Fabrication of Gelma-Fibroin Conjugated Materials with *Gymnema Syvestre* Extract to Target Colon Cancer and Acute Wound

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Abstract- Background: In the present study revealed that concentration of Leaf extract+GelMA+fibroin exhibited a greater result of anti-cancer activity, speed of wound healing, anti-inflammatory and cyto-compatibility. Methods: Further FT-IR analysis was performed to found the functional groups present in extract, Fibroin, GelMA and its complex. Morphometric characterization was achieved by SEM analysis. Results: The cytotoxicity of colon cancer cell lines were analyzed with all ingredients by MTT assay and found its IC_{50} at (24µg/mL) leaf extract, (16µg/Ml) GelMA, (12µg/mL) Fibroin and (3µg/mL) LE+GelMA+Fibroin. Thus, therapeutic agent of LE+GelMA+fibroin complex showed best results of faster re-epithelialization as well as increased collagen expression, due to better and rapid healing through synergistic effect of formulated biomaterial *in vitro* and *in vivo*. We found that the wound healing or closure in 7th day was no significant within individual or complex treatments. However, the wound healing was started. We found at day 14th -21st the wound contraction in wounds treated with Betadine was 5%, plant extract was 64%, GelMA was 69%, Silk fibroin was 78% and Leaf extract+GelMA+fibroin composite were 85%. This preliminary study using plant extract, silk fibroin and GelMA composition have a potential activity against post operated colon cancer cell line and rat. Conclusion: Thus, the present findings validated the low toxicity and high therapeutic potentials of LE+GelMA+fibroin, which may provide a convincing evidence of LE+GelMA+fibroin as new potential drug for colon cancer and acute wound. In future it will be a good combination to carry nanomedicine towards post operated wounds in various cancer models.

Key words: *Gymnema sylvestre,* GelMA, Fibroin, Colon cancer

Background

Emerging effective cancer treatments and a possible cure for cancer constitute some of the most difficult challenges facing modern medicine due to the complex nature of the disease. Colorectal cancer (CRC) has turned into a huge concern for human health. In several cases, a cancerous growth called colorectal cancer (CRC) can be found in the gastrointestinal tract. Presently, CRC is the third leading cause of death in the globe. the third most common malignancy in both sexes worldwide (Sung, H. et al. 2020) Each of these hurdles must be tactically addressed when developing effective treatments for particularly deadly cancers like colorectal cancer, which is the third most common cause of cancer deaths worldwide after breast and lung cancer (World Health Organization (2020), Global Burden of Disease 2019). Chemotherapy, which relies on chemical agents to kill cancer cells, is one of the most important tools available to treat cancer. However, in its usual form, chemotherapy is not sufficiently selective because it kills an unacceptable number of healthy cells while destroying malignant cells [4,5]. For example, the anticancer drug 5-fluorouracil (5-FU), which has proven to be effective in treating colorectal cancer, has limited clinical applications due to its toxicity to healthy cells and the resistance that cancer cells develop to this drug over time [6] [2]. For 2019, the predictable number of new cases of colon cancer and rectal cancer are 101,420 and 44,180, respectively, adding together to a total of 145,600 new cases, death for 51,020 of colorectal cancer [3]. Conversely, this pathology is diagnosed more frequently in younger patients, due to risk factors

such as obesity, sedentary, bad nutritional habits (high in fats and proteins) smoking, and the progressive aging of the population [4].

Medicinal plants can be taken into account as the potent and promising therapeutics for development of wound healing processes based on the variety of the active and effective components such as flavonoids, essential oils, alkaloids, phenolic compounds, terpenoids, fatty acids, and so on. These traditional medicines can be preferred over modern therapy due to the low cost, limited adverse effects, bioavailability, and efficacy [5, 6]. GS, taxonomic serial number 506007, is a plant belonging to the family *Asclepiadaceae*, order Gentian ales, that is used in Indian traditional medicine for patients with diabetes. It is a woody climber distributed throughout India at an altitude between 300 and 700 m [7, 8]. *G. sylvestre* also possesses potential anti-microbial property and the same has been practiced in folk medicine for various infections. *G. sylvestre* is an indigenous herb, belonging to the class dicotyledonous of the family *Asclepiadaceae*. The plant is a good source of a large number of bioactive substances [9]. Natural biomaterials are derived from animals, microbial, or plants. One advantage of natural biomaterials is that they are similar to materials familiar to the body [10]. In this regard the field of biomimetics, or mimicking nature, is growing. Natural polymers such as chitosan, collagen, elastin, and fibrinogen are biocompatible substrates that are similar to macromolecules recognized by the human body [11]. Silk fibroin is processed from mulberry silk after removal of the outer silk sericin which may potentially elicit an immunological response when it is associated with fibroin. Silk fibroin processes excellent biocompatibility, controllable biodegradability, remarkable mechanical strength, and low immunogenicity [12, 13].

