

ANTIMICROBIAL WOUND HEALING GEL USING FISH COLLAGEN

Submitted in partial fulfillment of the requirements for the award of Bachelor of Engineering / Technology degree in B.TECH Biotechnology.

by

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POOJA . V (39230031)



DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

MAY - 2022



SATHYABAMA

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DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Sheril Tabitha . Y (39230042)**, **Shobana . A (39230043)**, **Tiviya Thangaswamy (39230053)**, **Pooja . V (39230031)** who carried out the project entitled “ **ANTIMICROBIAL WOUND HEALING GEL USING COLLAGEN** ” under my / our supervision of internal and external guide from **26.03.2022** to **26.04.2022** .

Internal Guide

DR. RAMESH KUMAR .V

External Guide

DR. SINDHU DIVAKARAN

HEAD OF THE DEPARTMENT

DR. RAMESH KUMAR . V

Submitted for viva voce examination held on **29.04.2022** .

Internal Examiner

External Examiner

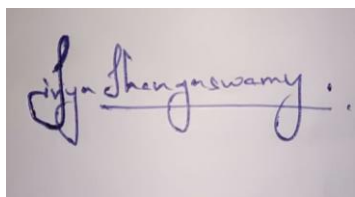
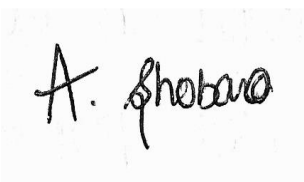
DECLARATION

We **Sheril Tabitha . Y, Shobana . A , Tiviya Thangaswamy, Pooja . V** hereby declare that the Project Report entitled “**ANTIMICROBAL WOUND HEALING GEL USING FISH COLLAGEN**” done by me under the guidance of Dr. Bala at Avigen Biotech Lab Pvt.Ltd ,Chromepet, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology, degree in Biotechnology.

DATE: 29.04.2022

PLACE: Chennai

SIGNATURE OF THE CANDIDATE:

Handwritten signature of Tiviya Thangaswamy in blue ink.Handwritten signature of A. Shobana in blue ink.Handwritten signature of N. Pooja in blue ink.Handwritten signature of Sheril Tabitha in blue ink.

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to Dr. -----., Dean, School of Bio and Chemical Engineering and Dr. Ramesh Kumar, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide Dr. Ramesh Kumar for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT :

Marine collagen has been identified as a potential protein that plays an important role in biomedical, pharmaceutical and cosmetic industries, its extracellular matrix (ECM) plays important roles in the physical integrity of cells, where it is involved in cell proliferation, differentiation, migration, and adhesion. It can also be used as a biomaterial as it is water soluble, metabolically compatible, and highly accessible. Collagen is a naturally occurring polymer characterized by its biodegradability, low toxicity, non-antigenicity, high nutritional value, high stability and binding capacity. It is also characterized by some unique properties such as high emulsification abilities, gelation, water binding capacity. Upon reviewing, it is evident that marine collagen is a versatile compound capable of healing skin injuries of varying severity, as well as delaying the natural human aging process. From in vitro to in vivo experiments, collagen has demonstrated its ability to invoke keratinocyte and fibroblast migration as well as vascularization of the skin.

In addition, Collagen can absorb and associate with nanoparticles, which can have significant impact on the biological behaviour of collagen and nanoparticles in a biological fluid , Nanoparticles like silver nitrate (AgNO_3), have become an attractive alternative source to combat microbes that are highly resistant to various classes of antibiotics, where Silver is a potent antimicrobial agent against a variety of microorganisms and once the element has entered the bacterial cell, it accumulates as silver nanoparticles with large surface area causing cell death. It is safe inorganic antibacterial and it has the ability to kill many types of disease-causing microorganisms . Furthermore, silver is bactericidal at minute concentrations, exhibiting an “oligodynamic” effect through the presence of toxic metal ions . Through this, the activity of silver facilitates its use as antifungal, anti-inflammatory and antibacterial agent.

PROTOTYPE:

EXTRACTION OF COLLAGEN FROM SKIN/ TENDON :

STEP:1

- The skin part alone was collected from the leatherjacket fish and 0.8 mol/L sodium chloride was treated to it at a ratio of 1:6 (w/v) for 10 mins thrice to remove off the impurities.



STEP: 2

- Then it was washed with cold distilled water to reduce its salinity.



