



Preparation of biocompatible polymeric bio-scaffold as wound healing activity: Characterization and In vivo analysis

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ABSTRACT

In this study, a combination of collagen (CL), gelatin (GL) and squid ink (SI) were used to produce a biocompatible polymeric bio-scaffold (CL: GL and CL: GL: SI) was examined for wound healing purposes. Biocompatible polymeric bio-scaffold (BPBS) plays an important role in wound care due to their cost-effective, good mechanical properties, bio-compatible and antimicrobial characteristics. Thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR) of the BPBS has showed the heat stability and nature of samples, respectively, and while scanning electron microscopy (SEM) images revealed the blending of CL fiber, GL, and SI pattern interface. BPBS displayed excellent antimicrobial activity against both *E.coli* and *S.aureus*. The inhibition zone of BPBS results obtained in 4.57 ± 0.30 mm against *E.coli* and 4.74 ± 0.15 mm against *S.aureus*. Mechanical characteristics of BPBS shows tensile strength of 20.88 ± 0.05 (Mpa), 17.74 ± 0.05 (%) as Elongation at break, 26.31 ± 0.21 (%) of flexibility and water absorption of 29.80 ± 0.05 (%). *In vitro* analysis of CL: GL: SI, displayed 100% biocompatibility and cell viability was found 3rd days. In vivo study using mice by excisional animal model examined the CL: GL: SI scaffold can significantly accelerate the process of wound healing.

1. Introduction

Collagen is a significant biopolymer of animal origin. It is the basic fundamental protein in the extracellular space in the different connective tissues (Veit et al., 2006). Collagen has numerous applications in prescription and makeup. In our study we demonstrate one of the strategies to acquire collagen films from collagen arrangements. These collagen arrangements were analyzed based on rheological estimations. Collagen was first solubilized and reconstituted, to keep up its underlying properties (Knapp et al., 1977). Industrial collagen of fresh skin origin has been used in functional foods, biomedical materials and cosmetics (Senthil et al., 2016). Type I collagen is through structural chain molecules include the dermis and migrating from the signal

sequence of amino acids with keratinocyte prospective intermingle with protein (Matthew et al., 2009).

Collagen, as one of the most common cationic biopolymer, has been demonstrate to have physical, chemical and biological properties that supports its use as wound dressing materials including tensile strength, fiber orientation, membrane semi permeability, low antigenicity and its positive effect on wound healing mice and hemostatic properties (Arian et al., 2020). Collagen is constitutionally contributed in all stages of the wound healing and a lead of collagen grounded dressing materials delivers numerous benefits for chronic and acute wound closure. Collagen, which constitutes about 40% of the protein in a living being, is the support substance for conjunctive tissue, and is necessary for the tissue to function. Collagen is the main constituent of extracellular matrices

Abbreviations: CL, Collagen; MHA, Muller Hinton Agar; GL, Gelatin; DMEM, Dulbecco's Modified Eagles Medium; SI, Squid ink; FBS, Fetal bovine serum; BPBS, Biocompatible polymeric bio-scaffold; NIH 3T3, Fibroblast cells line; TGA, Thermo gravimetric analysis; NaCl, Sodium chloride; FTIR, Fourier transform infrared spectroscopy; NaOH, Sodium hydroxide; SEM, Scanning electron microscopy; H₂SO₄, Sulfuric acid; SZM, Stereo zoom microscopic; *E.coli*, *Escherichia coli*; EG, Ethylene glycol; *S.aureus*, *Staphylococcus aureus*; MTT, (3-(4,5-dimethylazol-2-yl)-2,5-diphenyl-tetrazolium bromide).

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and having very high biocompatibility and wound healing properties that makes this proteinous component of the collagen to be used in various fields of the biomedical science and industries (Ting et al., 2020).

Biocompatible polymers are abundantly found in renewable resources, such as agro-industrial and marine waste such as fish skin, fish scale and fishbone (Senthil et al., 2016). Gelatin obtained by hydrolysis of the collagen of fish skin, fishbone, fish scale and connective tissues of land and marine animals is of low cost and is easily available. They find their use in many applications (Cao et al., 2007). Gelatins possess excellent biodegradability, biocompatibility, non-toxic and wound healing properties and have great potential as edible films and tried their use in a specific application in pharmaceutical, packaging and biomedical fields (Rajini et al., 2001).

Squid fish commonly live in seawater and belong to the family of Cephalopoda. Cephalopods are common and important in the Indian fisheries industry because of their economic values. Cephalopods are more amount of ink is produced as fish processing industry waste. Useful products produced from this squid fish would be an economical way of waste utilization. Cephalopod ink is used for many practical and commercial purposes such as particularly in medicine, millennia, art, cuisine, etc., and a potential source of good bioactive compounds (Hochberg, 1980). Since many patients suffer from skin injuries or burns, wound dressing is essential both the medical field and everyday life. The basic properties of wound dressings should be biocompatibility and nontoxicity. Furthermore, the complexities of the skin wound environment, as well as the need for rapid wound healing; necessitate a variety of wound dressing functions (Hadi et al., 2020).

There are different numbers of wound dressing material available in the market, in various forms such as gels, pastes, films, scaffold, membrane, polymer, oxidized regenerated cellulose, ethylene diamine tetra acetic acid and powder (Vig et al., 2017). Many authors have reported that collagen films prepared from chrome shavings leather waste possess hemostatic properties and can be a suitable material for wound dressing applications (Senthil et al., 2020). Several collagen wound dressing materials cover with antibacterial mediator has been protecting micro-organism with inside and outside of the wound (Rath et al., 2016). Several research studies report collagen-based wound dressing materials to produce enhanced fibroblast production, fibroblast permeation, migration of cells, deposition of oriented and collagen fibril by attracting fibroblasts (Cole et al., 2018).

To treat wound with bacterial infection, antibacterial therapeutics may be applied topically in the form of wound dressing, antiseptic cream or gel. As a results, for wound healing treatment, a delivery method that maintains antibiotic release to the wound area. Depending on the clinical presentation, several wound dressing has been used to chronic wounds (Ibrahim et al., 2020). In general, wound dressing materials, caused a more bacterial infection and high toxicity properties. Since recent research has focused on the development of antibacterial wound dressing materials as antibacterial agent reinforcements, this study attempted to apply this technology to the preparation of antibacterial wound dressing materials.

