were visualized after thawing under a field emission scanning electron microscope (Houreld and Abrahamse, 2007).

Determining the antibacterial activity of AMP conjugate coated wound healing material

Cell developed wound healing mesh coated with AMP conjugate were analyzed for antibacterial activity using a standard disc diffusion method as described by El-Rehewy et al., 2009). The antibacterial activity of AMP coated mesh was earlier presented in our previous publication (Deepak Tom Jose et al., 2020). Briefly, the mesh was cut (disc shaped-20mm) and placed over MHA plates pre-seeded with test organisms. Mesh without AMP conjugate was used as control samples. Zone around the AMP conjugate-coated mesh was measured after 24 hours of incubation and the results were presented as mean ± standard deviation

Analyzing the wound healing ability of developed AMP conjugates using *in vitro* wound scratch assay

L_{929} mouse fibroblast cells grown in 24 well plates at a density (1 X 10^5 cells per ml) with ~80% confluence was taken for the analysis. A small linear scratch was created in the confluent monolayer by gently scraping with sterile cell scrapper as per the method of Liang et al. (2007). After creating a scratch on L_{929} mouse fibroblast cell lines, the cell migration, cell proliferation and wound closure was measured for the selected concentrate (100µg) of AMP conjugates at different time periods (0^{th} hour, 12^{th} hour and 24^{th} hour). Migration of cells between the scratch site and the distance traversed by cells migrating into the denuded area which emphasize the self-healing was observed using Phase contrast microscope for each time period.

RESULTS AND DISCUSSION

L_{929} fibroblast cell proliferation and adhesion

L_{929} fibroblast cells attachment and its stage wise development on wound dressing mesh 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 (Fig. 5A). Partial cell growth was observed on 6^{th} hr (Fig. 5B). Cell attachment was found to be evident on 12^{th}hr (Fig. 5C) and proliferated cell covering the entire surface and interstices of the fibres was observed at 24^{th}hr (Fig. 5D). 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.

Antibacterial activity of AMPs conjugate coated mesh samples

Antibacterial activity of cell developed wound healing mesh coated with AMP conjugates were analyzed against different test bacteria. The antibacterial activity was expressed based on the inhibitory zones measured around each conjugate coated mesh samples.

Escherichia coli and *Staphylococcus aureus* exhibited inhibitory zones of 32.1 ± 0.56 mm and 31.3 ± 1.25 mm for the coated mesh samples. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* exhibited the inhibitory zones of 26.3 ± 1.04 mm and 29.3 ± 0.56 mm against their respective coated samples. *Acinetobacter baumannii* showed significantly 30.1 ± 1.25mm of inhibitory zones for the test samples (Table-1 and Fig. 4). The obtained results revealed that the antimicrobial conjugates increased the therapeutic effect against the test organisms. The mode of action of AMP 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.

Wound healing ability of developed AMP conjugates using *in vitro* wound scratch assay

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 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). Fig. 6 corresponding to self-wound healing ability of the developed AMP conjugates showed that, at 0^{th} hour, no cell migration and proliferation was observed for the known concentrate (100µg) including control (Distilled water). At 12^{th} hour, positive cell migration and cell proliferation was observed when compared to the control sample. After 24hours, more cell proliferation was evident indicating the wound closure. In Fig. 6, the control showed a little more cell proliferation than the treatment group after 24^{th} hour. This was due to a small delay in the healing process of the AMP conjugate samples on the developing L_{929} cell lines when compared to the control. The reason for this little difference was illustrated from the research

article of Peplow and Chattrejee, (2013). The researchers explained that many growth factors and cytokines enhanced migration of keratinocytes *in vitro*. This migration of keratinocytes may vary from one sample to another depending on these above factors. However, in our present study 100% wound closure was clearly evident in both the control group and the sample groups after the incubation study period. The results revealed that AMP conjugates could be used as wound healing agents for any biomedical cases like diabetic foot ulcers, boil wounds, bur wounds, accidental cuts/wounds, etc.

In vitro scratch assay could be recorded as an appropriate and inexpensive method for the wound healing potential of herbal composite used in the present research.

Similar *in vitro* wound scratch assay method was recorded from the literature survey. Srinivasa Rao Bolla et al. (2019) recently investigated the wound healing capacity of *Aristolochia saccata* leaf extract by using *In vitro* wound scratch assay, where proliferative and migratory capabilities of test compounds could be monitored through microscopy studies. L_{929} fibroblast cell line was used for the assay. Scratch assay showed 34.05%, 70.00%, 93.52% wound closure at 12hrs, 24hrs and 48hrs of incubation respectively. These results were similar compared to positive control which showed 37.60, 56.41 and 99.05% of wound closure. As there was similar wound healing abilities were noted for many antimicrobial compounds from the literature survey on L_{929} mouse fibroblast cell lines, it was proved that the developed AMP conjugates could be used for the development of novel tissue engineered wound healing materials.