STEP : 3

- The sample was then given alkali treatment with 0.1 mol/L sodium hydroxide at a ratio of 1:10 (w/v) for 2-3 days to remove the non-collagenous proteins and also prevent the activity of endogenous proteases on the sample. It is mandatory to change the NaOH solution everyday and then the skin was removed and washed with cold distilled water to bring down its alkalinity.



STEP : 4

- Now the skin was evenly ground to small pieces using a homogenizer.



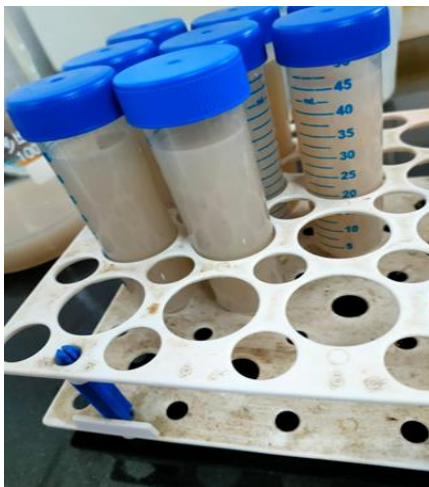
STEP : 5

- The acid soluble collagen (ASC) was prepared by dissolving in 0.5 mol/L of acetic acid for 3 days. And then the solution was centrifuged at 9000rpm for 20 minutes at 4°C.



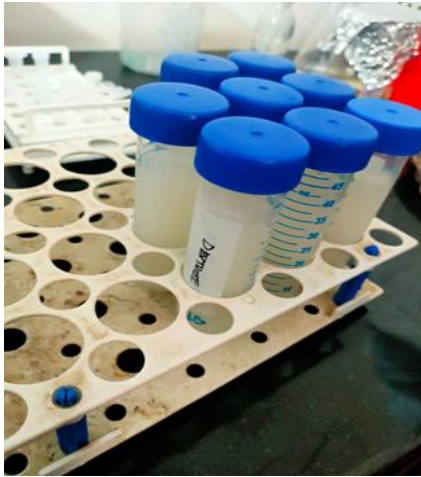
STEP : 6

- The supernatant was collected and salted out using 2 mol/L NaCl for 24 hrs at 4 °C.



STEP : 7

- The solution precipitates out and the precipitated collagen was centrifuged again at 9000 rpm for 20 mins at 4⁰ C. The residue was dialyzed under phosphate buffer at pH 7.2 for 3 days and further dialyzed again with acetic acid for two days.



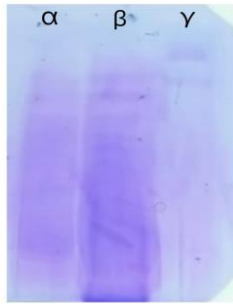
STEP : 8

- Now the samples were held frozen under lyophilization for further analysis.



STEP : 9

- The sample was confirmed as collagen by performing SDS-PAGE analysis.



• collagen is confirmed

EXTRACTION OF NANOPARTICLES FROM PLANT SAMPLES :

- Take 1 gm of four different samples has been taken in 30 ml of double distilled water.
- S1- red banana
S2- watermelon
S3- pomegranate
S4- sweet lime



- The samples were boiled in water bath for 20 mins at 70°C



- Filter the extraction of the above samples using filter paper.



SYNTHESIS OF NANOPARTICLES :

- Take 8 test tubes and wash it thoroughly with distilled

water.



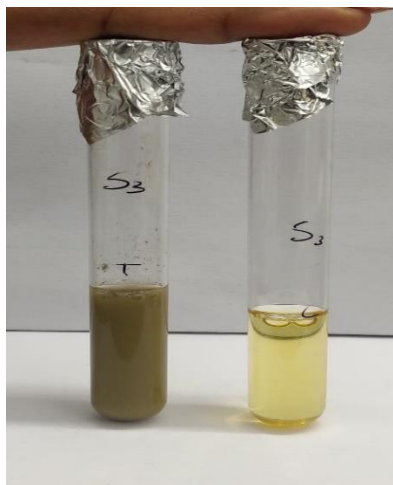
- 4 ml of distilled water is added to 1 ml of the sample in the test tubes for the synthesis of nanoparticles .