In this research study, BPBS was prepared from fishbone collagen, fish skin gelatin, squid fish ink. The BPBS was characterized for its physicochemical and mechanical properties. *In vitro*/*In vivo* and antimicrobial properties of the BPBS were also analyzed. All the experimental findings showed that these BPBS had a greater wound healing efficacy in the repair of incised skin tissue defects.

2. Materials and method

Fishbone of Bluefin Trevally (*Caranx melampygus*), fish skin of pufferfish (*Lagocephalus gloveri*) and ink of squid fish (*Cephalopoda*) were collected from Jeppiar fishing harbor, Muttom, Kanyakumari- 629202, Tamilnadu, India. NaCl (99.9%), acetic acid (99.85%), NaOH (99%), citric acid (100%) and H₂SO₄ (98%) were purchased from Sigma Alrich

India Co Ltd, (Chennai, India). Distilled water (purity 99.9% and conductivity 0.5 to 3 µmhos/cm) was obtained from Astrra chemicals, Chennai, India.

2.1. Preparation of squid ink (SI)

The squid ink was isolated according to the method of Ikram et al. (2009). *Cephalopoda* squid fish was identified and fresh squid fish were obtained and washed with clean water. The freshly obtained squid's ink portions from squid were dissected and ink glands was manually removed from the viscera and placing in a container and freeze-dried. The freeze-dried sample was minced to a fine powder using mortar and pestle. 1g of powder sample with 70% ethanol (v/v) was taken by using the orbital shaker (180 rpm) for a period of 12 h at 28 °C. Later, the mixtures were centrifuged at 3000 rpm for 1h and obtain a final content.

2.2. Preparation of collagen (CL)

Fish bones (*Caranx melampygus*) were well sized small pieces. The sized samples were washed with chilled water for 10 min. The cut bones were treated with chloroform: acetone in the rate of proportion 2:2 to remove fat content. The defatted sample was further treated with 50 g/L NaCl (v/w) at 4 °C to remove the non-collagenous materials and other residues, followed by extraction using acetic acid (2 g fishbone with 200 ml of 0.10 mol/L acetic acid) and pepsin (1,100 U/ML) at 4 °C for 48h. The extracted sample was using centrifuge on 8000 rpm apply for 10 min. The supernatant solution was collected, adding NaCl (0.7 mol/L). The collected collagen sample was purification using dialysis. Dialysis: 1% collagen supernatant solution in 0.1 M acetic acid with pH level 2.9 was prepared and placed in dialysis tubes with a 16 mm diameter by using the stirrer for 80 rpm at overnight.

2.3. Preparation of gelatin (GL)

Gelatins were prepared according to the method of Grossman and Bergman (1992). In brief, 500 g of fish skin was taken and cleaned with tap water. The fish skin was treated using 0.5% NaOH for 42h and washed with water and then subjected to acid incubation using 0.3% w/v sulfuric acid and then in a 2.0% w/v citric acid, followed by distilled water at 45 for 24 h. They were then extraction of gelatin was done and filtered using Whatman No.4 filter paper and preserved at 70 °C for 48 h. Water content was removed using the freeze-drying process.

2.4. Biocompatible polymeric bio-scaffold (BPBS)

BPBS was prepared using 3g of collagen was dispersed in 5 mL of distilled water, 1g of GL was dispersed in 2 mL of distilled water and 1 wt % SI using a homogenized in the presence of 400 µL of ethylene glycol (Muthukumar et al., 2013). The resultant colloidal solution of CL: GL: SI was cast (5 × 5 cm) into a scaffold and dried at 30 °C for 24h. Two other scaffolds (CL and CL: GL) were prepared as CL (5 mL of CL and 280 µL ethylene glycol (EG) with distilled water) and CL: GL (5 mL of CL, 2 mL of GL and 400 µL ethylene glycol (EG) with distilled water).

In brief, 2 g of collagen paste was dispersed in 20 mL of distilled water and homogenized in the presence of 500 µL ethylene glycol (EG). The resultant solutions were cast into films by drying at 30 °C–35 °C.

2.5. Characterization

The BPBS was analyses for its physico-chemical, mechanical, antimicrobial activity, *In vitro* and *In vivo*.

2.5.1. Antimicrobial activity

Escherichia coli and *Staphylococcus aureus* were testing the antimicrobial properties of the CL: GL: SI, CL: GL and CL scaffolds. Antibacterial properties was analyzed by the disc diffusion test make use of

Muller Hinton Agar (MHA), and zone of inhibition formed after incubation time was measured and recorded.

2.5.2. *In vitro* study

The culture cells (NIH 3T3 fibroblast cells) were kept up in DMEM-Dulbecco's Modified Eagles Medium with FBS (fetal bovine serum) 10% using 37 °C in a carbon dioxide humidified incubator. 3-(4, 5-dimethylazol-2-yl)-2, 5-diphenyl-tetrazolium bromide test was using to examine toxicity. NIH 3T3 fibroblast cells were added on CL: GL: SI, CL: GL and CL scaffold instead well plates (24 plates). Later, FBS medium was added and transferred to incubator for 1, 3 and 5 days at 37 °C after incubation visibleness was analyzed from microplate reader (spectra max M4). Culture plate in the absence of experimental samples was take hold as control. The % of cell viability was calculated comparing with the control (Jitendra et al., 2013).

2.5.3. *In vivo* experiment

Male mice weighing 150–180 g were maintained at room temperature and appropriate humidity in animal house. Models of wounds of dimension were 4 mm length was incised exposing the subcutaneous adipose tissue using a sterile surgical blade. The wound in the control mice group and experimental groups (CL, CL: GL and CL: GL: SI) was handled with twice in 3 days. The mice was divided through two category (n = 4), the control and experimental were maintained for period of days 2, 8 and 14.

2.5.4. Histology study

A section of cut sample from wound layer test from the individual batch was gathering a day on 2, 8 and 14 and histopathological assessment was go through the analysis. Tissue was arranged in neutral buffered formalin (8%), alcohol with installed paraffin wax. Section of 4 µm was get make use of microscopy after insert with scale and stained with hematoxylin and eosin.

3. Results

Marine food waste is one such potential waste which can be converted into valuable products. This present development attempts to prepare BPBS with wound healing properties from collagen, gelatin and squid ink. The BPBS possessed better cell viability, physio-chemical and antimicrobial properties. The present product deciphers a cost-effective technology for the production of BPBS and feasible technology for recycling of marine food wastes. The production of BPBS which is used in biomedical industries for a variety of purposes has gained significance. The more medical sector coming into existence to provide to the growing needs of the people, it is predicted that the demand for absorbable collagen scaffold. The schematic representation of the prepared BPBS is shown in Fig. 1.