CONCLUSION

Antimicrobial peptides extracted from *Streptomyces* sp and *Carangoides malabaricus* were developed as conjugate. The conjugate was mixed with PVA and coated with wound dressing mesh samples. The cell adhesion assay, antibacterial activity and *in vitro* wound healing assay of developed tissue engineered wound dressing mesh revealed a need for such a novel product for treating complicated diabetic foot ulcer infective cases. Based on the obtained wound healing ability of developed conjugates, it can be concluded that the developed tissue engineered wound dressing material could be useful to treat diabetic wounds. The fibroblast migration and proliferation of cells could able to favour in increasing the levels of cytokines, growth factors and keratinocytes at the wound site which results in effective wound healing in diabetic wounds. The results showed that the developed tissue

engineered wound dressing has commercial interest in pharmaceuticals companies for the manufacturing of such materials in near future with different types of drugs coating on it.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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Fig. 1: *Carangoides malabaricus* fish for extraction of AMP

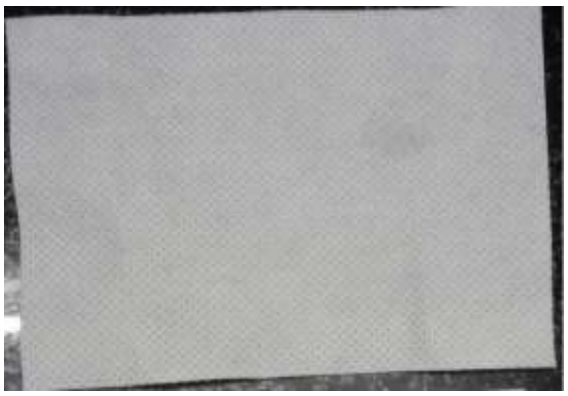


Fig. 2: Non-woven wound dressing mesh used in the study

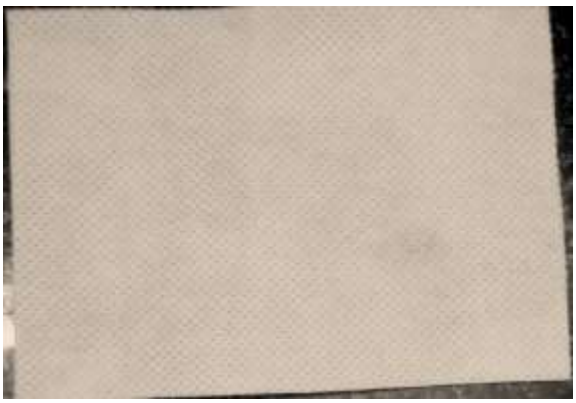
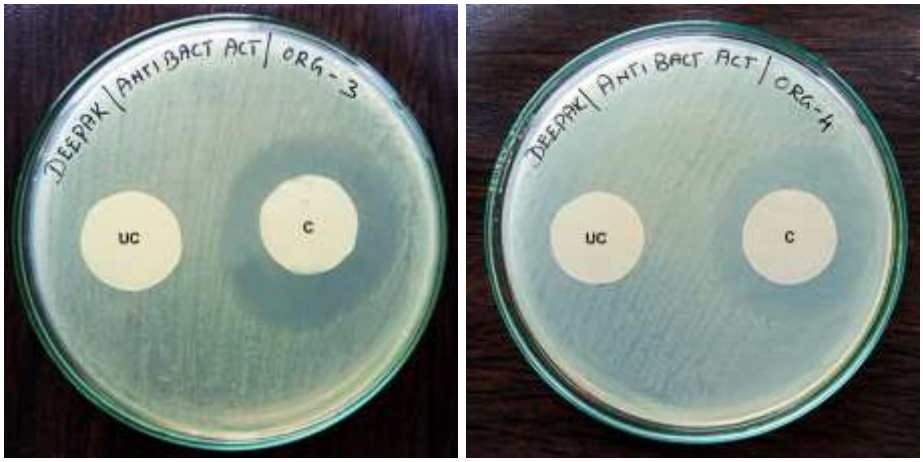