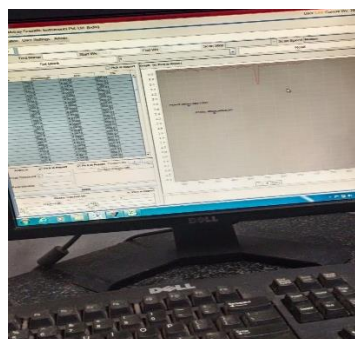
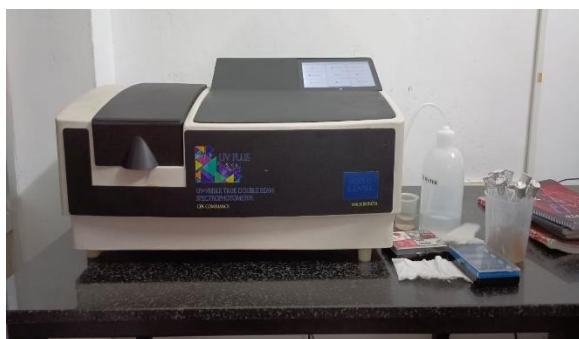
- In total of 8 tubes ,4 test tubes are marked as Control and the remaining 4 test tubes are marked as Test, they are named as c1,c2,c3,c4 and t1,t2,t3,t4.



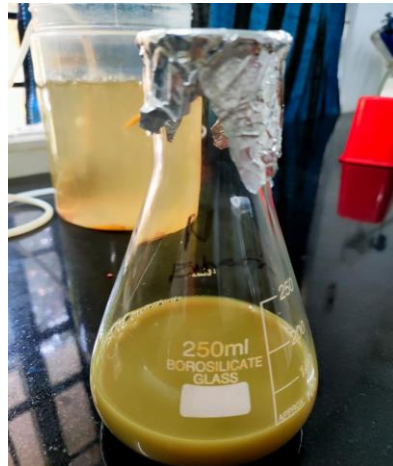
- 20 μ l of silver nitrate was added to only Test (4 test tubes) and went for dark incubation.



- The colour change and readings are noted the next day at 300 – 700 nm in spectrophotometer.



- The pomegranate sample (synthesized with AgNO_3) have shown the maximum colour change , hence bulk has been made for 50 ml using pomegranate sample .



INCORPORATION OF COLLAGEN AND NANOPARTICLE AgNO_3 :

CONJUGATION OF NANOPARTICLES WITH COLLAGEN USING STIRRING METHOD –

- 14 ml of collagen with 14 ml of synthesized nanoparticle (AgNO_3) is added to a 50 ml beaker and kept in magnetic stirrer for 2 hrs.
- The gel is/was formed.

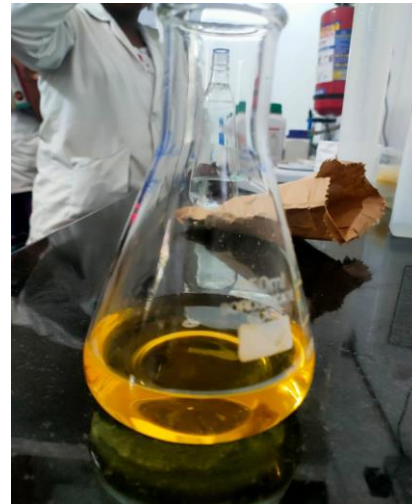


CHECKING ANTIBACTERIAL ACTIVITIES AGAINST WOUND PATHOGENS:

➤ PREPARATION OF NUTRIENT AGAR MEDIUM (150 ml) :

- Peptone : 0.75 mg
- NaCl : 0.45mg
- Yeast extract: 0.45mg

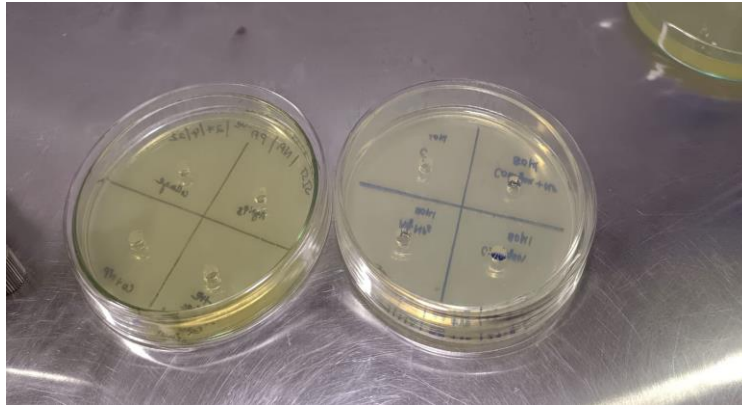
- Beef Extract: 0.45mg
- Agar-Agar: 3.075mg



- The medium was autoclaved along with four Petri plates.
- The medium was poured in all four petri plates and was allowed to settle for 20 mins.