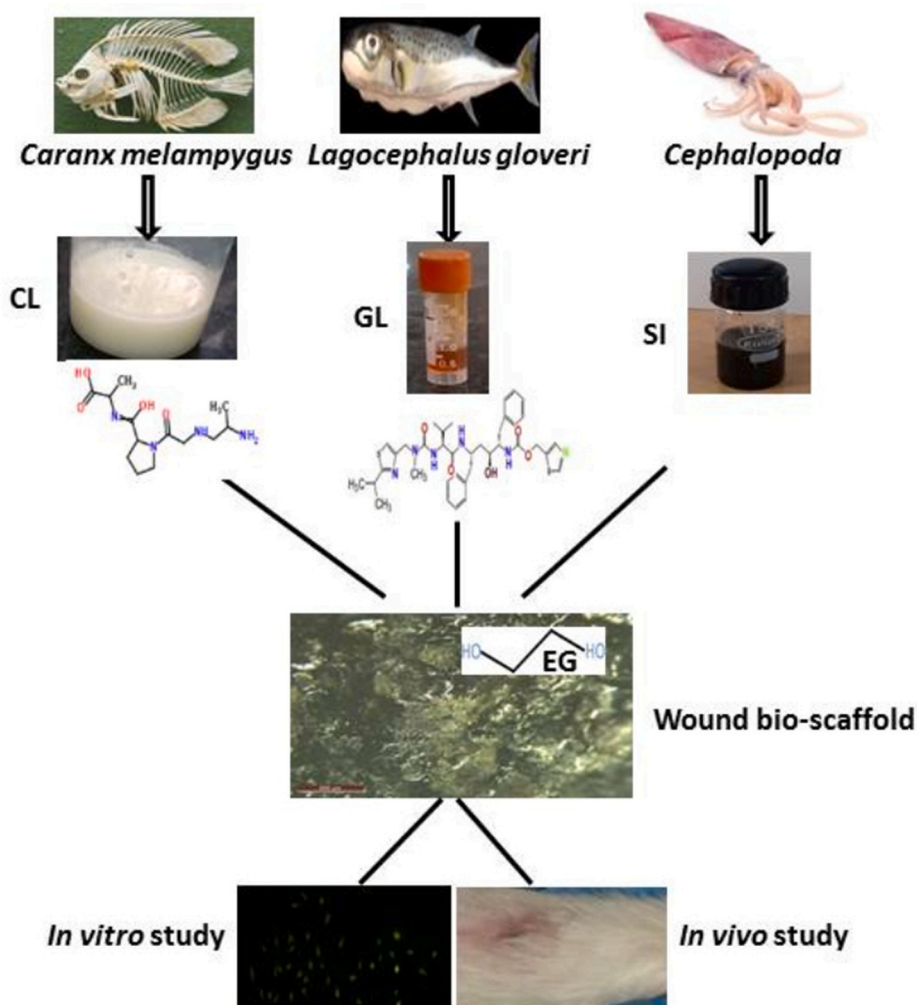


Fig.1. Schematic representation of biocompatible polymeric bio-scaffold.

3.1. Preparation of squid ink (SI)

The ultraviolet absorption spectrum of the SI fraction was shown in Fig. 2a. The fraction showed a strong absorption band at 390 nm. FTIR spectra of the SI were shown in Fig. 2b. The protein showed the presence of a characteristic group of amide II and III, wavenumber at 1547 and 1321 cm^{-1} , respectively. TGA was performed to assess the thermal stability of the SI. SI (Fig. 2c) two-step weight loss was first identified of around 9% at 93 °C and second was 70% at 380 °C and 13% final residue. The morphology of SI (Fig. 2d) explains the scanning electron microscopy and confirmed the porous nature due to the linking of protein and melanin. The antimicrobial activity assessment showed that SI exhibited antimicrobial properties on Gram (+) bacteria (*E.coli*) and Gram (–) bacteria (*S.aureus*) and revealed the inhibition zone of SI (Fig. 3 (a&b)).

3.2. Biocompatible polymeric bio-scaffold (BPBS)

3.2.1. FTIR

CL, CL: GL and CL: GL SI (Fig. 4a) was characterized for their functional group. The representative amide I, II and II connection present at 1629, 1541 and 1235 cm^{-1} could be identified as evidence for CL. In GL, the peak was found about 3,535 cm^{-1} .

3.2.2. TGA

TGA was performed to measure thermal characteristics of the CL: GL: SI, CL: GL (Fig. 4(b–d)) and CL scaffold as a purpose of bio-adhesion condition. In CL, initial degradation of about 84% between 225 °C and 550 °C. CL: GL scaffold exhibited degradation temperature between 255 °C and 580 °C. Major weight reduction (90%) noted between 180 °C

and 590 °C which could be due to the decomposition collagen, gelatin, and squid ink.

3.2.3. SZM

Stereo zoom microscopic investigation was executed (type LEICA M205A, Camera MC170HD, Make Germany) instrument. The results of the surface (low magnification) for CL, CL: GL and CL: GL: SI scaffold were analyzed by the stereo zoom microscopy and results are shown in Fig. 5(a–c). The CL, CL: GL and CL: GL: SI scaffold had a smooth surface and collagen fibers were visualized in all the samples.

3.2.4. SEM

The surface morphology of CL, CL: GL and CL: GL: SI was analyzed using scanning electron microscopy Fig. 5(d–f). Collagen fibers were observed as a perfectly smooth surface and they were spread fine in gelatin. The SEM images showed proper binding of gelatin and squid ink powder by collagen. CL: GL: SI was found to reveal smaller porosity with irregular texture and agglomeration of ethylene glycol.

3.2.5. Mechanical properties

Mechanical properties of CL, CL: GL and CL: GL: SI was given in Table 1. Tensile strength revealed the strength and elasticity of the samples. The wound dressing materials should be preferably strong and flexible. Three sample preparations differed significantly ($p < 0.05$) in mechanical strength. CL: GL: SI possessed high values of mechanical strength when compared to the other two samples (CL and CL: GL). These results indicated that CL, CL: GL were tough and hard, whereas CL: GL: SI was more soft and flexible.

