EXPRESSION OF INTEREST FOR TECHNOLOGY TRANSFER

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COLLAGEN BASED WOUND DRESSING LOADED WITH PHYTO-NANO COMPOSITE

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Name of the invention: COLLAGEN BASED WOUND DRESSING LOADED WITH PHYTO-NANO COMPOSITE

Patent Application & priority date / Patent Number & date of patent: 201941053468 filed on 23/12/2019

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Brief description of the patent (Abstract): (1-2 sentences)

This product development technology employs a combined approach to design a cost-effective, indigenous, non-toxic, anti-microbial, eco-friendly product. It represents a translatable approach for developing a skin substitute for medical applications

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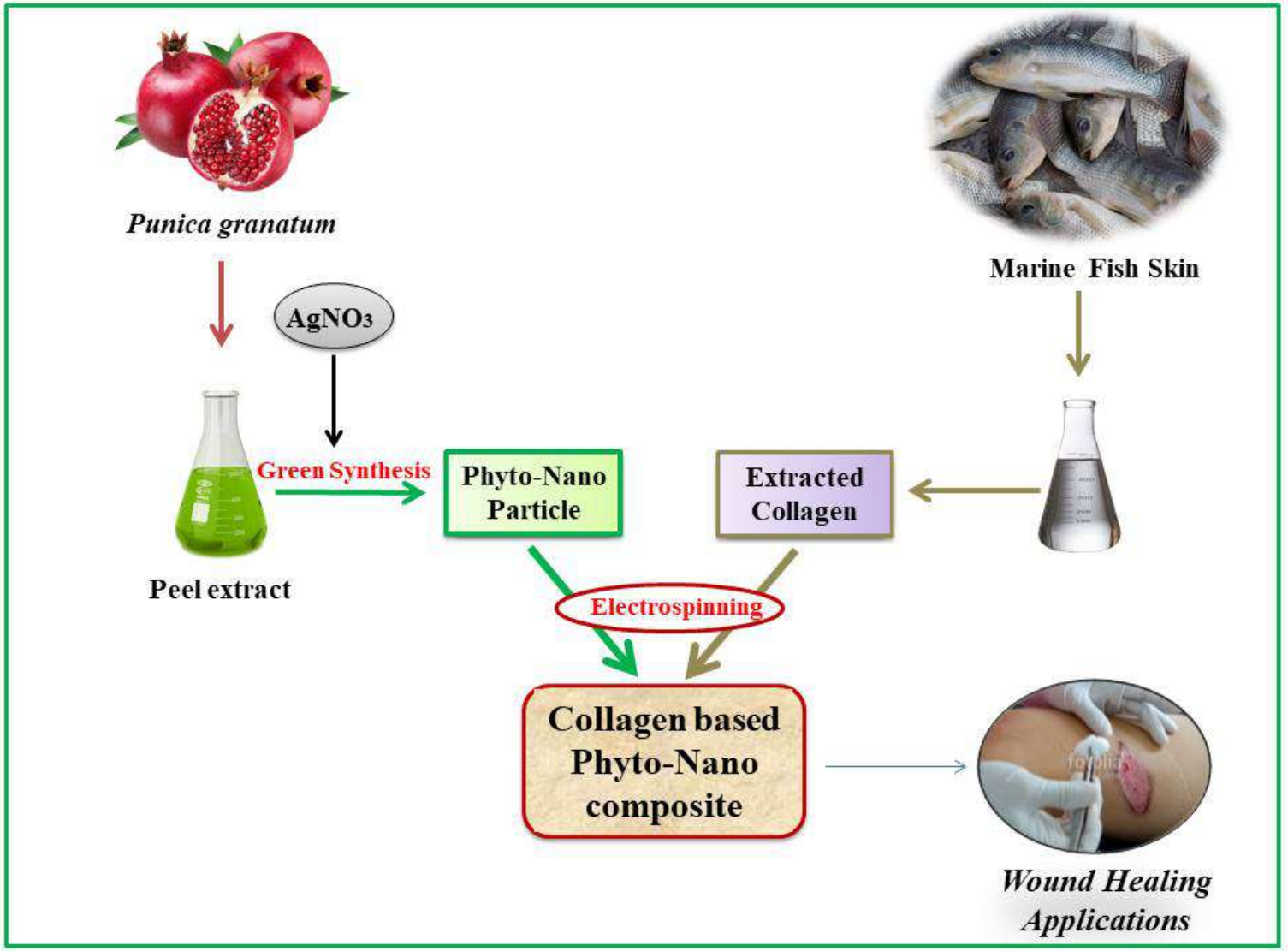
Graphical abstract:



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Novelty of the invention:

Phyto-nano composites were merged with native collagen from marine waste to create wound treatment scaffolds/dressings. We utilize *Punica granatum* peel extract to produce phytochemical capped silver nanoparticles, enhancing wound healing. These nanoparticles are seamlessly integrated into collagen scaffolds, offering antimicrobial and regenerative benefits. Our approach represents a pioneering blend of natural and technological innovation, promising effective treatment for chronic wounds and scar reduction.

Utility of the invention:

Effective Wound Treatment: It provides an advanced solution for treating wounds by incorporating phyto-nano composites and collagen scaffolds, enhancing the healing process.

Scar Reduction: The product aids in minimizing scarring, improving the overall aesthetic outcome of the wound healing process.

Antimicrobial Properties: By integrating silver nanoparticles, it prevents and reduces infections, ensuring better wound management.

Regenerative Action: The product promotes regenerative cellular actions, facilitating faster healing and tissue regeneration.

Sustainability: It utilizes marine waste and discarded *Punica granatum* peel extract, contributing to environmental sustainability by repurposing waste materials.

Cost-Effective: By utilizing natural and readily available materials, the invention offers a cost-effective alternative for wound treatment compared to conventional methods.

Versatility: It can be applied to various types of wounds, including chronic wounds, burns, and injuries, expanding its utility across different medical scenarios.

Non-obvious nature of the invention:

The non-obvious nature of this invention lies in its unconventional combination of materials and processes, such as phyto-nano composites, marine waste collagen, and *Punica granatum* peel extract-derived silver nanoparticles. These elements, along with their unique integration methods, offer synergistic benefits like antimicrobial properties, regenerative cellular actions, and scar reduction. Additionally, the incorporation of sustainability aspects, such as utilizing waste materials, adds another layer of novelty to the invention, distinguishing it from conventional wound care solutions.

Results: (proof for clause 2)

Raw materials for the product development

Figure 3: Pomegranate plant and fruit

Figure 4: Photographs and Certificate of Herbarium collection of Herbarium collection of Pomegranate plant (Voucher /Accession No: KUBH 11317)

Figure 5: Red snapper fish (*lutjanus argentimaculatus*)

Figure 6: Identification of fish-Red snapper From Department of Aquatic Biology, University of Kerala

Extraction and Evaluation of bioactive compounds in Pomegranate peel extract

Figure 7: Raw Pomegranate peel and shade dried powdered plant extract

Figure 8: Soxhlet extraction of Pomegranate dried powder to obtained the bioactive extract

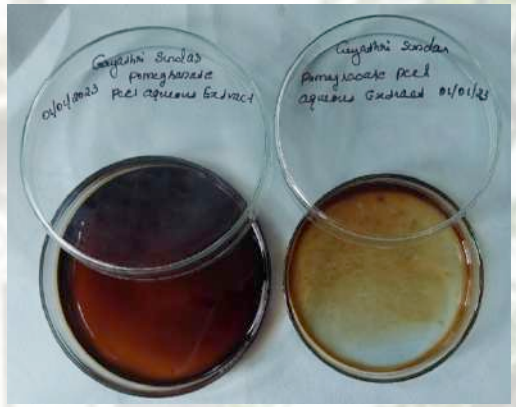


Figure 9: Plant Aqueous extract of Pomegranate peel after soxhlet extraction procedure and Condensed extract with rotary evaporator system for further experiments

Observation: 16 % Yield of plant extract obtained from the Soxhlet extraction method

Phytochemical Screening of plant Extract

Qualitative screening

Plant Constituent	Tests	Plant Extract
Terpenoids	Salkowski's test	++
Flavonoids	Alkaline Reagent test	++
Alkaloids	Mayer's test	+
Carbohydrates	Molisch's test	++
Protein	Biuret test	-
Phenolic Compound	Ferric Chloride test	++
Tannins	Lead Acetate test	++
Saponins	Foam test	++
Test for fixed oils and fats	Spot test	-

Table 1: Presence of Secondary metabolites of plant extract from phytochemical screening (- Absent, ± weakly present, + Present, ++ strongly present)

