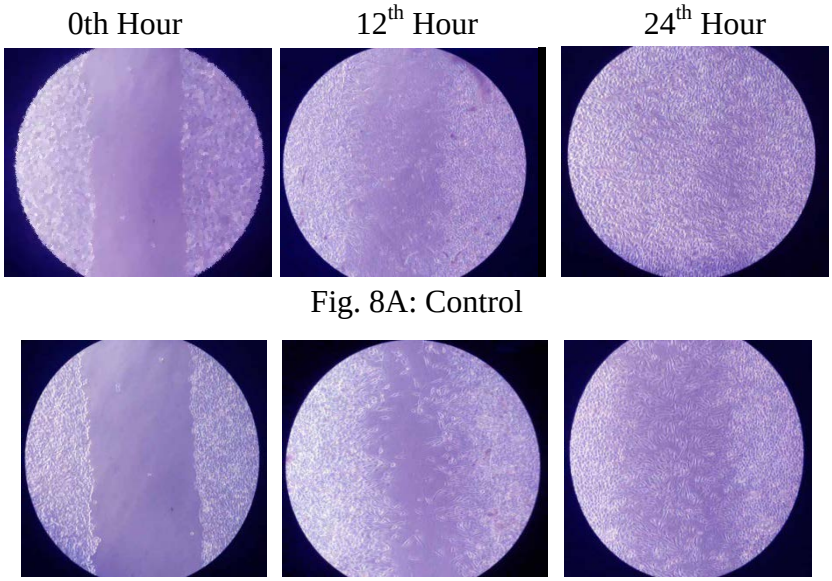


Fig. 8A: Control

Fig. 8B: Sample (100µg)