- The petri plates were marked.



- The Pathogens (*Streptococcus Agalactiae* , *Bacillus subtilis* , *Pseudomonas aeruginosa*, *Ecoli*) were swabbed in the petri plates on the medium.



- The wells were cut and the samples (Gentamicin, AgNPs, collagen, collagen+AgNPs) were loaded and incubated for 24 hours.



- The antimicrobial activity was viewed.





IN VITRO WOUND HEALING ASSAY:

- Preparation of cells (vero celline).
- 90 % of DMEM medium was added in 12 well plate.
- 10% FBS (fetal bovine serum) is added.
- PBS buffer is added.
- Scratches were made in streaks.
- The sample (collagen + AgNPs) was added.
- And was observed under inverted microscope at zero hour , 24 hours , 48 hours .

FINAL PRODUCT :



OUTCOMES:

BIOMEDICAL APPLICATIONS :

- The product is confirmed to have antimicrobial and wound healing qualities .
- The types of wounds which can be healed are
 - i. Diabetic wounds
 - ii. Burns
 - iii. Scratches / cuts.

REFERENCES:

- WWW.MDPI.COM
- WWW.SCIENCEDIRECT.COM
- WWW.NCBI.COM

IMAGE REFERENCES:

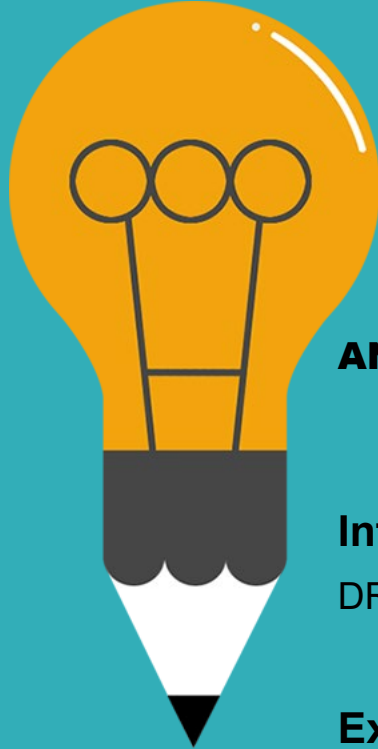
The images attached were taken during the lab sessions.

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND CHEMICAL ENGINEERING

COLLABORATING WITH BIOMEDICAL ENGINEERING DEPARTMENT



ANTIMICROBIAL WOUND HEALING GEL USING FISH COLLAGEN

Internal Guide

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POOJA . V (39230031)

SHERIL TABITHA . Y (39230042)

External Guide

DR. SINDHU DIVAKARAN

SHOBANA . A (39230043)

TIVIYA THANGASWAMY (39230053)

OBJECTIVE :- the aim of the project is to produce an antimicrobial wound healing gel loaded with biosynthesised silver nanoparticles to heal the wounds, decrease the pain, and protect the wound from microbes.

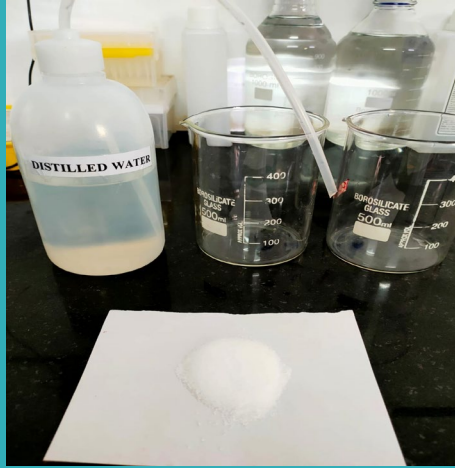
ABSTRACT :

- Collagen has been identified to have wound healing abilities.
- Nanoparticles like AgNO_3 have antimicrobial properties.

EXTRACTION OF COLLAGEN:



Fish skin is washed, cleaned thoroughly.