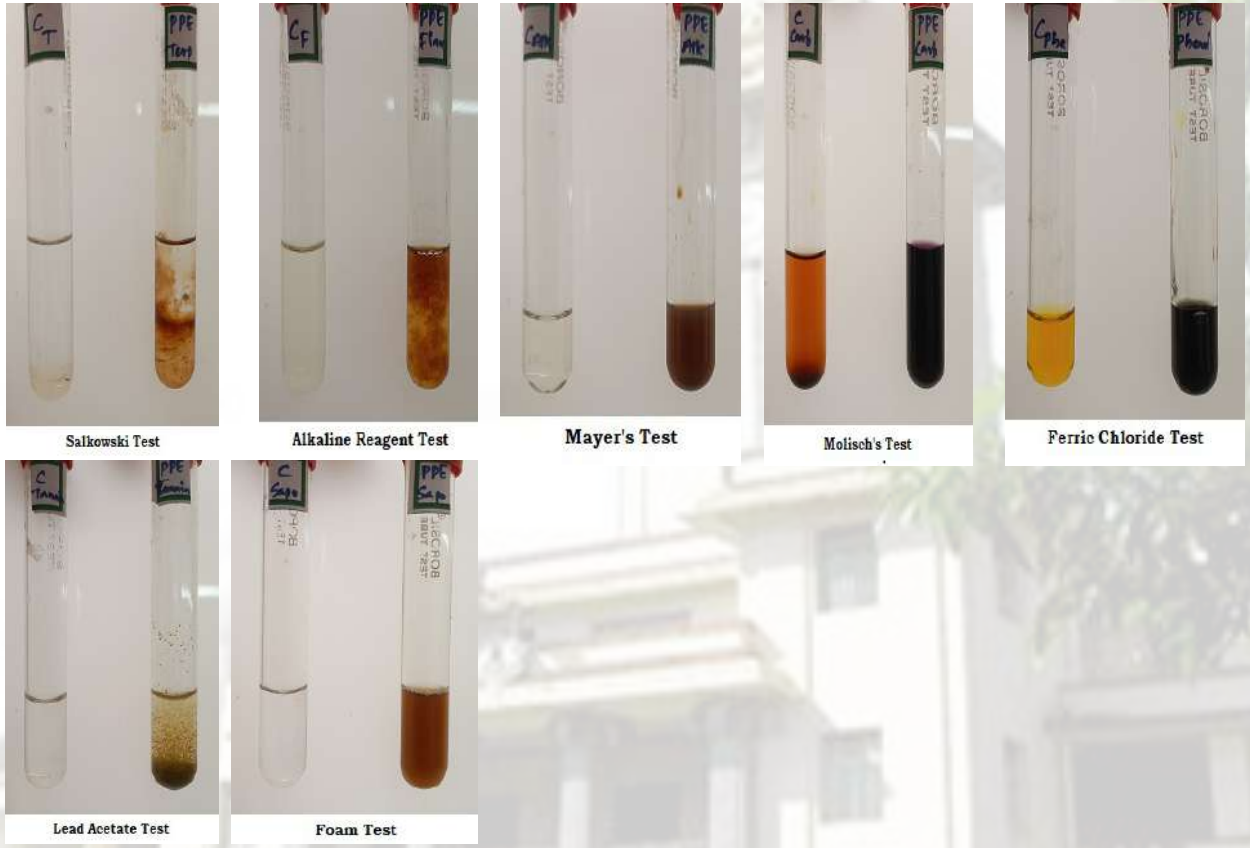
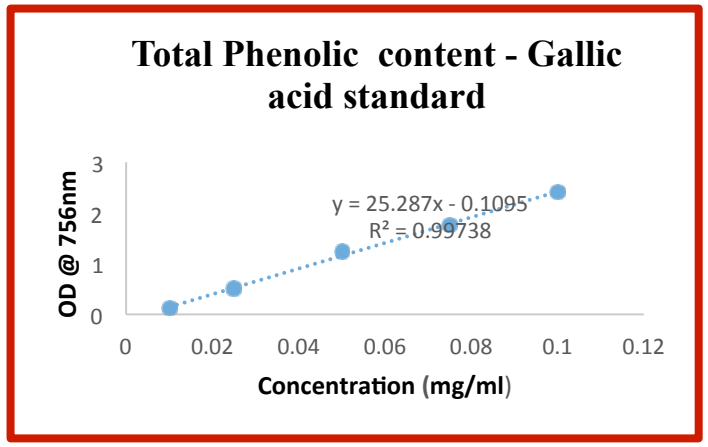
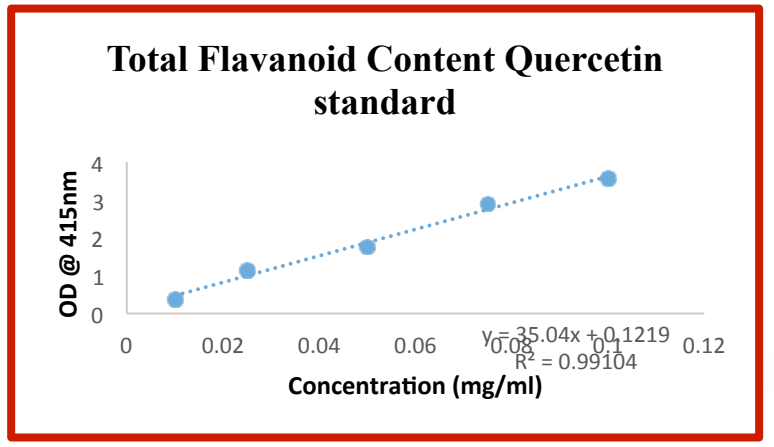


Figure 10: Photographs of phytochemical Screening

Observation: Presence of Terpenoids, Flavonoids, Alkaloids, Carbohydrates, Phenolic Compound, Tannins, Saponins identified in the plant Samples

Quantitative Analysis





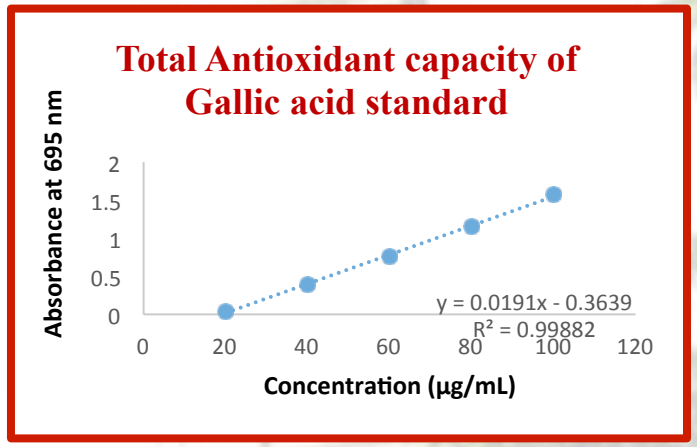


Figure 11: Quantification of Total Phenolic Content, Total Flavanoid Content and Total Antioxidant Capacity of plant extract

Observation: Presence of Phenolic content, Flavanoid content and total antioxidant Capacity is quantified with their standard in plant extract

Identification of Bioactive Compound in plant extract by mass spectral analysis

GC/MS Analysis

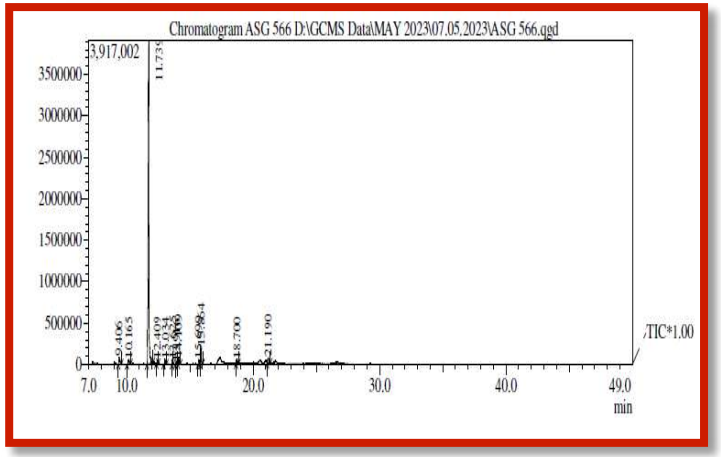


Figure 12: Chromatogram of plant extract in GC/MS analysis

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.406	521751	2.66	89502	1.90	Pyranone	101.05
2	10.165	325501	1.66	53248	1.13	2-Furanone, 3,4-dihydroxytetrahydro	56.00
3	11.739	16217174	82.77	3909669	83.02	5-Hydroxymethylfurfural	97.05
4	12.409	110972	0.57	43165	0.92	3,5-Dimethyl-3-heptene	126.05
5	13.034	208689	1.07	58605	1.24	N,N'-DI-N-PROPYL THIOUREA	55.00
6	13.625	156534	0.80	66235	1.41	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.05
7	13.916	125927	0.64	34039	0.72	Larixic acid	126.05
8	14.109	239667	1.22	65378	1.39	2-HEPTYL HEXANOATE	55.00
9	15.699	229271	1.17	55736	1.18	1,2,3-BENZENETRIOL	126.00
10	15.864	715039	3.65	216164	4.59	1,3-DIHYDROXY-4-HEXENE	71.00
11	18.700	307482	1.57	50980	1.08	1,6-ANHYDRO-BETA-D-GLUCOPYRANOSE	60.00
12	21.190	434049	2.22	66334	1.41	1,6-Anhydro-.beta.-D-glucofuranose	73.05
		19592056	100.00	4709055	100.00		

LC/MS Analysis

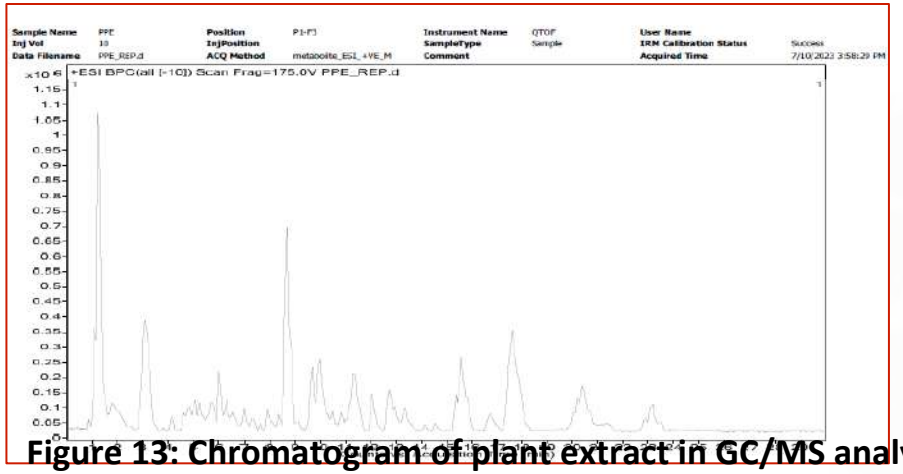


Figure 13: Chromatogram of plant extract in GC/MS analysis

O-Caffeoyl-(b-D-glucose 6- O-sulfate)
1-O-Caffeoyl-(b-D-glucose 6- O-sulfate)
Pallidol 3,3''-diglucoside
Punicalin
Punicalin
Punicacortein D
Punicalin
Punicacortein D
Punicacortein D
Punicacortein D
Punicacortein D
Sanguiin H9
Punicacortein B
(±)-Flufenprox
4-Methylumbelliferyl sulfate
4-Methylumbelliferyl sulfate
Naringenin
1-O-Caffeoyl-(b-D-glucose 6-O-sulfate)

Table 2: List of bioactive compounds present in the plant extract with their charge

Table 3: List of major wound healing responsible compounds present in plant Extract

Fourier-transform infrared spectroscopy (FTIR)

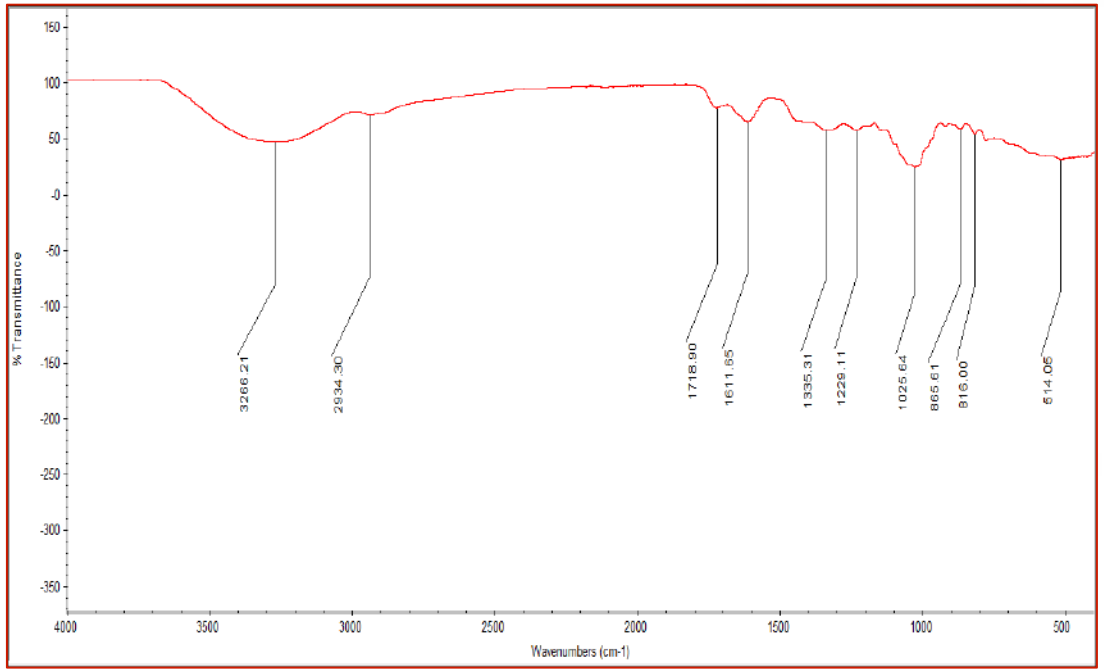


Figure 14: FTIR Images of plant extract identified functional groups present in the plant extract