Hydrogels dressings can be formulated to provide controlled, targeted release of antimicrobial agents that are facilitated by bio adhesive, stimuli (wound)-responsive characteristics. Additionally, controlling the amount of agent delivered to the wound bed can avoid overloading at the site of infection while allowing effective antimicrobial activity. This may reduce possible side effects due to localized toxicity [14]. GelMA hydrogels are derived from collagen, the most abundant protein in the body, and have been shown to support the growth of a range of cell types seeded within or on their structures. Ease of synthesis and versatility has made GelMA one of the most commonly used materials to form hydrogels for biomedical applications [15].

In the present study novel type of biomaterial *G. sylvestre* leaf extract, GelMA, Fibroin and LE+GelMA+Fibroin has been synthesized and characterized to target breast cancer and post-operate wound healing performance. The synthesized biomaterials low cytotoxicity, control degradability, high adhesion strength *in vitro* and *in vivo*. The LE+GelMA+Fibroin acculturated wound healing and anti-cancer properties, which evaluate cytotoxicity, apoptotic activity, advantage of adhesion strength tunable degradability and *in vivo*. Wound healing rat model the biomaterial based adhesives night. Provide a new clinical option for wound closure. As LE+GelMA+Fibroin composite are very stable in a must environment, which also may help the wound healing process and could be beneficial in wound healing and surgical cases.

MATERIALS AND METHODS

Chemical and Reagents

Isopropanol and ethanol were purchased from Medox Pvt. Ltd. Sodium dodecyl sulfate (SDS, 99%) was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Polyethylene glycol diacrylate were obtained from HiMedia (Mumbai, India). Gelatin (type A, 300 bloom from procine skin) obtained from Himedia Laboratories Pvt, Ltd., Mumbai, India. Methacrylic anhydride (MA) and 3-(trimethoxysilyl) propyl methacrylate (TMSPMA) were purchased from Sigma-Aldrich (Wisconsin, USA). The deionized water (D.I.) was generated using a Millipore Milli-Q Biocel system (Billerica, MA). All other chemicals and reagents were of analytical grade and purchased locally.

Preparation of (*Gymnema sylverstre*) Ethanol leaf extract

Gudmar leaf extracts were prepared as per the method adopted by. Gudmar leaf powder (500g each) was extracted with 70 % ethanol and aqueous by boiling on water bath at 700 C for 60 mins and cooled extracts

were filtered through vacuum filtrations unit, evaporated to dryness on rotary film vacuum evaporator. The dried extracts were kept in refrigerator for future use [16, 17].

Synthesis of gelatin methacryloyl (GelMA)

Gelatin methacrylate was synthesized as described previously [18]. Briefly, 10 g of procine skin gelatin was dissolved in 100ml of DPBS at 50° C in 1hr. Then 8 ml of MA was added to the gelatin solution at a rate of 0.5 ml min^{-1} under stirring condition added very slowly and drop wise under stirring first solution. This gelatin solution was kept in magnetic stirrer at 50°C and 230 rpm at 3hr. After 3hr, the reaction was stopped following a 1:5 dilution using worm phosphate buffered saline at 50° C and allowed to react for 1hr [19]. The mixture was allowed to react for 3hr at 50° C. Then dialyzed against distilled water by using a dialysis membrane (MWCO=12-14KDa) for 7 days at 40° C to remove salt and methacrylic acid. Photocross linking was achieved by exposing the GelMA prepolymer to 6.7 mW cm^{-2} UV light (360-480 nm; using an Omni Cure S2000 UV lamp (Lumen Dynamics) for 20s at room temperature. The dialyzed GelMA solution were frozen at -80°C the solution was lyophilized for 1 week to generate a light white porous foam and stored at -80°C in room temperature until further use [20] .