Fig. 3: Wound dressing mesh coated with AMP conjugates and PVA

Escherichia coli *Staphylococcus aureus*



Klebsiella pneumoniae *Pseudomonas aeruginosa*

Acinetobacter baumannii

Fig. 4: Antibacterial activity of AMPs + PVA coated mesh samples

(UC: Uncoated C: Coated)

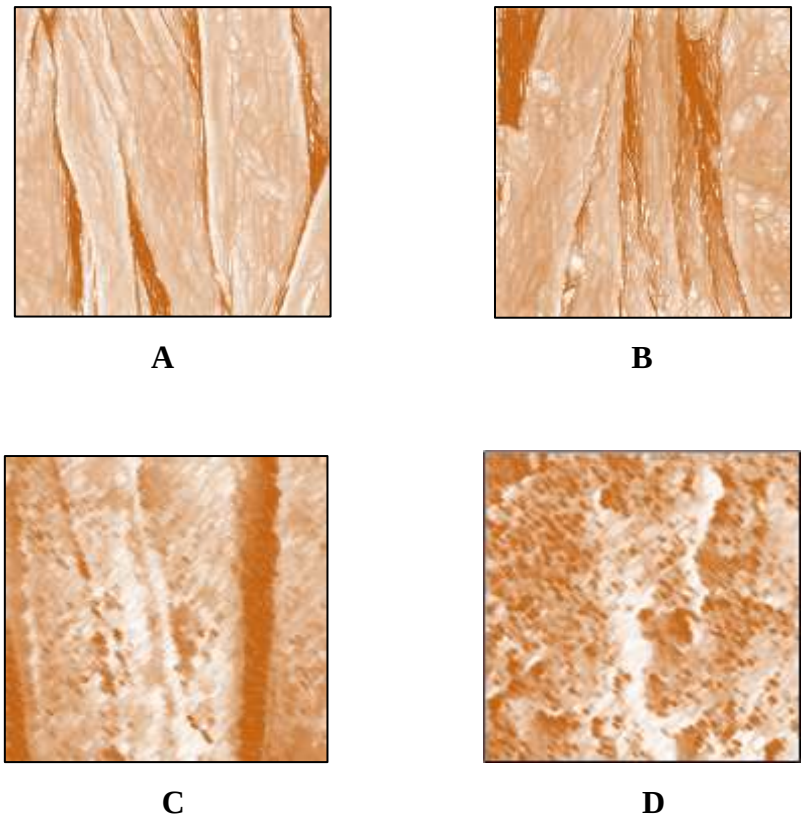


Fig. 5: L_{929} fibroblast cell proliferation and adhesion

A: L_{929}fibroblast cell development on wound dressing mesh at 0^{th} hour

(No cell growth)

B: L_{929}fibroblast cell development on wound dressing mesh after 6^{th} hour

(Partial cell growth)

C: L_{929}fibroblast cell development on wound dressing mesh after 12^{th} hour

(Cell attachment evident)

D: L_{929}fibroblast cell development on wound dressing mesh after 24^{th} hour

(Proliferated cell covering the entire surface and interstices of the fibre)

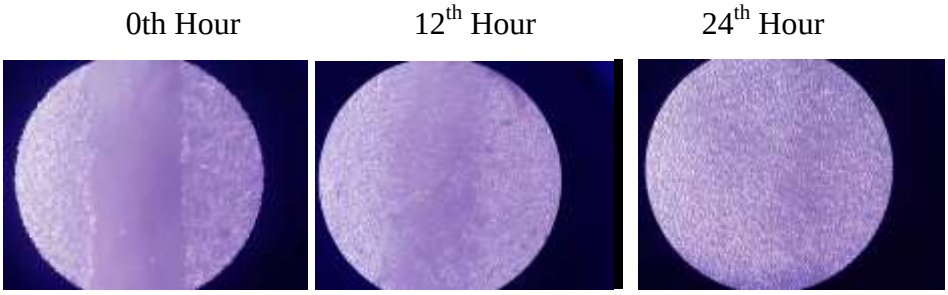


Fig. 6A: Control (Distilled water)

Fig. 6B: Sample (100µg)

Fig. 6: Self-wound healing scratch assay: *In vitro* Wound Scratch Assay

Table-1: Qualitative antibacterial activity of AMPs + PVA coated materials

S. No	Test organism	Zone of inhibition (mm)
		AMPs + PVA coated*
1	*Escherichia coli*	32.1 ± 0.56
2	*Staphylococcus aureus*	31.3 ± 1.25
3	*Klebsiella pneumoniae*	26.3 ± 1.04
4	*Pseudomonas aeruginosa*	29.3 ± 0.56
5	*Acinetobacter baumannii*	30.1 ± 1.25