Functional Group	Frequency (cm-1)	intensity
alcohol OH stretch	3266	strong
-C-H stretch	2934	weak
C=O ketone	1718	strong
NO2 stretch	1335	strong
C-O-C stretch	1229 several	strong
C-F	865	strong
C-Cl	814	strong

Table 4: Infra red frequency table of plant extract

Observation: Major wound healing responsible bioactive compounds and secondary metabolites are identified from the Mass spectral analysis

Antimicrobial Evaluation

Mueller Hinton agar (MHA) test was used to evaluate the anti microbial activity of plant samples against organisms: *Staphylococcus aureus* (MTCC 96) and *Pseudomonas aeruginosa* (ATCC 27853)

Plant Extract Concentration (mg/ml)	Zone of inhibition (mm in diameter)	
	Pseudomonas aeruginosa	Staphylococcus aureus
Streptomycin	13	17
DMSO	NA	NA
0.5	19	9
1	22	10
2	25	12

Table 5: Measurement of Zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Staphylococcus aureus ***Pseudomonas aeruginosa***

Figure 15: Photographs of zone of inhibition against gram negative and gram positive bacteria

Observation: Plant extract having strong anti bacterial activity in different concentrations against gram positive and gram negative bacteria

Synthesis and characterization of Pomegranate peel extract based Silver nanoparticles

Figure 16: Synthesis of nanoparticles by green synthesis method, mixing of plant extract and silver nitrate solution and kept for hours to synthesize nanoparticles

0th Hour of Nano synthesis 24th Hour of Nano synthesis 48th Hour of Nano synthesis 72th Hour of Nano synthesis

Figure 17: Different time interval to take the synthesis of phyto nanoparticles

Figure 18: Synthesized by green synthesis method using Plant extract and Silver nitrate solutions- Phyto based silver nanoparticles

Observation: Phyto nanaoparticles are successfully synthesized using plant extract by bioreduction/green synthesis method

Characterization of phyto nanoparticles

UV-Vis Spectroscopy at different time intervals

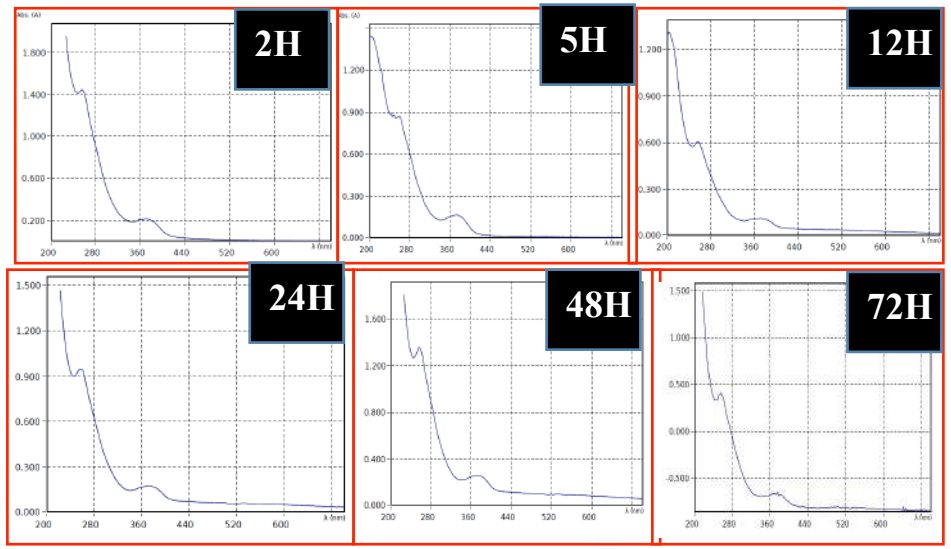


Figure 19: Confirmation of the synthesis phyto nanoparticles by UV spectral analysis at different time intervals

Functional Group	Frequency (cm-1)	intensity
carboxylic acid OH stretch	2996	strong
-C-H stretch	2915	weak
stretch	1990	variable
C=O amide	1686	strong
C=C aromatic	1442	weak
CH2 bend/CH3 bend	1339	medium
C-F	1191	strong
C-Cl	923	strong

Table 6: Infra red spectral table of nanoparticles

FTIR analysis

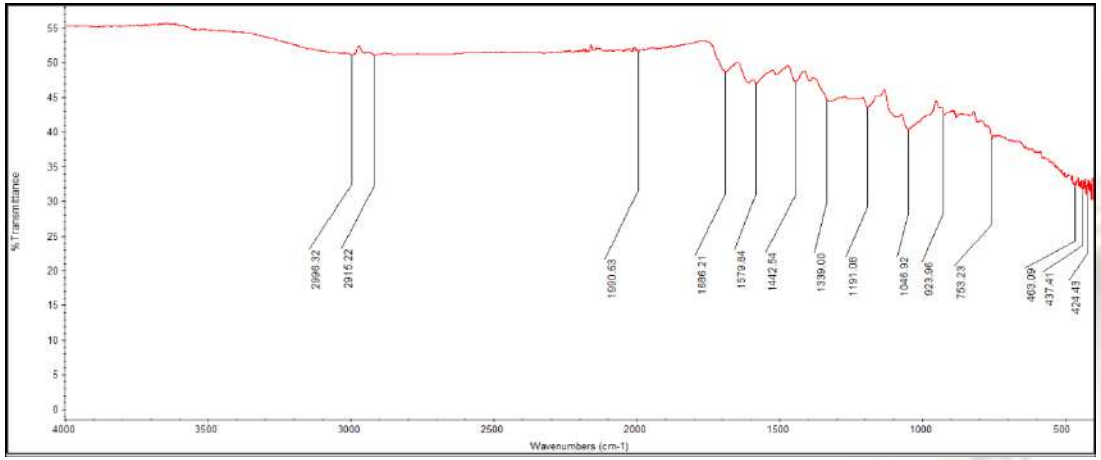


Figure 20: Functional groups present in the phyto nanoparticles

Dynamic light scattering analysis (DLS Analysis)

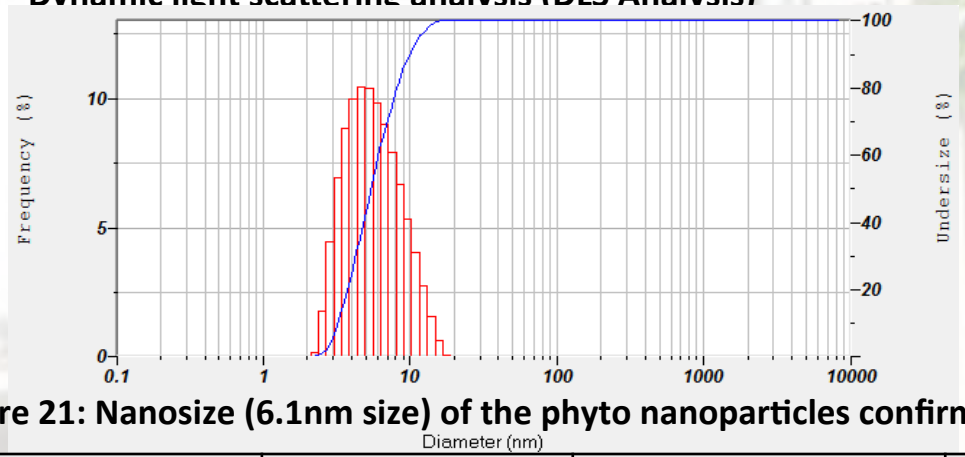


Figure 21: Nanosize (6.1nm size) of the phyto nanoparticles confirmed by DLS Analysis

Measurement Type	Sample Name	Scattering Angle	Size (Median)(nm)	Mean(nm)
Particle Size	PGSN	90	5.4	6.1

Zeta potential Analysis

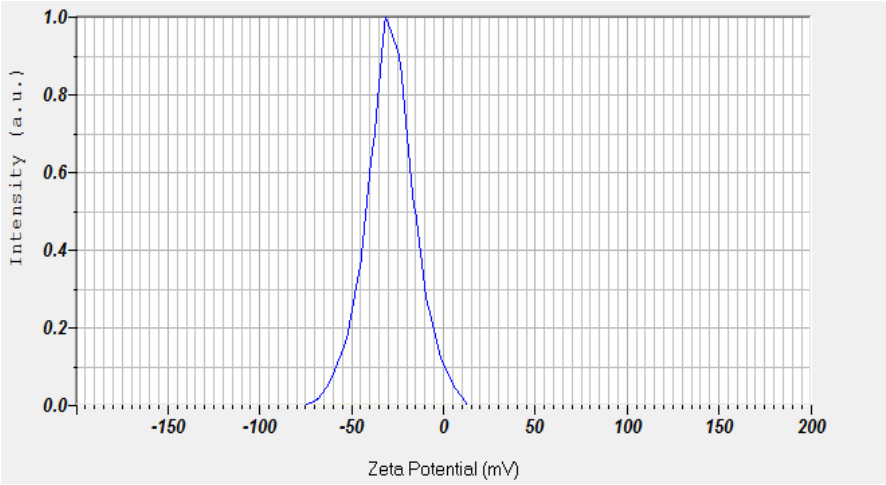


Figure 22: Stability of nanoparticles confirmed by Zeta potential Analysis of phyto nanoparticles

Atomic Force Microscopy (AFM Analysis)

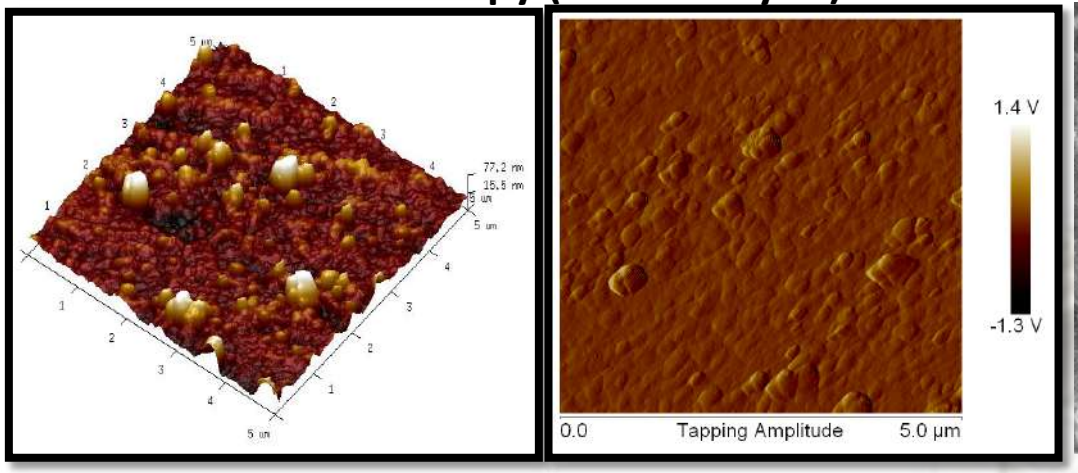


Figure 23: Surface roughness and bioactive molecule presence confirmed by AFM Analysis