Isolation of sericin using cocoon from silkworm Bombyx mori

Cocoons of *Bombyx mori* were obtained from local sericulture farm, Salem, Tamil Nadu.
Only cocoons that look undamaged should be used in the process. Clean cocoon shells were weighed, chopped into small pieces. The aqueous silk fibroin solution was prepared in silk fibroin protein was extracted from raw silk with an aqueous solution containing 0.02 M Na_2CO_3 at 87-92°C for 30 mins rinse fibers for 20 mins total of three times, squeeze out excess water and allow to dry overnight and then 9.3 M LiBr on top of silk fibers and incubate at 60°C for 4 hr [21, 22]. The silk fibroin solution was dialyzed against distilled water using dialysis bags (M_W: 8000 Da) at room temperature for 48 hr and remove silk solution from dialysis bags and then centrifuged at 4°C for 10 mins. The supernatants were collected in store at 4°C and then father study use

GC/MS analysis

GC/MS analysis was performed on GC–MS-QP (Shimadzu) equipped with a VF-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film coating). The injector temperature was set at 260 ˚C. Helium was used as carrier gas at a constant flow rate of 1.51 ml/min through the column. The column temperature was initially kept at 70 ˚C for 2 mins, and then increased from 70 to 300 ˚C at 10 ˚C/mins, where it was held for 10 mins. The MS ion source temperature was set at 200 ˚C and the ion inlet temperature was 240 ˚C. Full-scan mass range of 40–1000 m/z was acquired. Sample components were identified by matching their mass spectra with those recorded in NIST08s, Wiley-8 and FAME Library [23, 24].

Characterization of *Gymnema sylverstre*

The visual properties were observed by UV-visible spectrophotometer (UV-1800, Shimadzu) at room temperature in the range between 200-800 nm. Dried powder of methanolic extract was used after performing KBr pelleting. The sample was loaded onto FT-IR spectroscope used in the Temet GASMET FT-IR CR- series is a SiC ceramic at a temperature of 1550 K. The morphology and structure of synthesized of was investigated by Scanning Electron Microscopy (SEM) (EM TECNAI microscope).

Cell culture and culture conditions

FHC cells were maintained in DMEM, HCT-5 cells were maintained in RPMI 1640 media, HT-29 cell line were cultured in Dulbecco's Modified Eagles medium supplemented with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM nonessential amino acids, and 1.0 mM of Sodium pyruate. The cultures were maintained in t-25 flask with the growth condition maintained at 37 °C and 5% CO_2 in an air jacketed CO_2 incubator. After the cells attained 70–80% confluency following trypsinization, they were seeded in 96-well plates or 60-mm petriplates of tissue culture grade for experiments.

In vitro cell culture and cytotoxicity analysis

The cytotoxicity analysis of the dissolution content of the tissue glue was measured using a quantitative MTT assay. RAW 264.6 mouse macrophages were cultured in DMEM containing 10% FBS and 10 units mL^{-1} pen/strep at 37 °C in a 5% CO_2 humidified atmosphere. Cells were seeded in a 24 well-plate at a concentration of 40,000 cells per well for 24 hr. The tissue glue (30 μL) was added into the Trans well chamber. The cells cultured in DMEM were set as a control. After incubation for 24 hr, the Trans-well chamber and the medium were removed and replaced with 50 μL of MTT solution (1 mg mL^{-1} in PBS), and the cells were incubated for another 4 hr. Finally, the MTT solution was removed, and 100 μL of DMSO was added per well to dissolve the crystals completely. The absorbance of each well at 570 nm was measured using a Multiskan FC microplate reader (Thermo Fisher, USA). For the microscopy image of RAW 264.7, first, a solution of LE+GelMA+Fibroin in the Tris buffer (pH = 8.5, 2 mg mL^{-1}) was added into the 24 well-plate and coated for 24 hr. After the solution was removed, the polymers were coated on the bottom of the plate. Then, the cells were seeded and cultured for 24 hr before taking the image.

Estimation of protein expression levels in wounded skin tissue homogenate

For biochemical analysis of wounded skin tissue take out from all group of mice at 7, 14 and 21 days post wound was also processed. The 100 mg excised skin tissue was homogenized in 1 mL of PBS (1M) by homogenizer. The homogenated skin was centrifuged at 10,000 rpm for 10 min at 4 ºC and collected supernatants were stored at −80 ºC until analyzed. The homogenates were estimated for TNF, TGF-beta, IL-1, IL-10, COX-2, Collagen I and Collagen III proteins contents using a specific ELISA kits. The particular absorbance wavelength was noted, and then estimates the amount of these factors at specified time intervals to study about the consequence of LE+GelMA+Fibroin treated groups as compared to control groups on the wound healing actions.

In vitro wound scratch assay

To evaluate the *in vitro* wound scratch cell migration assay in NIH-3T3 cells were cultured in six-well plates. The cell monolayer was worn out in a straight line by 200-μL pipette tip, and the cell debris was washed three times with PBS. After that, cells were treated with LE+GelMA+Fibroin in 5% FBS DMEM. Wounded areas were photographed at time zero under phase-contrast microscope. After 7 hr, 14 hr, and 21 hr of incubation, photos were taken from the same areas as those recorded at time zero. Experiments were performed at least three times in quadruplicate.