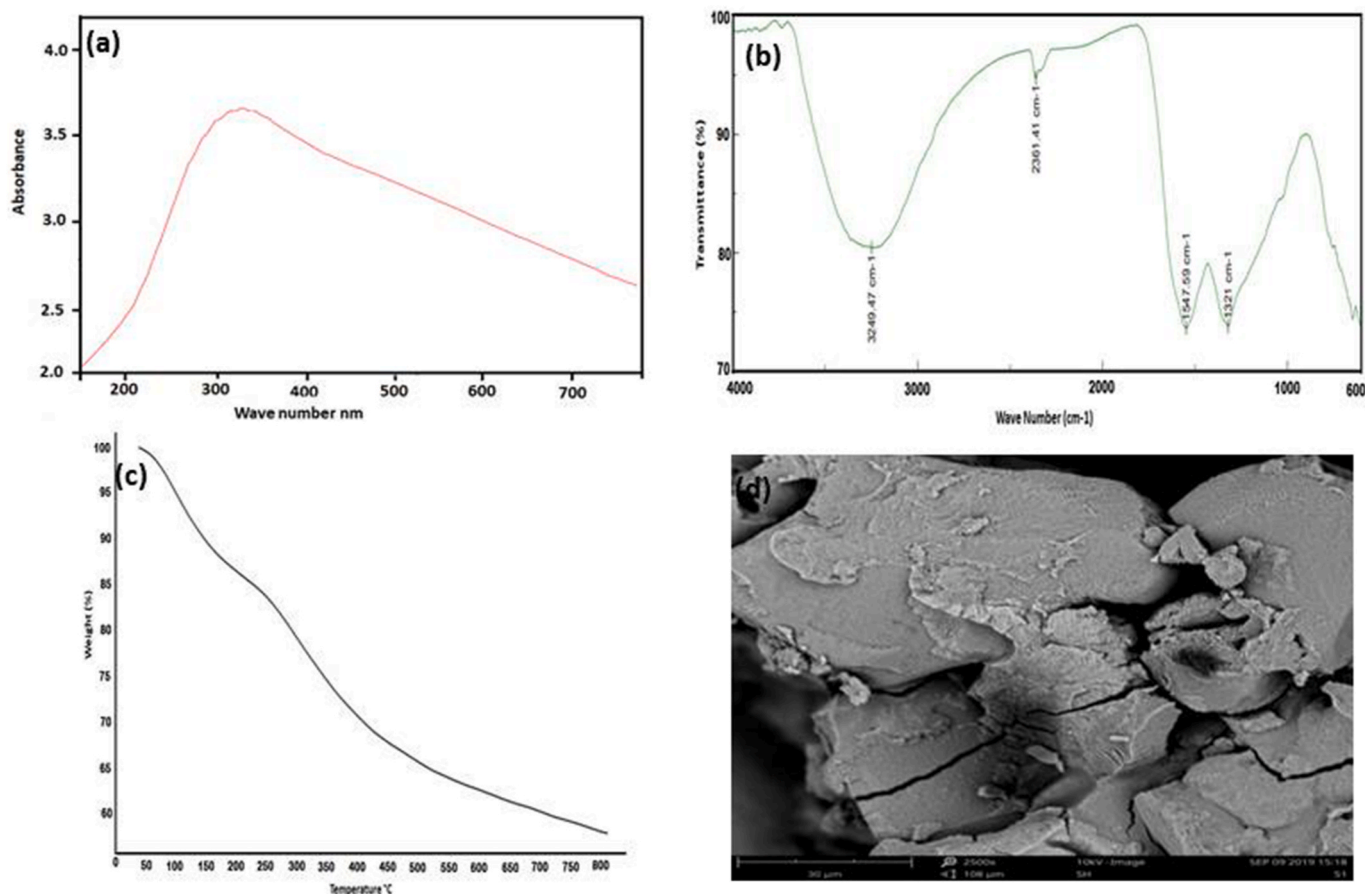


Fig. 2. (a) UV of SI (b) FTIR of SI (c) TGA of SI (d) SEM of SI.

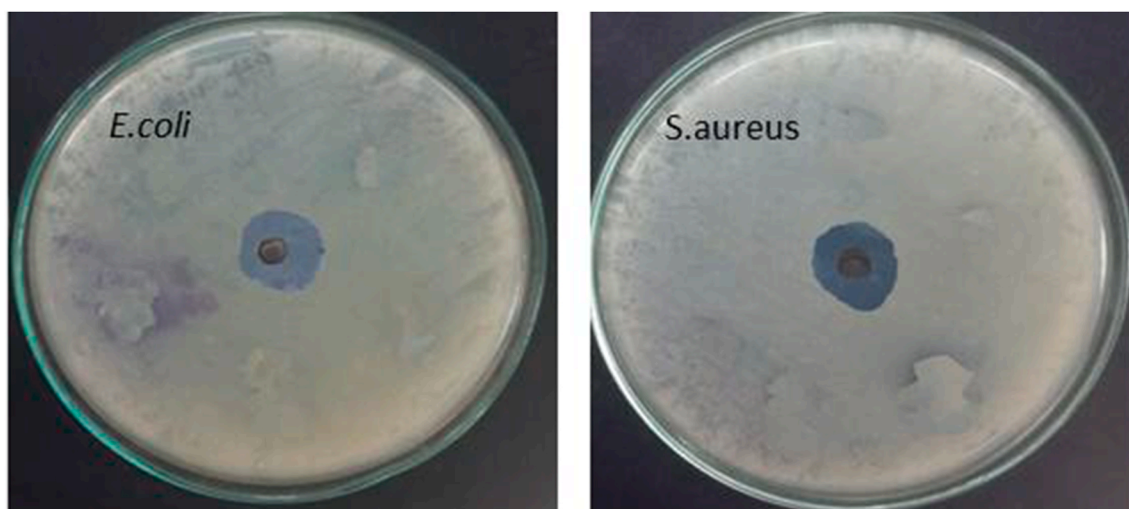


Fig.3. Antimicrobial study (e) Inhibition zone of SI against *E.coli* (f) Inhibition zone of SI against *S.aureus*.