Scanning Electron Microscopy

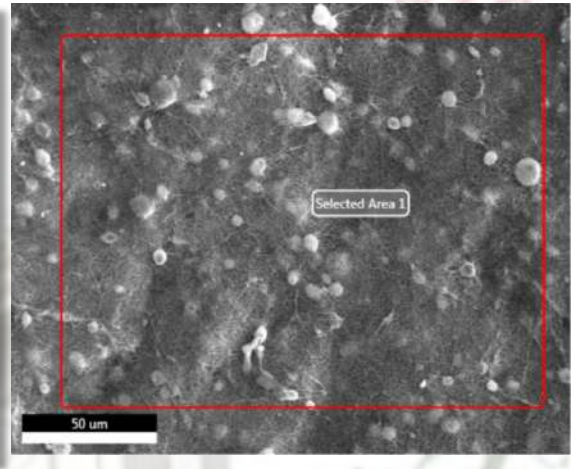
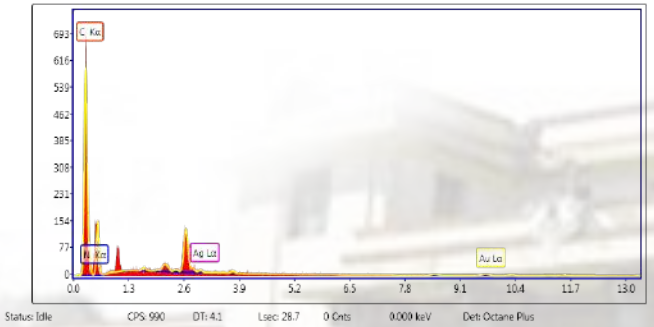


Figure 24: Surface roughness and nanosize of phyto nanoparticles confirmed by SEM analysis

Measurement Type	Sample Name	Zeta Potential (Mean) (mV)	Electrophoretic Mobility mean (cm2/Vs)	Conductivity (mS/cm)	Temperature of the holder (°C)	Electrode Voltage (V)
Zeta Potential	PGSN	-28.9	-0.00022	0.126	25	3.4

Table 7: List of Zetapotential value and measurements of Phyto nanoparticles

Energy dispersive X-ray (EDX analysis)



Element	Weigh t %	Atomic %
C K	54.21	60.58
N K	40.74	39.04
AgL	0.56	0.07
AuL	4.50	0.31

Figure 25: Elemental composition confirmed by EDX presence of Ag is confirmed the silver nanoparticles and N is indicated the presence of phyto compounds in nanoparticles

Observation: Phyto nanoparticles nanosize shape, incorporation od phyto compounds in nanoparticles and surface roughness are confirmed by different analytical techniques

Fish Collagen Extraction Procedure






Figure 26: Different steps of fish collagen isolation from fish skin waste

Extracted fish collagen shaped as 1 cm diameter

Characterization of Extracted Collagen

FTIR Analysis

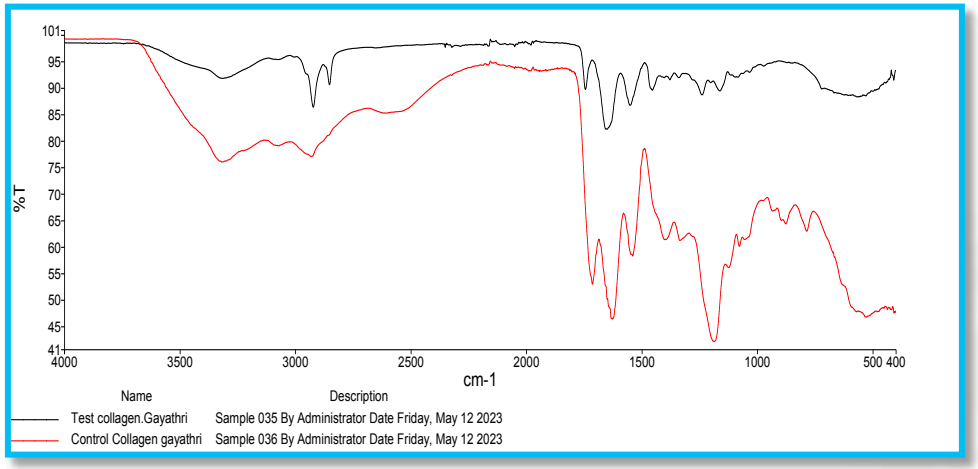


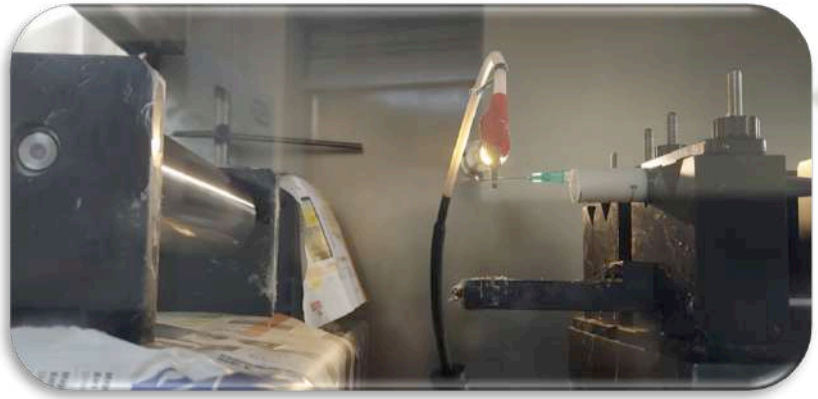
Figure 27: FTIR spectrum, confirmed the purity of Extracted collagen with commercial control collagen

Observation: Fish collagen is successfully extracted from fish skin waste and purity of extracted collagen is confirmed by different analytical techniques

Fabrication of phyto based nanoparticle incorporated collagen scaffold by electrospinning method

Extracted collagen is used to fabricate the wound dressing by electrospinning method with and without the incorporation of phyto nanoparticles

Fabrication of Wound dressing



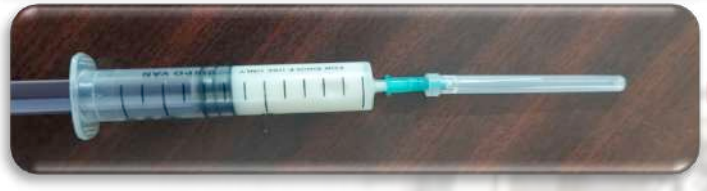


Figure 28: Electrospinning apparatus under the wound dress making process

Figure 29: collagen based wound dressing for burns

Observation: Nano fibrous continuous Phyto nano incorporated fish collagen wound dressing is successfully synthesized by electrospinning method

Characterization of wound dressing

FTIR Analysis

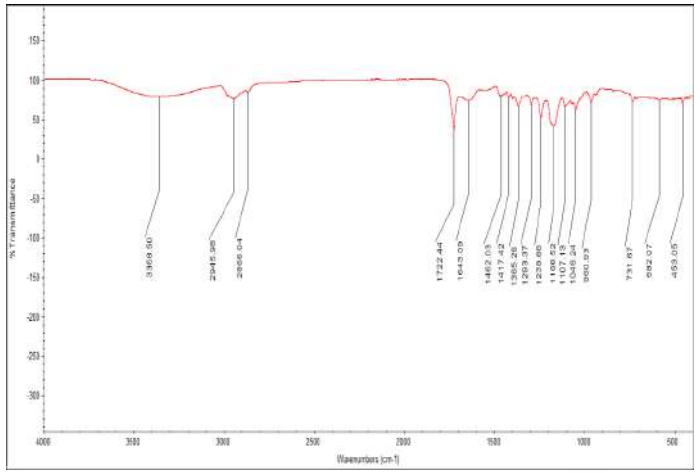


Figure 30: Presence of phyto nanoparticles and collagen is confirmed by FTIR Analysis

SEM Analysis

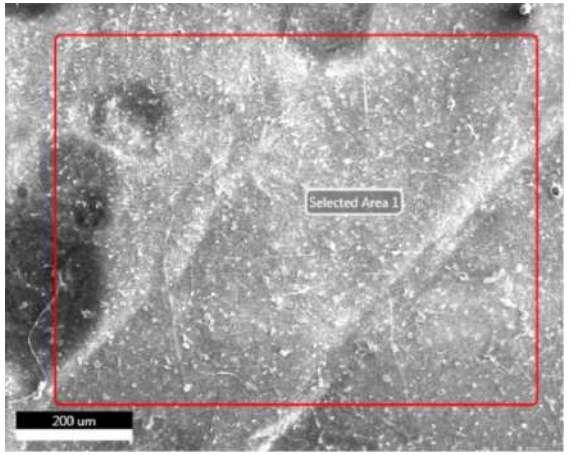


Figure: 31: Surface morphology of wound dressing with nano fibrous continuous structure observed

Energy Dispersive X-ray analysis (SEM-EDX)

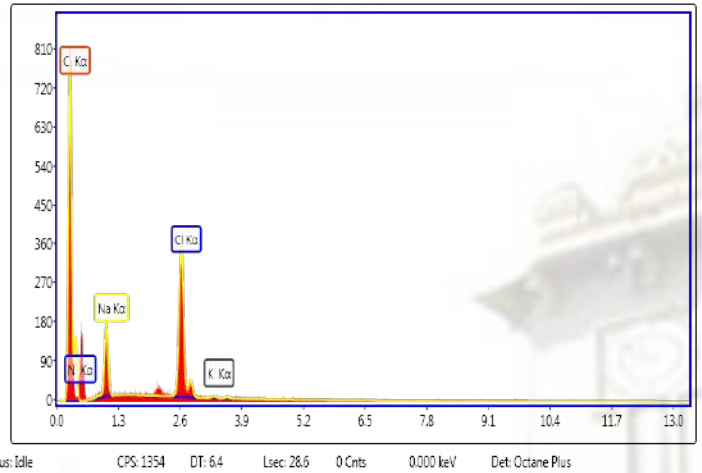
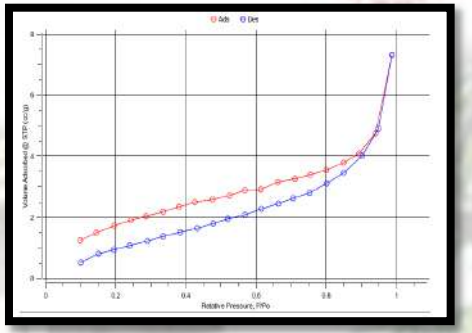