Histological examinations of Re-epithelialization

The wound samples were taken from euthanized mice at days 1, 7, 14 and 21 days were cut in half, this all samples were fixed in 10% formalin for 24 hr, processed, and embedded in paraffin. Then, the vertical sections were set to glass slides and added to H&E stain to examine tissue morphological characteristics, collagen evidence, vascularization, macrophage activity and neutrophil infiltration, respectively. Tissue slides were examined by under light microscopy with an Olympus BX51 microscope.

In vivo wound healing experiments

Twenty four male BALB/c rat, 16 weeks old, with an average weight of 20-23g body weight were purchased from TANVAS Chennai and weighting about 20–30 g (16 weeks old) were placed in clean polypropylene cages with access to food and water. These cages were maintained in an air-conditioned animal house at 20 to 24° C, 50–60 % relative humidity and 18:6 hr light–dark cycle. The animals were randomized equally into control and experimental groups. As per the institutional Animal Ethical Committee (IAEC) of Periyar University were carried out “Accordance” with the relevant guidelines of CPCSEA with the regulatory approval PU/IAEC/085/PO/C/07/CPCSEA/Zool/05/2016).

Statistical analysis

All measurements were made in triplicate and all values were expressed as the mean ± standard error of the mean. The results were subjected to an analysis by Bonferroni’s multiple comparison tests And Data are presented as means ± SD. Statistical Significances were determined using the Student’s t-test and *p*-values of <0.05 were considered statistically significant.

RESULTS

Analysis of UV-Visible spectroscopy

The UV–Vis absorbance spectra of *G. sylvestre* leaf extract and Silk Fibroin (SF) were wavelength scanning in the range of 200–800 nm, shown in (Fig. 1a). The presence of compound showed strong absorption band peak at 413nm, 417nm and 669nm [17]. The alkaloids, phenols, flavonoids, sterols and tannins reported that a wide range of biological activities, such as antioxidant activity for plant extract. The absorption wavelength of SF has shown (Fig. 1b). UV absorption of SF amino acid, a clear decreased distinct peak was observed at 344 nm, which indicate that the conformation structure and fibroin has been UV resistant capacity of the silk-based material would have a great effect on the UV absorption ability.

Analysis of GC

The GC analysis of methonolic extract of *Gymnema sylvestre* leaf showed that there were totally 44 different biologically active compounds. In which presence of 5 major peaks in methanolic extract corresponding to compounds 1-Methyl-5-Fluorouracil (0.44%), Catechol (1.09%), 2-Furancarboxaldehyde and 5-(Hydroxyme) (5.79), Galactitol (2.11%) and Phytol (7.24%) were identified [23] The results of GC analysis have illustrated (Fig. 2). The sample was extracted with methanol because of the effect of biological activities, anti-diabetic and anticancer activity in this solvent. GC analysis also provides the spectrum for the methanolic extract. The phyto chemical screening showed that the leaves were amino acids and secondary metabolites such as alkaloids, flavonoids. They have both therapeutic and physiological value and exhibit *Gymnema sylvestre* leaves of relative concentrations of various compounds with a function of retention time were illustrated (Table 1 &2).

Analysis of FT- IR

FT-IR analysis was used to identify the functional group of active components based on peak values in the region of infrared radiation. FT-IR spectroscopy of *G. sylvestre* leaf extract, GelMA, Silk Fibroin and LE+GelMA+Silk Fibroin were shown (Fig. 3) [25]. The stretching of vibrations were recognized and assigned to various functional groups of *G. sylvestre leaf* extract could be observed in the range of 3899 cm^{-1} band at 3899 cm^{-1} due to the presence of C-H stretching was shifted to the lower frequency (2928 cm^{-1} and 2873 cm^{-1}) 2337. 33 cm^{-1} at O=C=O Stretching bond of Carbon dioxide, 1623. 80cm^{-1} at C=C Stretching bond of Conjugated alkene, 1507. 11 cm^{-1} at N−O stretching bond of nitro compound) 1406. 03cm-1 at S=O stretching bond of Sulfonyl chloride, 1319. 51cm^{-1}at C−N Stretching bond of Aromatic amine, 1264. 55cm^{-1} at C−O Stretching bond of Alkyl aryl ether, 1040. 64cm^{-1} at CO−O−CO Stretching bond of Anhydride and 883. 63 cm^{-1} C=C bending in alkene. [26] Min, *et al.* investigated that effect of silk fibroin film was first evaluated in a small animal model (rabbit full-thickness skin defects).The silk fibroin show that 3463 cm^{-1} due to O−H Stretching of Alcohol, 1770 cm^{-1} due to C=O Stretching of Vinyl/ phenyl ether, 1387 cm^{-1} due to C−H bending of aldhyde, 1348 cm^{-1} due to O−H stretching of Phenol, 1040 cm^{-1} due to C−N stretching of Amine, between 1634 cm^{-1} and 838 cm^{-1} and 667 cm-1 and 521 cm^{-1} due to C−l Stretching of halo compound were due to C=C Stretching of conjugated alkene and alkene.