Sample is rinsed in NaCl and distilled water.



NaOH is added and incubated for 24 hrs.

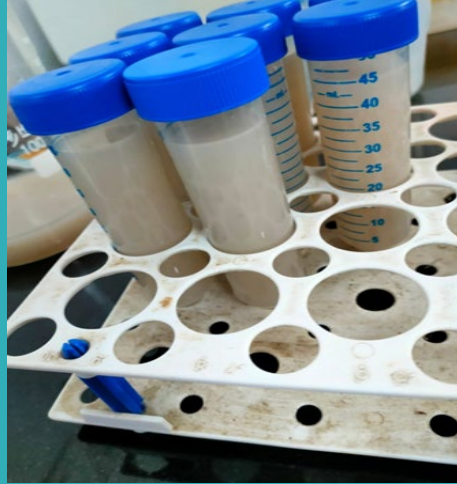


Sample was kept in Homogenizer.

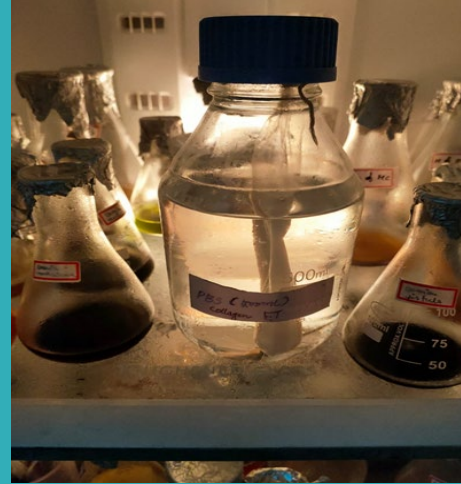
EXTRACTION OF COLLAGEN:



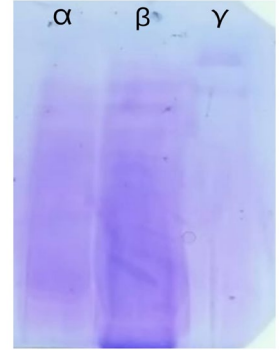
Sample is Centrifuged at 9000rpm.



Supernatant is discarded, pellet is collected.



Sample is dialysed in PBS solution and acetic acid.



- collagen is confirmed

SDS- PAGE is performed.

EXTRACTION OF NANOPARTICLES:



Four plant extracts are taken and Distilled water is added.



Heated in water bath for 20mins.



The extract is filtered and sample is Collected.

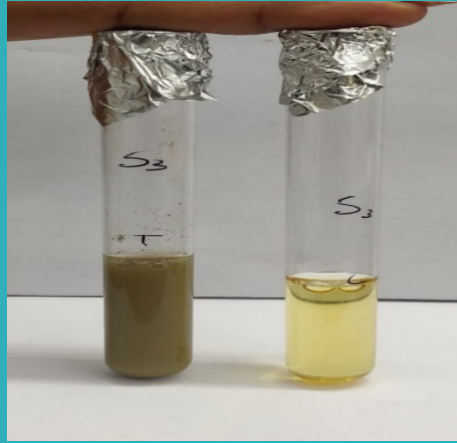


The sample were taken in 8 test tubes , marked TEST and CONTROL.

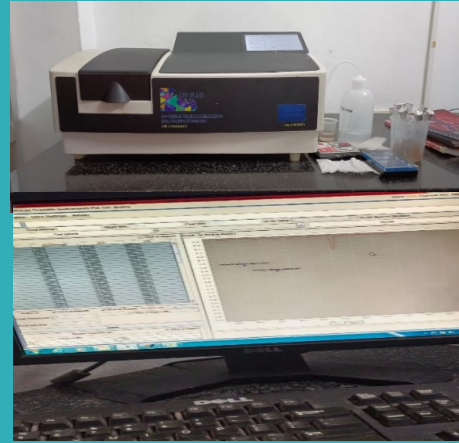
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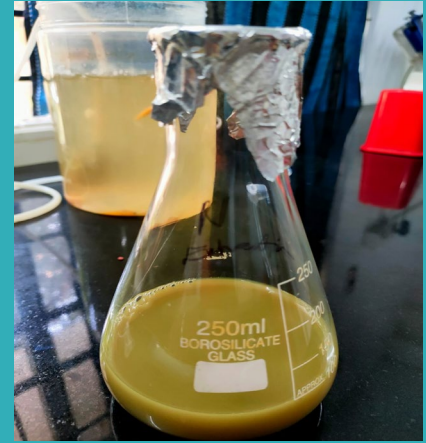
AgNO₃ is added for synthesis of nanoparticles.