Figure: 32: Elemental composition of wound dressing with phyto nanoparticles

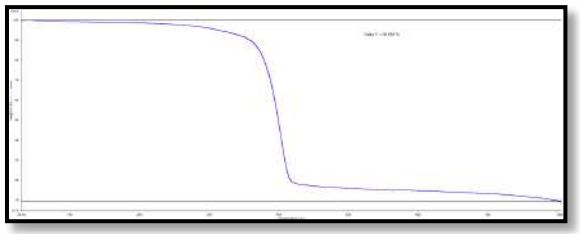
BET Analysis



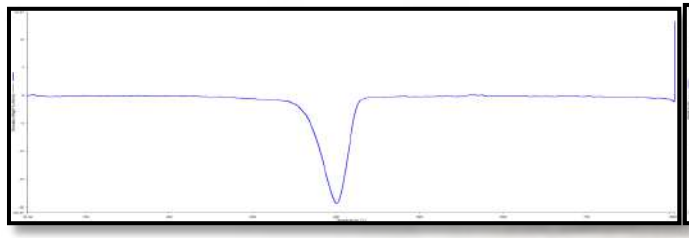
Surface area	Average pore Radius	Total pore Volume	Pore size
6.987m²/g	3.249+00nm	1.1329e-02cc/g	75.24 nm

Figure 33: Details of incorporation of phyto nanoparticles in wound dressing

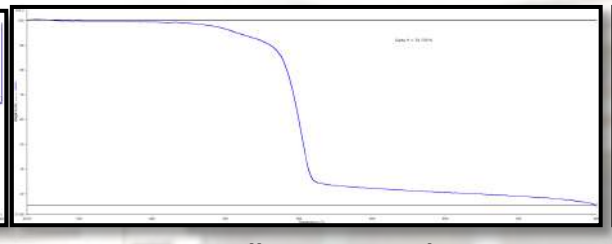
TGA (Thermal Gravimetric Analysis)



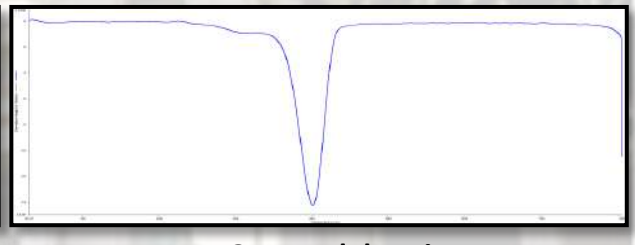
TG% Collagen Control



TG% wound dressing



DTG Collagen Control

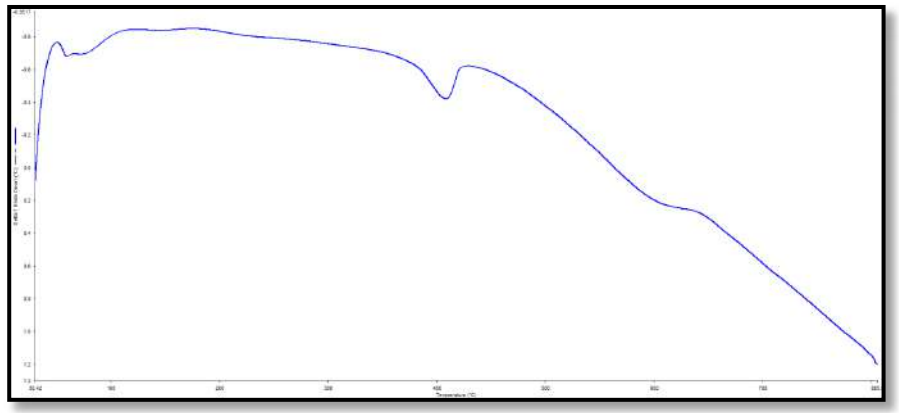


DTG wound dressing

Figure 34: Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a sample is heated at a constant rate.

DTA (Differential Thermal Analysis)

DTA Collagen Control



DTA Wound dressing

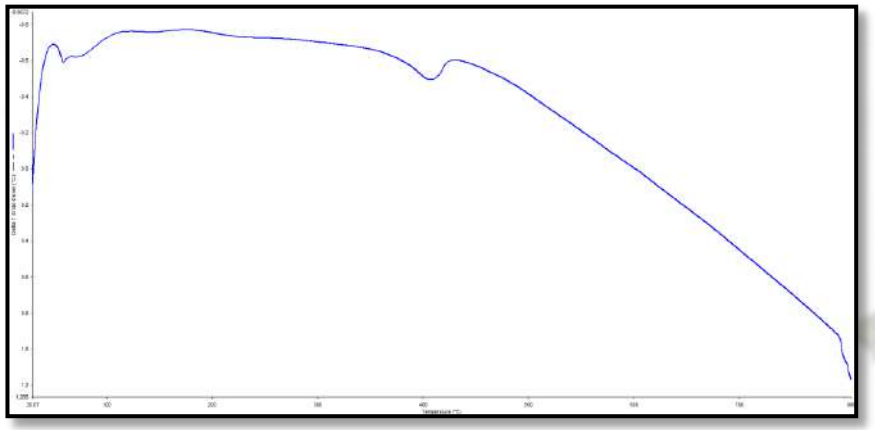
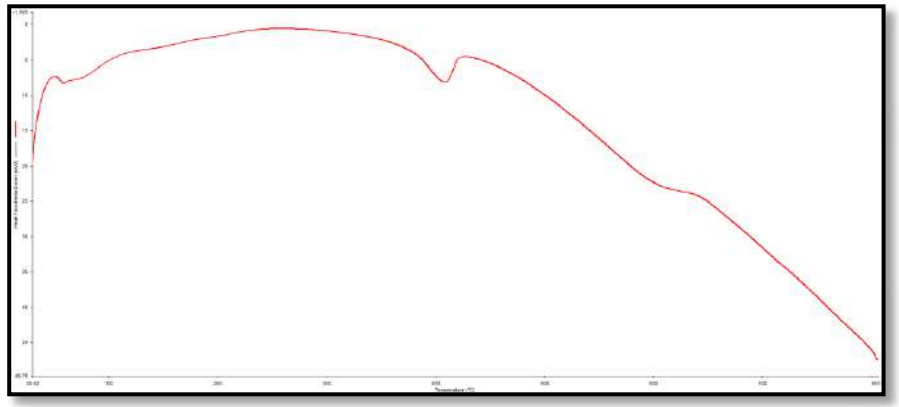


Figure 35: Differential Thermal Analysis of wound dressing with the comparison of collagen control

DSC (Differential Scanning Calorimetry)

DSC Collagen Control



DSC Wound dressing

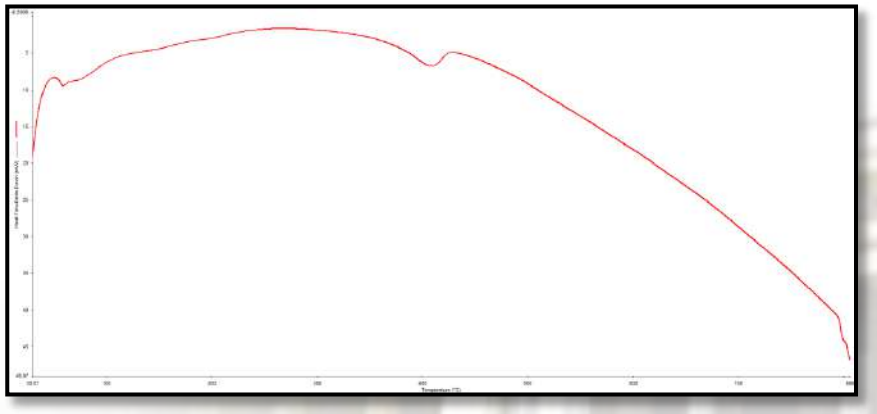


Figure 36: Differential Scanning Calorimetry of wound dressing with the comparison of collagen control

Observation: Wound dressing is well characterized related its stability, thermal degradation, incorporation, surface morphology and mechanical stability. All result indicates that the better performances might be showed as wound dressing

Cytocompatibility evaluation (*In vitro*) of collagen burn wound dressing

Direct contact assay on L929 Fibroblast cell lines

Figure 37: Cytocompatibility evaluation on L929 Fibroblast cell lines, Result indicates that the proliferation of cells increased in the wound dressing as a control

Direct contact assay on HaCaT Keratinocytes Cell lines

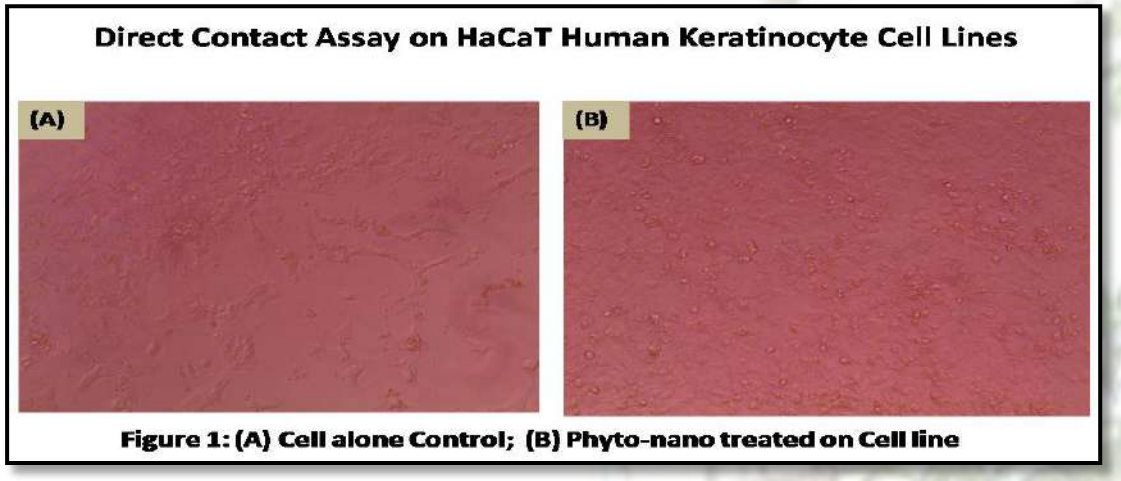


Figure 38: Cytocompatibility evaluation on HaCaT Keratinocytes cell lines, Result indicates that the proliferation of cells increased in the wound dressing as a control

Scratch wound assay

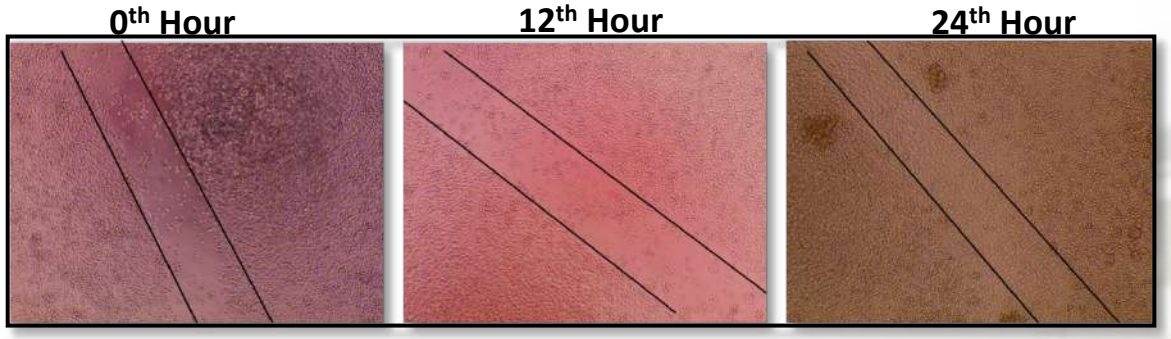


Figure 39: In vitro wound healing ability of wound dressing in different time intervals