FT-IR spectrum was further drawn-out to confirm the successful mixing loading of Leaf extract, GelMA and Fibroin band at 3388 cm^{-1}, 3402 cm^{-1} and 3463 cm^{-1} were shifted in to a higher frequency range of 3501 cm^{-1}, and fibroin range 1634 cm^{-1} shifted in to lower frequency range 1623cm^{-1} [27] Hu, *et al.*, previews reported that procedures which directly influence the safety of the final product were rigorously controlled. The whole results validate the successful loading of leaf extract, GelMA, Silk fibroin on the LE+GelMA+Fibroin complex [28, 29].

Scanning electron microscopy

The morphological structures of the Fibroin, GelMA and GelMA+Fibroin were analyzed by SEM [30, 31]. The morphological structure of the complex was shown (Fig. 4). The image shows the degummed silk fibroins appears in normal fiber structure of diameters of 200 μm (Fig. 4a) The morphology of GelMA Presence of uneven network structure and many small particles visible on the upper region of GelMA (Fig. 4b) The present of Fibroin with GelMA show normal fiber structure and surface of the fibroin minute particles are present. These clarify the mixing of fibroin GelMA (Fig.4c).

Cytotoxicity assay

MTT assay results conform the *in vitro* cytotoxicity of synthesized biomaterial. a) Cytotoxicity effect of leaf extract, GelMA and Fibroin. b) Cytotoxicity effect of LE+GelMA+Fibroin. The detected IC_{50} concentrations were leaf extract, GelMA, Fibroin and (3µg/mL) LE+GelMA+Fibroin for HT-29 cells at 24 hr [32]. We found that at very low concentration of complex can effectively reduce the cell death, although at increasing concentration showed cytotoxic affect *in vitro*. They have minor effect on target cancer cell as concentration. The LE+GelMA+Fibroin greatly decreased the cell viability at the low concentration (3µg/mL), which is comparaed to the cytotoxicty effect of LE+GelMA+Fibroin (Fig. 5). Data expressed as mean ± SD of three experiments. Percentage of cytotoxicity is expressed relative to untreated control (*significant $p<0.05$).

Morphological characterization

The morphology of the different LE+GelMA+Fibroin was investigated by fluorescence microscopy image. The morphological changes were noticed in LE+GelMA+Fibroin. Were test at different concentration (24µg/mL) leaf extract, (16µg/Ml) GelMA, (12µg/mL) Fibroin and (3µg/Ml) LE+GelMA+Fibroin against HT-29 cells [33]. The most characteristic morphological changes of LE+GelMA+Fibroin the cells detected in this experiment were nuclear condensation, cell shrinkage and aggregation of nuclear chromatic membrane. Hence, the morphology fifty percentage of cell death. (Fig. 6) This data replicated and compared with MTT assay used to assess the effect of LE+GelMA+Fibroin on proliferation of HT-29 cells.

In vitro strach assay

Cell migration incursion through the vault membrane are important steps in wound healing process. The different biomaterials such as control and LE+GelMA+Fibroin had been treated individually touching 3T3 cells to establish wound healing properties. The wound healing assay results depicted in (Fig. 7) microscopic images show the treatment LE+GelMA+Fibroin; reveal a larger cellular migration thickness was reduced in control group. Statistical analysis of five independent experiments exposed a radically increased density of migrating cells after LE+GelMA+Fibroin was present at the time of culture medium. To establish the 3T3 cell motility with long term effects of LE+GelMA+Fibroin perform 72 hr cellular migration assays and comparable as in the scratch assays be followed the untreated cells. In this result clarify the control and LE+GelMA+Fibroin treated cells, major level of cellular migrations occurs in LE+GelMA+Fibroin treated. Hence, the LE+GelMA+Fibroin complex were migration of cells into significantly higher to wound closure while compared to the control. These *in vitro* experimental data significantly confirm that our product (LE+GelMA+Fibroin) has been efficient to wound healing properties. The low incorporation of gellan doesn't change the morphology of gelatin hydrogels.