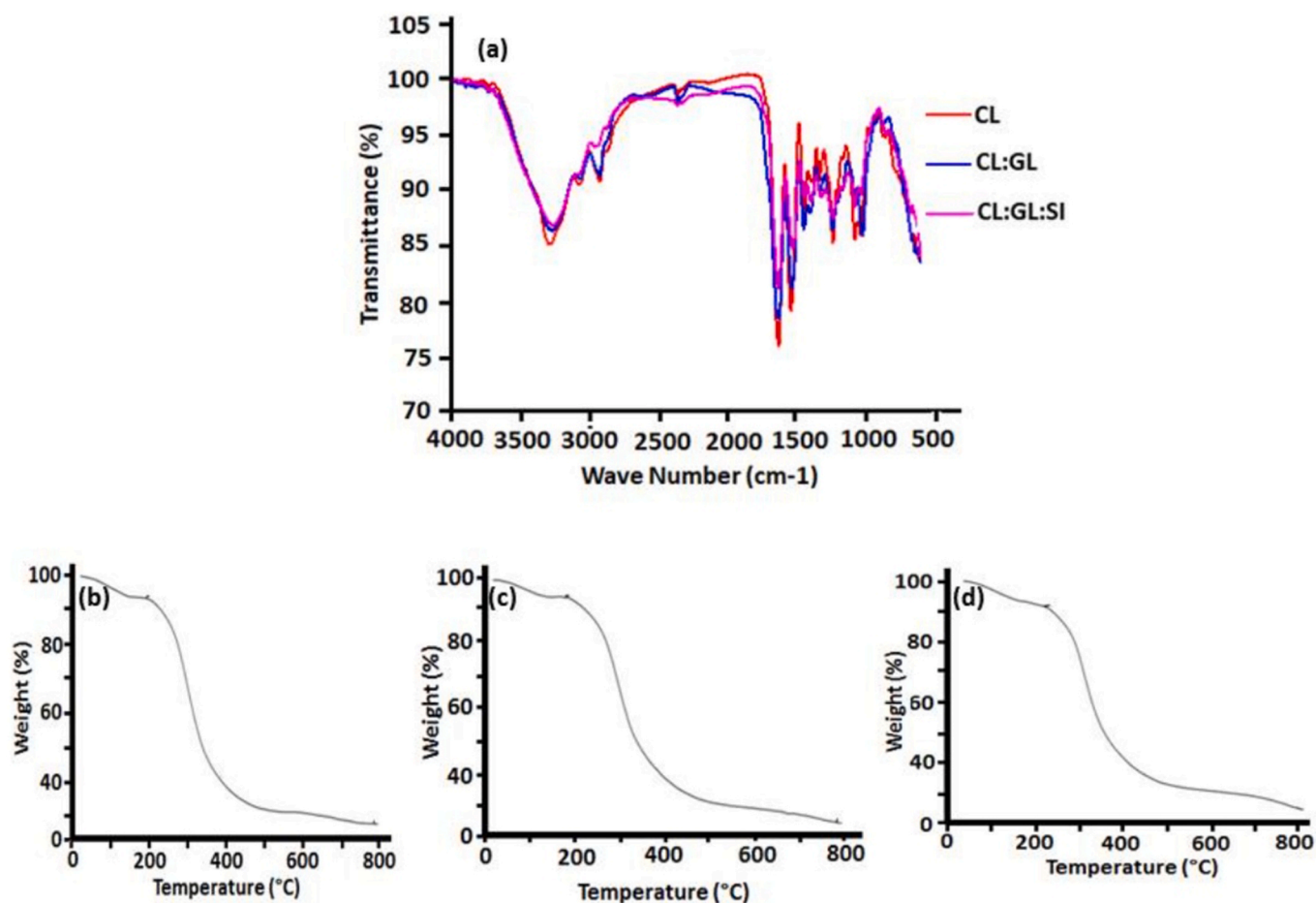


Fig.4. (a) FTIR of CL, CL: GL and CL: GL: SI. TGA of (b) CL (c) CL: GL (d) CL: GL: SI.

3.2.6. Antimicrobial activity

The antimicrobial activity of CL, CL: GL and CL: GL: SI scaffold was evaluated by using a good diffusion method against *S.aureus* and *E.coli* cultures. CL: GL: SI scaffold showed a better antibacterial effect comparison of CL, CL: GL scaffold (Fig. 5 g&h). Presented in Table 2, displayed results of all types of scaffolds were statistically significant in $p < 0.05$ values.

3.2.7. In vitro study

The cell culture assay using NIH 3T3 cells was performed to assess the viability of cells in CL, CL: GL and CL: GL: SI scaffolds were examined using fluorescence microscopy and MTT (3-(4,5-dimethylazol-2-yl)-2,5-diphenyl-tetrazolium bromide) analysis, respectively. Fluorescence microscopy images of viable of NIH 3T3 cell appearance of CL, CL: GL and CL: GL: SI was shown in Fig. 6a. The results were also compared with the