AFM Analysis of L929 Cell seeded wound dressing

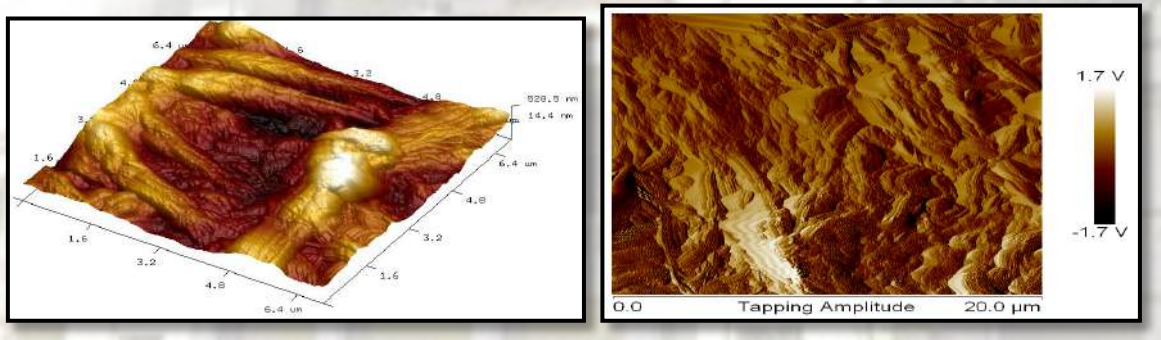
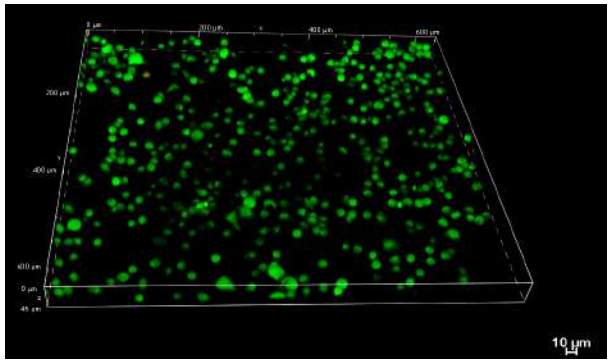
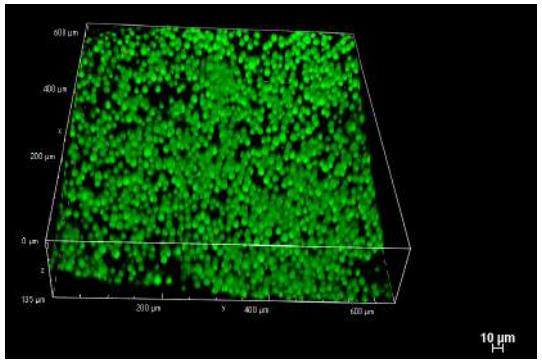


Figure 40: Cell seeded wound dressing surface morphology and cellular shape retained in AFM Results

LIVE/DEAD Assay on L929 Fibroblast Cell lines



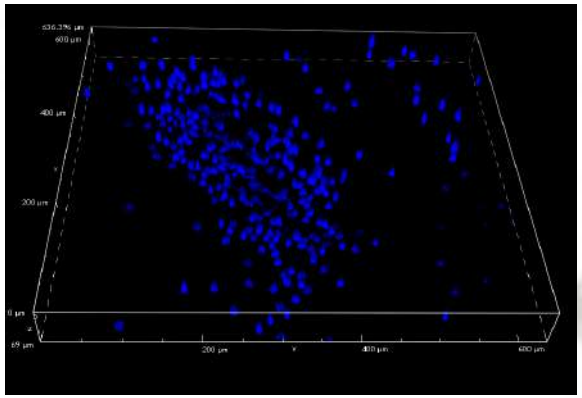
Collagen Control



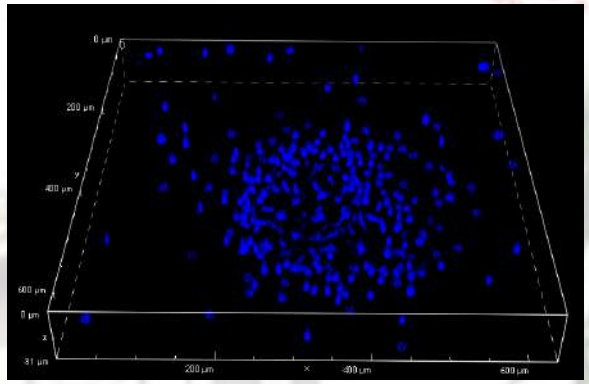
Wound dressing

Figure 41: Maximum viable (Green) cell appeared on Wound dressing than control

DAPI Assay on L929 Fibroblast Cell lines



Collagen Control



Wound dressing

Figure 42: Cell viability on L929 Fibroblast cell line observed, Blue colour indicated the viable nucleus of the cell

SEM Analysis of L929 Cell seeded wound dressing

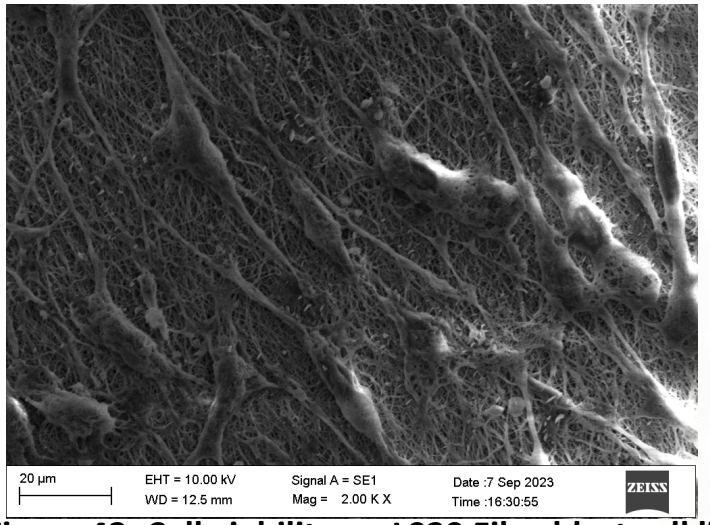


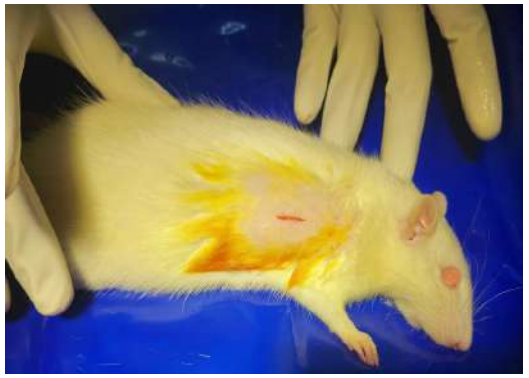
Figure 43: Cell viability on L929 Fibroblast cell line observed by SEM Analysis

Observation: Cells are successfully seeded on the wound dressing for three days, results indicates that the cell viability on wound dressing is perfect than the control

Biocompatibility evaluation (*In vivo*) of collagen burn wound dressing Sprague dawley rat animal model

Biocompatibility evaluations of wound dressing

Samples for Implanatation



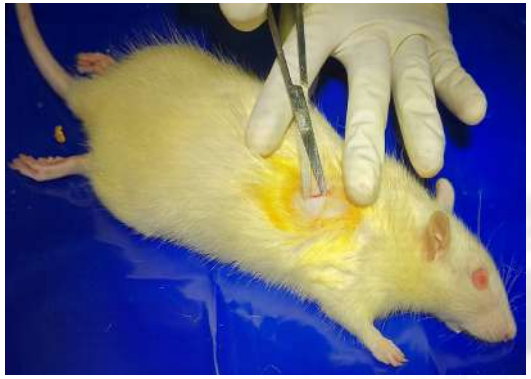


Figure 44: Creation of pouch to implant wound dressing Subcutaneous implantation of wound dressing Suturing of wound after implantation

Evaluation of wounds after implantation

Normal rat after 7th Day

Collagen Control after 7th Day

Wound dressing after 7th Day

Figure 45: Evaluation of wounds after implantation

Collection of implanted samples

Figure 46: Samples collected after implantation

Serum analysis

Sl. No	TEST	Normal Control (N1)	Collagen Control (C2)	Wound dressing (T1)
1	Urea	28	32	34
2	Creatinine	0.4	0.5	0.5
3	SGOT	148	117	167
4	SGPT	42	28	56

Table 8: Serum analysis of biocompatibility evaluation in 7th Day

Sl. No	TEST	Normal Control (N1)	Collagen Control (C2)	Wound dressing (T1)
1	Urea	28	28	31
2	Creatinine	0.4	0.5	0.6
3	SGOT	148	72	122
4	SGPT	42	24	40