In vivo wound healing analysis

Macroscopic photographs delegate rat wound were taken post-operative days d 0, 7, 14 and 21. The full thickness wound model in rat and wounds were applied topically treated with Bedatine, leaf extract, GelMA, Fibroin and LE+GelMA+Fibroin. Once, a day with betadine (5% a commercially available wound healing drugs in India) as the positive control. On the 7 day post-operation, the entire wound treated were still visible, and the wound sizes were comparable. Similar differences in wound healing among the Bedatine, leaf extract, GelMA, Fibroin and LE+GelMA+Fibroin were observed on days 14 post-operative (Fig. 8a) and it was found that the wound size was decreased 21st wounds treated with composite film has shown faster wound healing than wounds treated with leaf extract+GelMA+Fibroin composite film.

Wound closure was assessed by morphomatric analysis of wound areas. The percentage of wound contraction in untreated and treated groups was measured on 7th, 14th, 21st, post wound day and the results are shown in (Fig. 8b) 92% of wound concentration was observed in untreated control. Wound contraction in wounds treated with Betadine was 5%, plant extract was 64%, GelMA was 69%, Silk fibroin was 78% and Leaf extract+GelMA+fibroin composite were 85%. Wound contraction was significantly increased in wounds treated with composite film when compared to wounds treated with Leaf extract+GelMA+fibroin and untreated wounds, which indicated that the composite film has shown

more wound healing property than Leaf exract+GelMA+fibroin. The wound residual areas were determined (n=6). LE+GelMA+Fibroin incressed rat wound healing obviously. **p<0.01 indicate significant difference from the control.

HISTOLOGICAL EVALUATIONS OF WOUND HEALING

Histological evaluated the repaired tissue with hematoxylin and eosin (Fig. 9). On day 7 post operations, a thick scab but slight epithelium was present in the defect region of all groups. Immature granulation tissue was evident with loose collagen matrix and severe lymphocyte penetration. Enlargement of epidermis was observed on day 14 post-operation but varied extensively between groups. Epidermal regeneration was poor and incomplete in moreover Bedatine or control group which was characterized by absence of epidermis but presence of thick crusting in most wound sites. More collagen matrix was synthesized but still not well prearranged. Especially, in the silk fibroin group, the minority hair follicles can be observed in the newly formed tissue closest to the wound margin, which was absent in the other three groups. All groups achieve inclusive and good epidermal regeneration on day 21 post operation characterized by clear epithelial layers. Grown-up regenerated tissue, well-formed collagen matrix mutually with obvious skin appendage characterized the complete remodeling in the leaf extract+GelMA+Fibroin groups, which were significantly enhanced than individuals in the Bedatine and control untreated groups. Among these processes, inflammation is the first step in the healing response after tissue injury.

DISCUSSION

The increase in demand in industrially developed countries to use alternative approaches to treat diabetes, such as plant-based medicines, is also due to the side effects associated with the use of insulin and oral hypoglycaemic agents [34]. Previously, reported that extracts of *G. sylvestre* with chloroform, Ethyl acetate and 95 per cent ethanol possessed anticancer activity evaluated against MCF 7 (epithelial cells of human breast cancer) and A 549 (epithelial cells of human lung cancer) by MTT assay [32] Srikanth *et al.*, reported that significant effect of *G. sylvestre* leaf extract on treatment of diabetic foot ulcer was proved. All the three extracts exhibited IC_{50} value concentration dependently and at 50 and 100µg/ml exhibit IC_{50} value similar to that of standard drug etoposide. Based on studies, several of the constituent exposed by GC–MS are biologically active compounds. They were confirmed to have pharmacologic tricks which may give to the curing possible of the plant. Phytol was proven to reveal antioxidant and antinociceptive effects [35, 36]. Silk fibroin is widely used in the tissue engineering and regenerative medicine field due to its good biocompatibility, controllable bio-absorbability, excellent mechanical strength, and low immunogenicity. Despite mounting basic investigations on silk fibroin wound dressing material, however, translational studies involving large animal and randomized controlled human trials have been seldom reported [37]. [38] Hu, *et.al* reported that procedures which directly influence the safety of the final product were rigorously controlled. The effectiveness of the degumming process was confirmed by multiple evaluation approaches including picric acid and carmine staining, weight loss measurement, TGA and FTIR analysis, and SEM imaging. The complete removal of sericin from our silk fibroin film potentially reduces the immunological responses when used *in vivo*. To increase the stability of the silk fibroin film, the green treatment TCWVA was used and modified, avoiding the commonly used organic solvent methanol treatment. In addition, wounds covered with the silk fibroin film exhibited faster re-epithelization, better angiogenesis, and more hair follicles than the other three groups. The present results are consistent with those reported earlier in Min's study which demonstrated the advantages of silk fibroin biomaterial in skin repair.