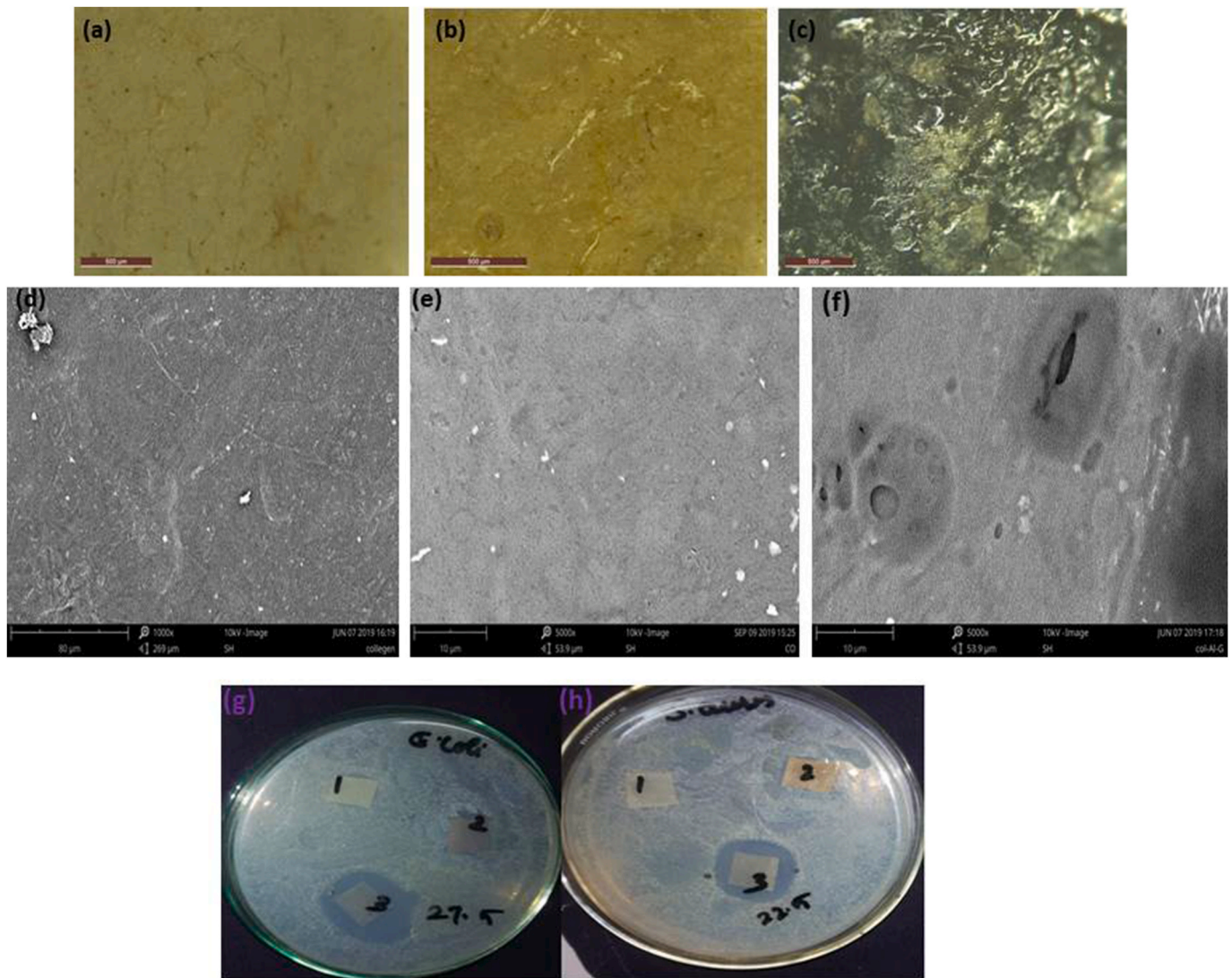


Fig. 5. (a–c) SZM of CL, CL: GL and CL: GL: SI, (d–f) SEM of CL, CL: GL and CL: GL: SI and antimicrobial activity (g) Inhibition zone of CL, CL: GL and CL: GL: SI against *E. coli* (h) Inhibition zone of CL, CL: GL and CL: GL: SI against *S. aureus*.

Table 1
Mechanical properties of CL, CL: GL and CL: GL: SI (BPBS).

Samples	Tensile strength (MPa)	Elongation at break (%)	Flexibility (%)	Water absorption (%)
CL	15.32 ± 0.20	14.21 ± 0.12	22.43 ± 0.09	25.74 ± 0.27
CL:GL	18.40 ± 0.37	16.11 ± 0.09	24.99 ± 0.10	29.13 ± 0.01
CL:GL:SI (BPBS)	20.88 ± 0.05	17.74 ± 0.05	26.31 ± 0.21	29.80 ± 0.05

The data are presented as mean ± SD of three individual experiments.
* $p < 0.05$, as compared to CL, using Duncan's multiple range analysis.

Table 2
Antimicrobial activity of BPBS.

Organism	Zone of Inhibition, BPBS
<i>E. coli</i>	4.57 ± 0.30
<i>S. aureus</i>	4.74 ± 0.15

The data are presented as mean ± SD of three individual experiments.

control sample. Fig. 6b MTT assay results on the cell viability and revealed no toxicity on the CL: GL: SI on 1, 2 and 3 days were noted.

3.2.8. In vivo experiment

Wound closure was showed in experimental groups (CL, CL: GL and CL: GL: SI) in comparison with the control group and results are shown in Fig. 7 (a&b). On day 8, the wound healing effect of control and experiments- CL, CL: GL and CL: GL: SI groups were 45%, 65%, 75% and 88% of wound contraction respectively. In CL: GL: SI treated groups, the wound size, and complete wound healing was observed from 14th days, whereas incomplete wound closure was detected in the control groups. Fig. 7b shows illustrative wound closure rate (%) of control and CL, CL: GL and CL: GL: SI groups on days 2, 8 and 14. Microbial environment and inflammatory response are significant mechanisms in wound healing and hence our results established that collagen and gelatin wound dressing materials could be a natural process with better wound healing.

3.2.9. Histology study

To evaluate the role of control, CL, CL: GL and CL: GL: SI was histopathological examined (Fig. 6c). The section of the samples was found by experimental and control mice days on 2, 8 and 14. On day 2nd in the control wound, no epithelial lining was observed over the ulcer. Day 8th,

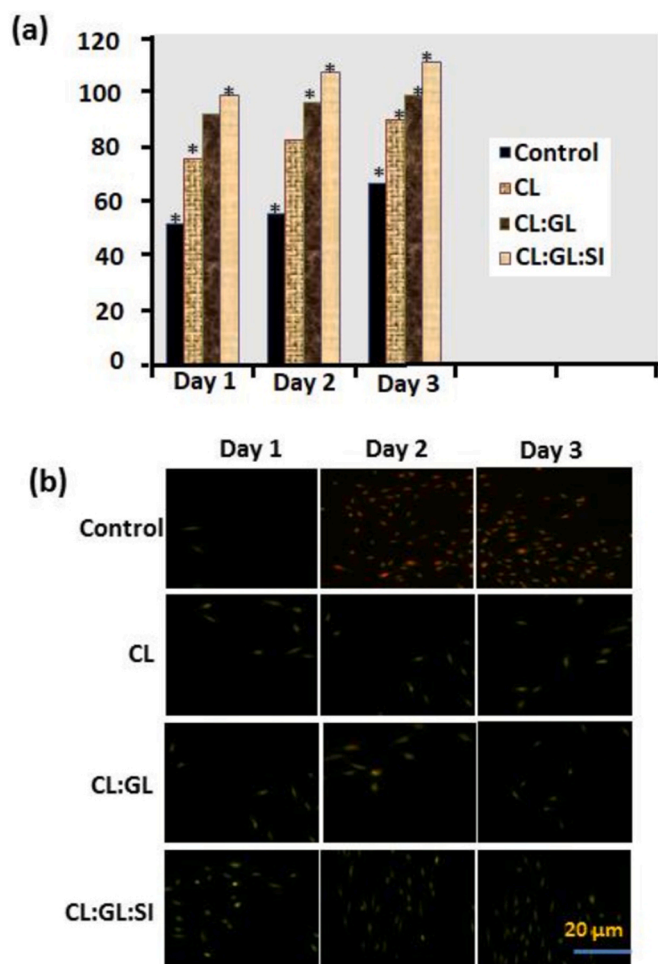


Fig. 6. (a) MTT assay demonstration of CL, CL: GL and CL: GL: SI on NIH 3T3 fibroblast cells. The asterisks (*) indicate statistically significant differences compared to the control $p < 0.05$ (b) Fluorescence micrographs (20X) of NIH 3T3 fibroblast cells cultured on days 1, 2 and 3.