Table 9: Serum analysis of biocompatibility evaluation in 21st Day

Histopathology evaluation of samples

Tissue samples

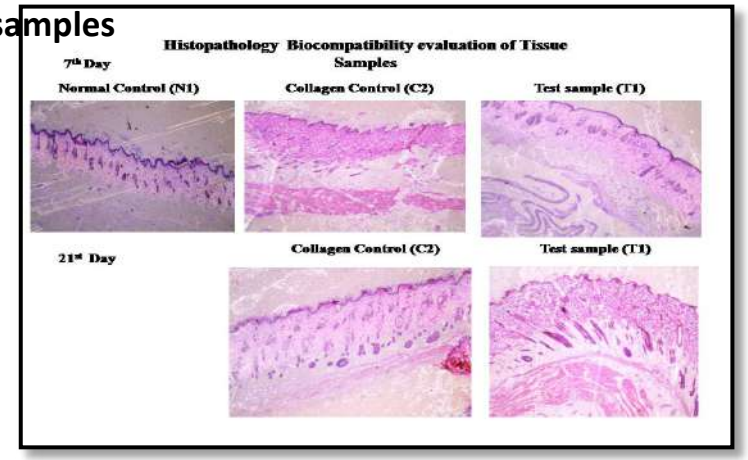


Figure 47: Histopathology evaluation of tissue samples

Kidney samples

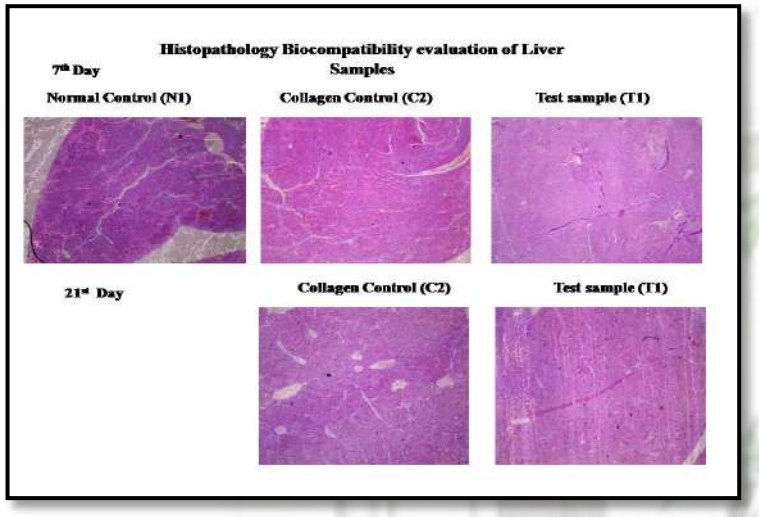


Figure 48: Histopathology evaluation of Kidney samples

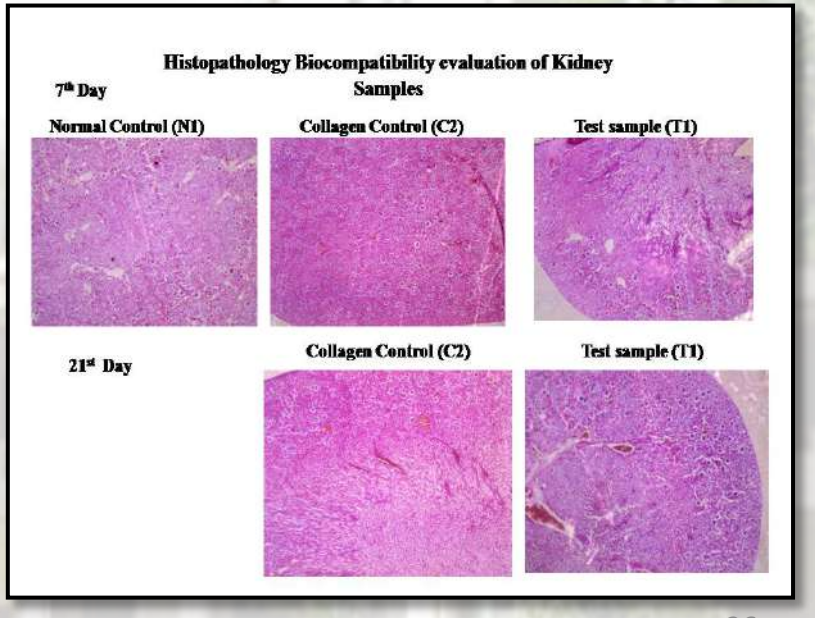
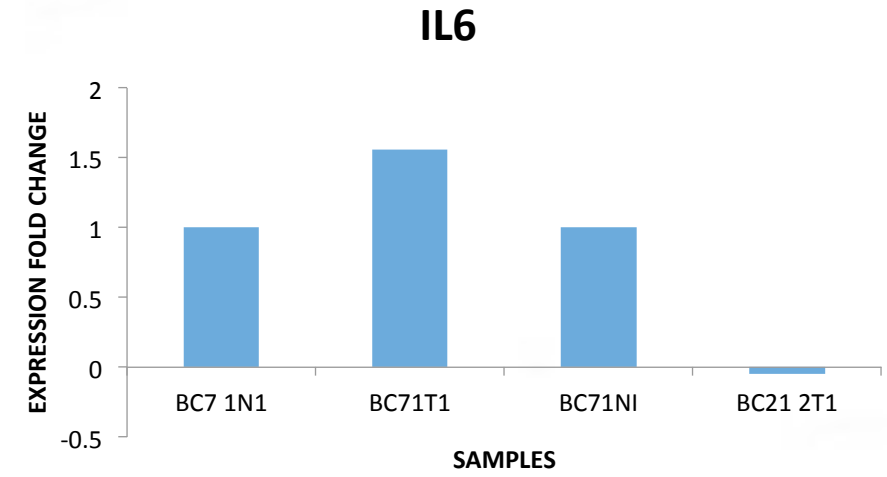
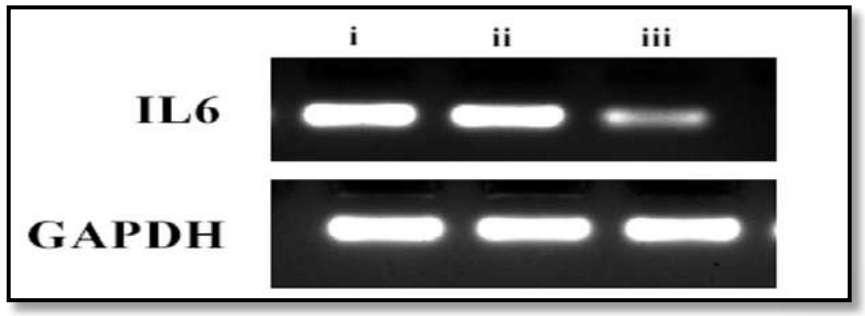


Figure 49: Histopathology evaluation of Liver samples

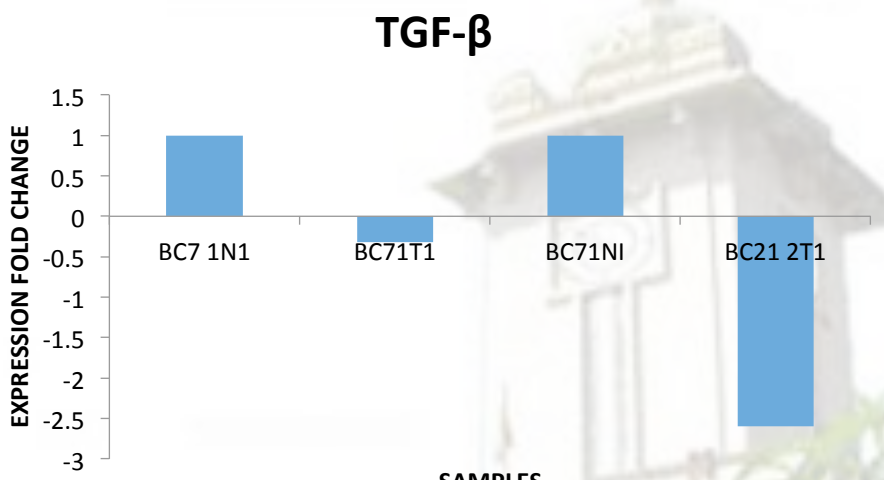
Molecular expresión of cytokines

Pro inflammatory genes: IL6





inflammatory genes: TGF Beta



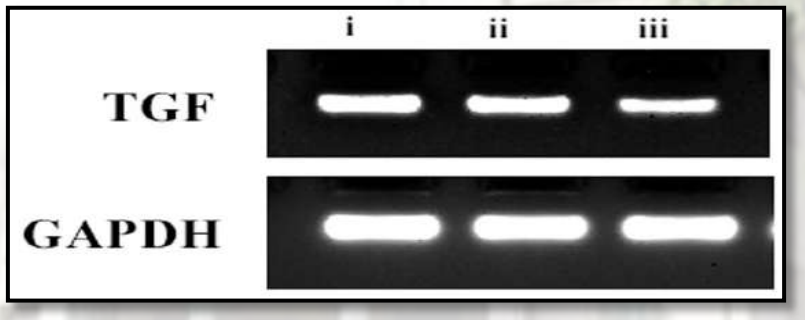


Figure 50: Gene expression of pro inflammatory and anti-inflammatory expression of tissue on 7th Day and 21st Day

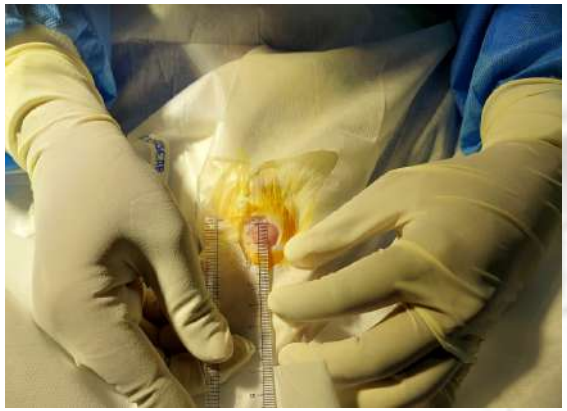
Observation: wound dressings are subcutaneously implanted and local effects after implantation is evaluated on rat, result confirmed the wound dressings are biocompatible

Wound healing evaluation of (*In vivo*) of collagen burn wound dressing in Sprague dawley rat animal model

Burn creation

Figure 51: Burn creation apparatus

Burn creation



Measuring the wound area

Implantation of wound dressing

Burn Evaluation

Burn wound healing on 7th Day

Sham Control (S1)

Commercial Control (C1)

Collagen Control (C2)

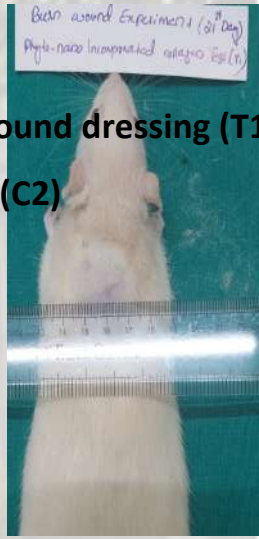
Wound dressing (T1)

Burn wound healing on 21st Day

Sham Control (S1)

Commercial Control (C1)

Collagen Control (C2)



Wound dressing (T1)

Serum analysis

Sl. No	TEST	Normal Control (N1)	Sham Control (S1)	Commercial Collagen Control (C1)	Collagen Control (C2)	Wound dressing (T1)
1	Urea	28	34	38	37	28
2	Creatinine	0.4	0.5	0.5	0.5	0.5
3	SGOT	148	111	97	134	145
4	SGPT	42	40	34	44	34

Table 10: Serum analysis of burn wound evaluation in 7th Day

Sl. No	TEST	Normal Control (N1)	Sham Control (S1)	Commercial Collagen Control (C1)	Collagen Control (C2)	Wound dressing (T1)
1	Urea	28	29	29	23	22
2	Creatinine	0.4	0.5	0.5	0.5	0.6
3	SGOT	148	88	75	81	91
4	SGPT	42	31	29	31	29