Balakrishnan *et al.* [39] investigated an oxidized alginate- and gelatin-based hydrogel for wound dressing application via *in vivo* study in a rat model. Their hydrogel dressing shows promising results with relatively low water vapor transmission rate compared with commercially available wound dressing products and good water absorptivity. The improved water retention facilitated the development of a moist environment that is conducive to wound healing; the alginate- and gelatin-based hydrogel was shown to enhance cell migration and re-epithelialization. At 15

days, the wound defects in the rat model filled up to 95.3% treated

Hydrogel-based dressings also absorb wound exudates, which in turn promote fibroblast proliferation, keratinocyte migration, and the eventual re-epithelialization of the wound, adhesives and biologically derived fibrin glues have been shown to exhibit poor adhesion to wet tissues, and are not able to support tissue regeneration [40.41]. [34] Yu, *et al*, investigated that *in vitro* studies have shown that nonwoven SF is biocompatible with human cells and can support the growth of a variety of human cell types including epithelial cells, fibroblasts, glial cells, keratinocytes, osteoblasts, and endothelial cells. [42] Yan *et.al* demonstrated that sericin recruits inflammatory cells at a low level similar to alginate and fibroin, but much less than chitosan and sericin was actually able to recruit regeneration-promoting cells, such as vascular endothelial (progenitor) cells. Furthermore, sericin did not trigger an allergenic reaction, and exhibited low and acceptable immunogenicity. Wound healing is a series of processes that involves control of inflammation, proliferation, and new tissue remodeling [43, 44].

The viability studies revealed that combining of the leaf exract, GelMA, Fibroin shows different cytotoxic effect on colon cancer cells, which indicating that the cytotoxicity is mainly based on the concentration dependent effect [45]. Among these processes, inflammation is the first step in the healing response after tissue injury. In addition, cell proliferation and migration are essential responses for re-epithelialization and skin remodeling during the healing process [42, 46]. Wound closure was assessed by morphomatric analysis of wound areas [47, 48]. In addition, cell proliferation and migration are essential responses for re-epithelialization and skin remodeling during the healing process. Wounds treated with the silk fibroin film exhibited reasonable to complete epidermal club with well-structured epithelial layers, while Wounds treated with plant extract showed less epidermal layer with shortened monolayer of epidermal cells [49]. Recently, researchers have been focused on the fabrication and development of the effective wound dressing that can help to treat wounds better and sooner. Hydrogel-based biomaterials are one of the most effective wound dressings that promote wound healing process [50, 51].

CONCLUSION

In this present study explains that the anti-cancer activity in colon cancer and wound healing ability of *G. sylvestre* leaf extract+GelMA+Fibroin for *in vitro* and *in vivo* rat model Meanwhile methonic leaf extract was prepared and bioactive compounds were identified through GC-MS analysis and also pure protein of silk fibroin was isolated from cocoons of Bombyx mori. Further FT-IR analysis was performed to found the functional groups present in extract, Fibroin, GelMA and its Complex. Morphometric characterization was achieved by SEM analysis. We found that the wound healing or closure in 7th day was no significant within individual or complex treatments. In our results concluded that concentration of Leaf extract+GelMA+fibroin exhibited a greater result of anti-cancer activity, speed of wound healing, anti-inflammatory and cyto-compatibility. Therapeutic agent of LE+GelMA+fibroin complex showed best results of faster re-epithelialization as well as increased collagen expression, due to better and rapid healing through synergistic effects of formulated biomaterial *in vitro* and *in vivo*. Thus, the present findings validated the low toxicity and high therapeutic potentials of LE+GelMA+fibroin, which may provide a convincing evidence of LE+GelMA+fibroin as new potential drug for colon cancer and acute wound.

Abbreviations

LE= Leaf extract, GelMA = (gelatin methacryloyl), FT-IR= Fourier-transform infrared spectroscopy, SEM= Scanning electron microscope), MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor, GS= *Gymnema sylvestre*, DPBS= Dulbecco's phosphate buffered saline, MA= methacrylic anhydride, GC/MS=Gas chromatography/ Mass spectroscopy, UV=Ultra Visible, DMEM= Dulbecco's Modified Eagles medium, RPMI= Roswell Park Memorial Institute, FBS= Fetal bovine serum, MCF=Michigan Cancer Foundation-7.

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Author Contributions

DM, LB and SK designed the work; KV and DM performed experiments. All authors participated in data analysis and writing.

# These are contributed equally.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol and experiments were approved by Animal Ethical Committee (IAEC) of Periyar University

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Consent for publication

Not applicable.

Competing of interests

The authors declare that they have no competing interests.