tissue reconstruct of having border of CL: GL: SI applied animal was visible and the layer of the epidermis grew thick and granular tissue with full cells and the artery with regenerating epidermis fibrocollagenous tissue completely occupied the wound in the experimental group. On the 14th day, experimental wound that was enlarged inflammation, granular settled and completely covered by regenerating epidermis, but control wound was not fully restored and inflammatory cells in the scar was observed. Control group of wound there is no epithelial observed in the skin of wound. Similarly, wound of CL: GL: SI treated mice relieve wound was fully envelope by epidermis, mutually accompanied grown collagen.

4. Discussion

Marine food waste is one such potential waste which can be converted into valuable products. This present development attempts to prepare BPBS with wound healing properties from collagen, gelatin and squid ink. The BPBS possessed better cell viability, physio-chemical and antimicrobial properties. The present product deciphers a cost-effective technology for the production of BPBS and feasible technology for recycling of marine food wastes. The production of BPBS which is used in biomedical industries for a variety of purposes has gained significance. The more medical sector coming into existence to provide to the growing needs of the people, it is predicted that the demand for absorbable collagen scaffold.

The absorption peak at $3300\text{--}3400\text{ cm}^{-1}$ for --OH stretching, --NH in pyrrole (3300 cm^{-1}) and C–H vibration of stretching aliphatic groups ($2850\text{--}2920\text{ cm}^{-1}$) from amino acid (Veeruraj et al., 2015), this forming variety were connected with the antimicrobial and antioxidant properties. Squid ink of *Ioligo duvauceli* showed antimicrobial activity properties as previously described (Girija et al., 2014).

The comprehensive peak at $3300\text{--}3500\text{ cm}^{-1}$ in collagens was due to N–H stretching (Wang et al., 2009). The FTIR spectra attributed to hydrogen bonding providing solidity to GL. The representative absorption peak at $3200\text{--}3600\text{ cm}^{-1}$ was essentially due to the N–H and O–H stretching vibration of a phenolic and carboxylic acid by way of aromatic amino functions present in the indolic and pyrrolic arrangements (Bernsmann et al., 2010). Chen et al. (2016) reported the FTIR peak of collagen from the fish skin of tilapia. The primary spectra comprised amide I, II and III were observed at 1700 , 1550 , 1250 cm^{-1} , respectively. Zhang et al. (2014) reported that amide links of a protein in most orderly confirmation such as α -helices and β -sheet show the characteristic supernatural link outstanding the specific directions of the chromophores contained in the protein backbone. The random coil conformation of the polypeptide chain can be combined with collagen molecules as the significance of breaking the intermolecular and intramolecular ionic bonds, hydrogen bonds and noncovalent crosslinking bonds (Sarka et al., 2014).

Miles & Ghelashvili (1999) reported the consequence of hydration on the thermal stability of collagen matrices and collagen fibers. The thermal stability of the collagen scaffold showed a denaturation peak at $350\text{ }^{\circ}\text{C}$ (Leikina et al., 2002). The second phase of weight reduction in the range 200 and $500\text{ }^{\circ}\text{C}$ and related to the change of gelatin chain and breakage of thermally stable structures (He et al., 2011). Earlier studies had shown that collagen/gelatin blended scaffold with improved smooth surface after blending with gelatin (Wang et al., 2016). Yongping et al. (2020) stated that injectable antimicrobial conductive hydrogels from glycidyl methacrylate functionalized quaternized chitosan, gelatin methacrylate and graphene oxide has on uniform pore size and distribution. Therefore, the cells connect to the small fibrous molecules form of the gelatin with various polymer based injectable solutions assembles and form themselves in the way of hydrogels, follow in a good outspread morphology.

Ethylene glycol could be used as a universal solvent and has been employed as a plasticizer to improve the flexibility of the scaffold (Lai and Padua, 1997). One of our previous studies (Senthil et al., 2018) stated that the collagen wound scaffold plays a significant role in wound healing application. Collagen-based biopolymer through excellent mechanical, biodegradability and amazing biocompatibility properties, hence promising bio-based materials for wound scaffold. Pure collagen scaffold possesses excellent biological properties but poor structural stability and mechanical properties. Collagens blend with bio polymeric and binder can be used to reach intermolecular of collagen scaffold for better mechanical properties (Senthil et al., 2019).

Wound normally delivers an encouraging atmosphere for the development of microorganisms, which causes infection and stays the wound healing process (Paul and Sharma, 2004). In recent research reported that SI had antimicrobial activity and is used for the food and agriculture industry, biologically active dietary supplement, production of functional food, cosmetics, and medicine industry (Besednova et al., 2017). Biocompatibility characterization has been useful method for cell attachment and proliferation. Moreover, the MTT examination, it is noticeably evident that the BPBS was had good biocompatibility and nontoxic of NIH 3T3 cells, while the biocompatibility study of nanocomposites have been reported in many studies (Xin et al., 2018).