Table 11: Serum analysis of burn wound evaluation in 21st Day

Histopathology evaluation of samples

Tissue Samples

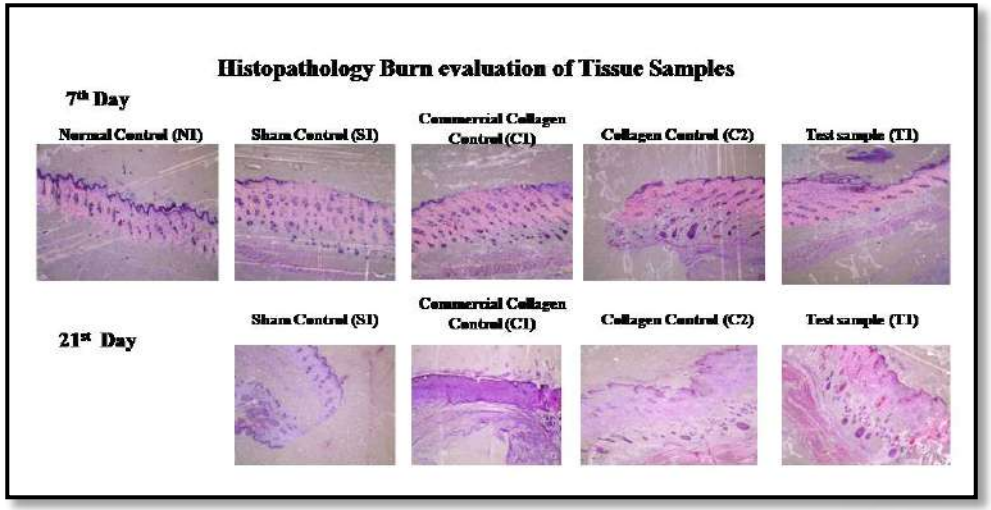


Figure 52: Histopathology evaluation of tissue samples

Kidney Samples

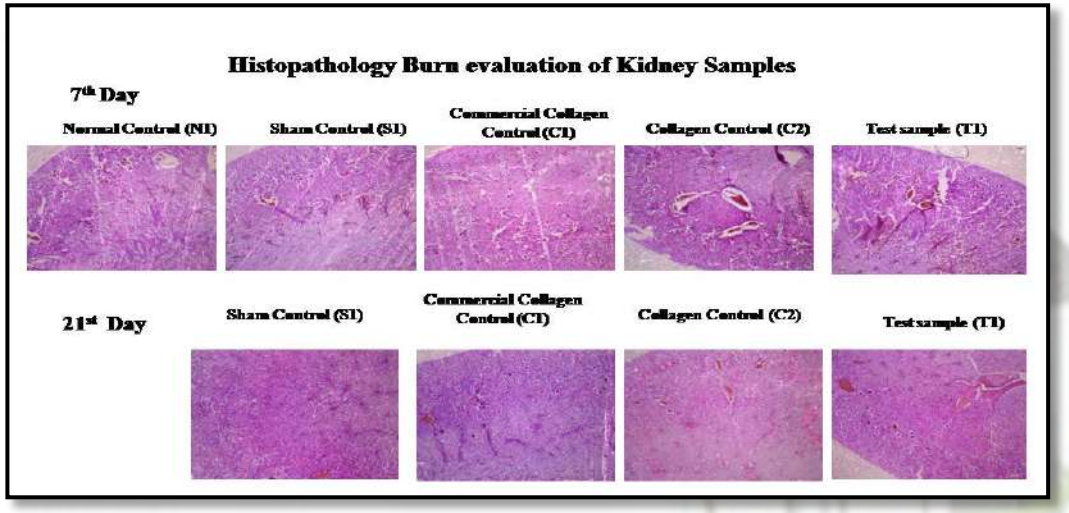


Figure 53: Histopathology evaluation of Kidney samples

Liver Samples

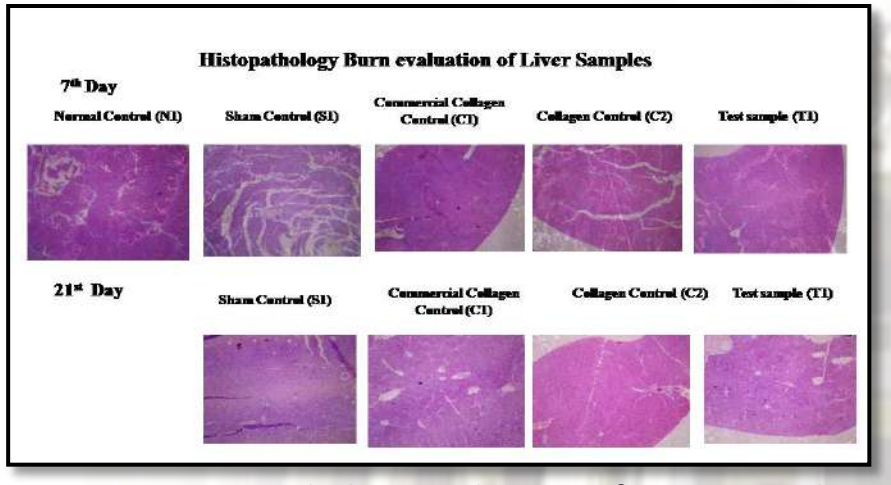
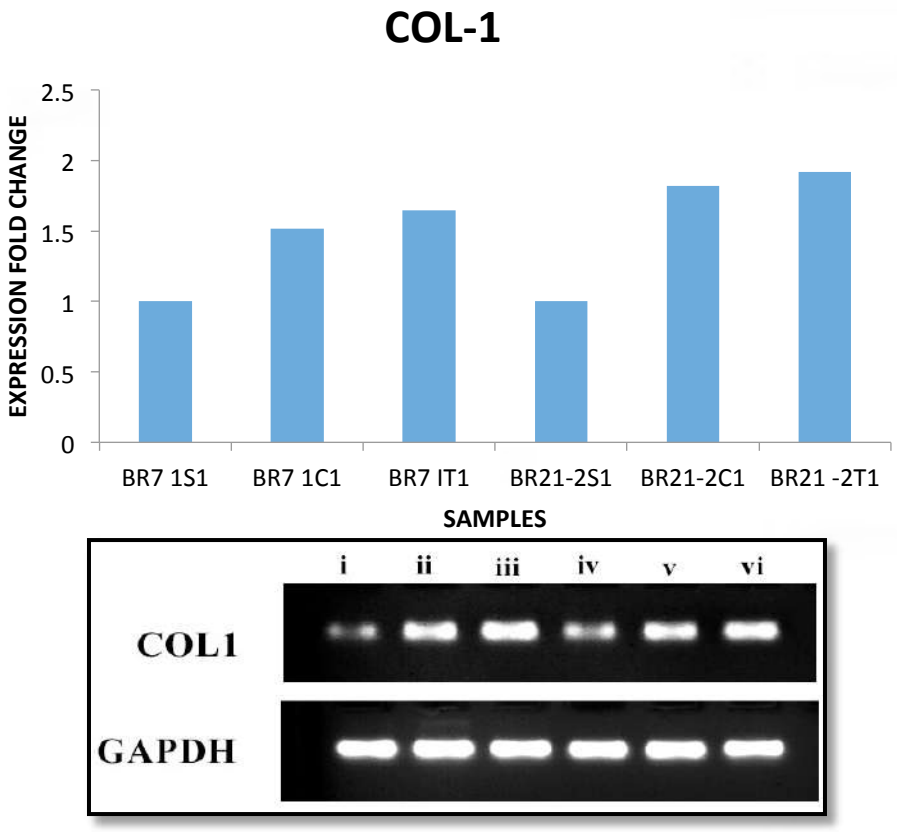


Figure 54: Histopathology evaluation of tissue samples

Molecular expresión of genes
Expression of Collagen 1



Expression of VEGF

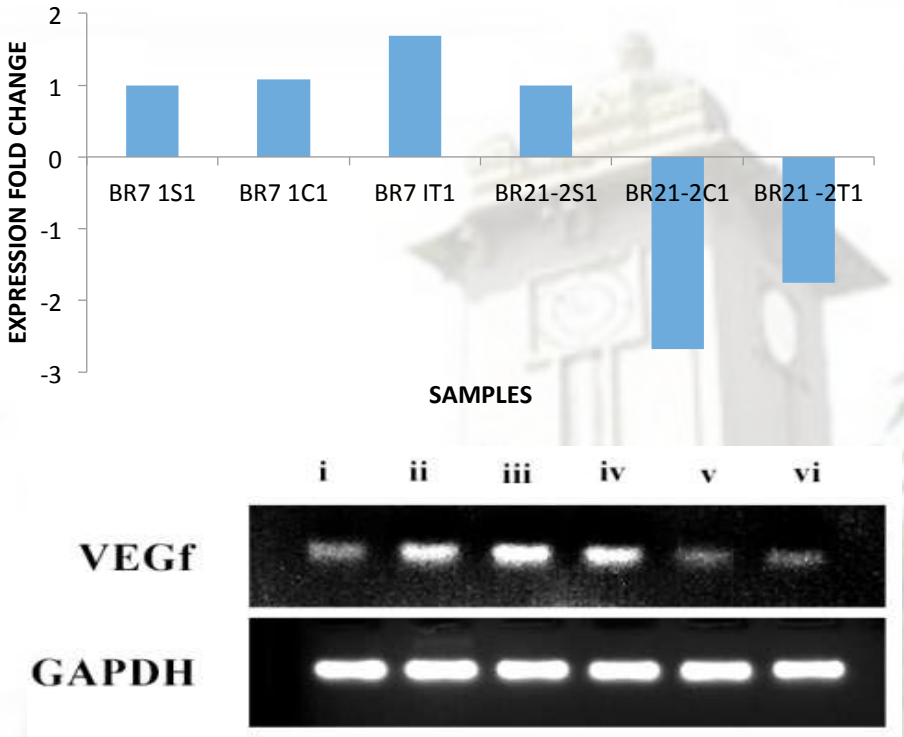


Figure 55: Gene expression of pro collagen 1 and VEGF expression of tissue on 7th Day and 21st Day

Observation: burn wound evaluations carried out in rat model, results indicate that the wound dressing enhanced the healing of wounds other than control

Clauses applied for /protected (for granted patents):

1. Collagen based phyto-nano composite scaffold is biocompatible and promotes wound healing.
2. Scaffold reduces hypertrophic scar formation during healing process.
3. Phyto-nano composites exhibit antimicrobial and anti-inflammatory scenario to favour wound healing.
4. Collagen based phyto-nano composite scaffold promotes the regeneration and repair of skin and surrounding tissues.
5. Collagen based phyto-nano composite scaffold is a chemo attractant and promotes the growth of fibroblasts and keratinocytes at the wound site.
6. Above all, phytochemical incorporated collagen scaffold may be proposed as an ecofriendly, and cost-effective alternative in wound healing applications.
7. In comparison with other commercially available wound dressings, this product has the added benefits of being economical and indigenous, antimicrobial, biocompatible and biodegradable. Furthermore, a waste disposal system may be built for collection of peel extract and fish waste beneficial of mankind.
8. The development of collagen based phyto-nano composite product expenditure will be 80% less than the wound dressings available in the Indian medical market with enhanced wound healing properties.
9. The collagen based phyto-nano composite scaffold has longer ‘shelf life’ compared to similar wound dressing products in clinical use.

Fields where the patent finds application:

Medical, Pharmaceutical, Cosmeceuticals, Nanotechnology, Environmental Science, and Biotechnology

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Whether the work has been published:

(Authors, year, title of publication, Journal name, volume, page no)

- Sundar, G., Joseph, J., C, Prabhakumari, John, A., & Abraham, A. (2021). Natural collagen bioscaffolds for skin tissue engineering strategies in burns: a critical review. International Journal of Polymeric Materials and Polymeric Biomaterials, 70(9), 593–604. https://doi.org/10.1080/00914037.2020.1740991

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