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Figure legends

Fig. 1a UV–Vis spectrum of leaf methonolic extract of *G. sylvestre*. The absorption peak at 417 nm indicates the presence of flavonoids and 669 nm in the visible region which exhibited the absence of chlorophyll. This character should make a positive contribution to determine the active compound present in *G. sylvestre*.

Fig. 1b UV absorption spectrum of fibroin. The absorbance peak 344 nm state that the presence of amino acids in fibroin and also conform that fibroin has been UV resistant capacity.

Fig. 2 GC- Chromatogram of methanolic leaf extract of *G. sylvestre*. In this results showed that totally 45 different biologically active compounds were present. A most important component like 1-Methyl-5-Fluorouracil (0.44%), Catechol (1.09%), 2-Furancarboxaldehyde and 5-(Hydroxyme) (5.79), Galactital (2.11%) and Phytol (7.24%) are identified.

Fig. 3 FT-IR transmittance spectra of G. sylvestre leaf extract, GelMA, Fibroin and LE+GelMA+Fibroin. FT-IR spectrum was further drawn-out to confirm the successful mixing loading of Leaf exract, GelMA and Fibroin band at 3388 cm^{-1}, 3402 cm^{-1} and 3463 cm^{-1} were shifted in to a higher frequency range of 3501 cm^{-1}, and fibroin range 1634cm^{-1} shifted in to lower frequency range 1623cm^{-1}. The on the whole results validate the successful loading of leaf extract, GelMA, Silk fibroin on the LE+GelMA+Fibroin complex.

Fig. 4 SEM images of a) Fibroin, b) GelMA and c) Fibroin GelMA. a) Exposed lyophilized the surface morphology of degummed fibroin. The shape of the degummed silk fibroin appears in normal fiber structure. b) The morphology of GelMA. Presence of uneven network structure and many small particles visible on the upper region of GelMA. c) Fibroin with GelMA show normal fiber structure and surface of the fibroin minute particles are present. These clarify the mixing of fibroin GelMA.

Fig. 5 MTT assay results conforming the in vitro cytotoxicity of synthesized biomaterial. a) Cytotoxicity effect of leaf extract, GelMA and Fibroin. b) Cytotoxicity effect of LE+GelMA+Fibroin. The detected IC50 concentrations were leaf extract, GelMA, Fibroin and (3µg/mL) LE+GelMA+Fibroin for HT-29 cells at 24h. Data expressed as mean ± SD of three experiments. Percentage of cytotoxicity is expressed relative to untreated control (*significant $p<0.05$).

Fig. 6 *In vitro* cell viability of formulated biomaterial LE+GelMA+Fibroin. a) Control b) leaf extract c) GelMA d) fibroin and LE+GelMA+Fibroin. The effects of LE+GelMA+Fibroin on proliferation of HT-29 cells. Fifty percentage of cell death, which determines the inhibitory concentration IC50 rat synthesized biomaterial against MCF-7 cell hold at (24µg/mL) leaf extract, (16µg/Ml) GelMA, (12µg/mL) Fibroin and (4µg/Ml) LE+GelMA+Fibroin.

Fig. 7 LE+GelMA+Fibroin induced migration and invasion of 3T3 fibroblastic cells. 3T3 fibroblastic were scratched with a pipette tip, separately treated with each of the following, namely control, LE+GelMA+Fibroin, migrating cells were analyzed by phase contrast microscopy. Quantified levels of cell migration of untreated relative to the basal migration the mean ± SE of 2 independent experiments in each cells treated with control.

Fig. 8a Macroscopic photographs delegate rat wound were taken post operative days d 0, 7, 14 and 21. The full thickness wound model in rat and wounds were applied topically by IC50 concentration of leaf extract (24µg/mL), GelMA (16µg/mL), Fibroin (12µg/mL) and LE+GelMA+Fibroin (4µg/mL) once a day with betadine (5% a commercially available wound healing drugs in India) as the positive control. Compared with control LE+GelMA+Fibroin had a significant enhancing effect on the skin wound repair of rat.

Fig. 8b Wound closure was assessed by morphomatric analysis of wound areas. The wound residual areas were determined (n=6). LE+GelMA+Fibroin increased rat wound healing obviously. **$p<0.01$ indicate significant difference from the control.

Fig. 9 Histopathological evolutions of skin biopsies taken on postoperative day 0, 7, 14 and 21. This images shows that histopathological analysis, 14 days after surgery the epidermis slightly thickened, uniform

thickness of renascent skin formed due to the rat topically treated with LE+GelMA+Fibroin (4µg/mL), synthesized biomaterial promote the regeneration and maturation of new epidermal tissue.