Biopolymers such as gelatin, collagen, fibrin, chitosan, etc., were found in the extracellular matrix. An earlier study has revealed that NIH 3T3 mouse embryonic fibroblast cells, Human keratinocyte cell line (HaCaT) and osteosarcoma cell lines MG-63 viability of cells grow more in this collagen scaffold (Kehe et al., 1999). The presence of pore size in the BPBS suggest that the hydrophilic and hydrophobic nature, which is

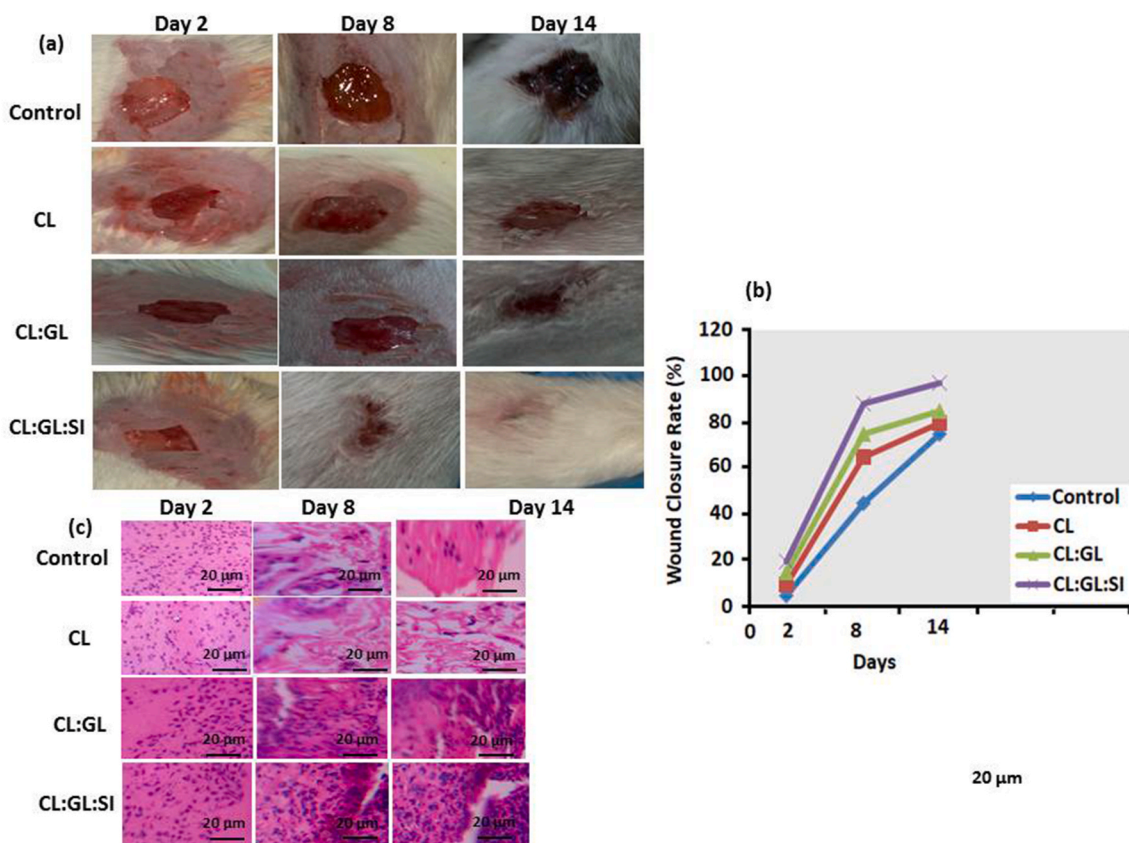


Fig. 7. (a) Animal studies: Control and Experimental group on day 2, day 8 and day 14 (b) Wound closure rate (%) of control and experimental group on day 2, day 8 and day 14 and (c) H & E staining. Control groups days 2, 8 and 14. CL, CL: GL and CL: GL: SI treated groups days 2, 8 and 14.

suitable for cell migration and infiltration (Simin et al., 2020). Collagen and gelatin are highly hydrophilic; they can increase the interface of cells and the capability to generate biological signals to support proliferation and cell adhesion (Lin et al., 2011). The cell attachment can be categorized into three stages: moving for the material surface, cell membrane surface and cell growth (Kim et al., 2005). Histological characterization was performed the inflammatory cells in filtration, fibroblast immigration, connective tissue synthesis and re epithelization. During the maturation of the collagen takes place by the formation of intermolecular crosslinks, therefore, the wound healing increased (Jiahui et al., 2020).

Earlier reports stated that the evolution of wound was healing covers three stages, inflammation, cell proliferation and tissue remodeling (Senthil et al., 2020). Advanced wound dressing materials such as collagen, gelatin and growth factors have focused on keeping a moist wound environment; maintaining the macro environment and treatment of wound exudates. Free radicals scavenging is large responsible for the wound healing process, since it play a important role in hemostasis, inflammatory, proliferative and maturation (Meng et al., 2019). The evaluation of the wound healing method to identified three steps: inflammation, cell proliferation and tissue remodeling (Haichao et al., 2020). Histological study displayed that the cutting wound the infiltrated neutrophils the wound location is reliable with an earlier study (Huang and Yang, 2008). To determine the character of BPBS in wound healing, the tissue samples were histological analyzed. Xin et al. stated that the although process of histological examination to assess inflammation, vascularization and skin regeneration (Xin et al., 2021).

Previous research also proved that extra neutrophils decreased the wound closure level and loss to normal cells (Dovi et al., 2003). Biopolymeric composites has been widely accepted as a healing process for noninvasive wounds and enrichment, platelet adhesion, excellent

mechanical strength, antioxidant, easy removability and good antibacterial performance and also researchers have developed some tissue adhesives like tissue adhesive sponges, tissue adhesive hydrogels and tissue adhesives. The development of scar tissue and a repair wound is evident in the BPBS treated mice on day 14th and is not detect the control group (Meng et al., 2020).

5. Conclusion

In this study, BPBS was produced from the marine resource using fishbone, fish skin and fish glands and were assessed as innovative wound healing scaffold. BPBS had a smooth nature by possessed better mechanical characteristic and displayed significant antibacterial properties against bacteria *E.coli* and *S. aureus*. *In vitro* study using NIH 3T3 cell lines displayed excellent biocompatibility. The BPBS showed good wound healing properties on experimental mice, compared to the control group. The BPBS was found cost-effective, eco-friendly and non-toxic. The results provide that BPBS can be used as a wound dressing material that better wound healing process.

Ethical approval statement

Animal experiments were conducted on 16 male mice after approval of the animal ethical committee of Central Leather Research Institute-CSIR (466/01a/CPCSEA-2bc/2014). All applicable National, International and Institutional guidelines for the care and use of animal were followed.

Author contribution statement

Senthil Rethinam, Contributed towards over all experimental design

layout, writing up of draft and final manuscript and funded the project. Tamilselvi Alagumuthu, Visualization. Kumaran Subaramaniyan, Statistical analysis. A.Wilson Aruni, Software. Serdar Batikan Kavukcu, Formal analysis.

Declaration of competing interest

The authors of this manuscript have no conflicts of interest related to the content of the study.

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