Sample is incubated in the dark and color change was observed.



The samples were loaded and reading were noted using UV-Spectrophotometer.

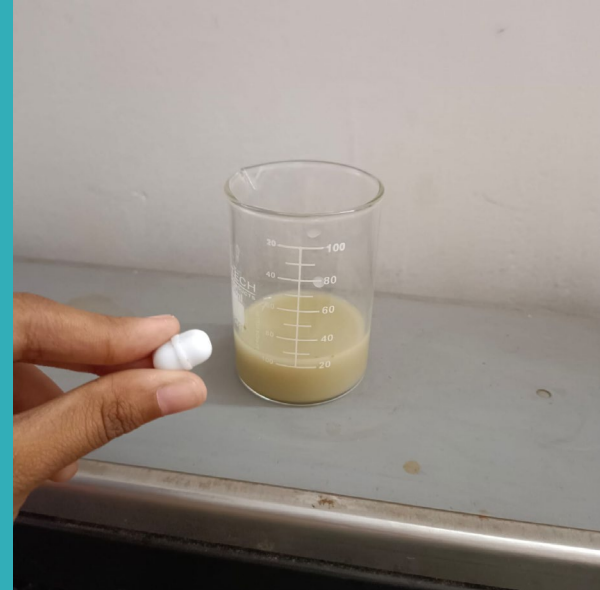


Maximum color change is observed in S3 (pomo) and bulk is prepared.

INCORPORATION OF COLLAGEN AND AgNPs :

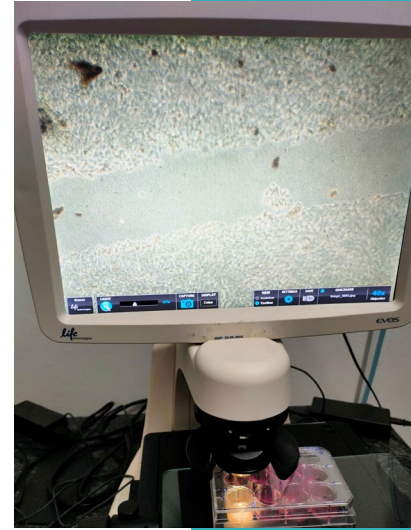
STIRRING METHOD :

- 14ml of collagen and 14ml Of AgNPs were stirred in magnetic stirrer.
- The gel was formed .

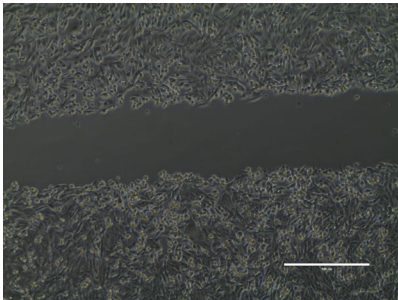


WOUND HEALING ASSAY :

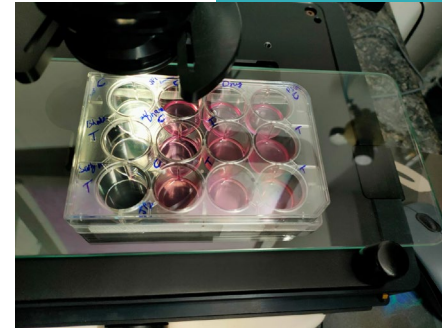
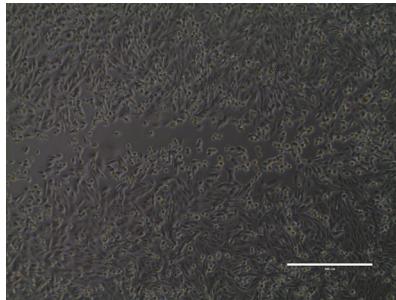
- Preparation of cells (normal vero cell line).
- 90 % of DMEM medium was added in 12 well plate.
- 10% FBS (fetal bovine serum) is added.
- PBS buffer is added.
- Scratches were made in streaks.
- The sample (collagen + AgNPs) was added.
- And was observed under inverted microscope at zero , 24 hours , 48 hours .



BEFORE-

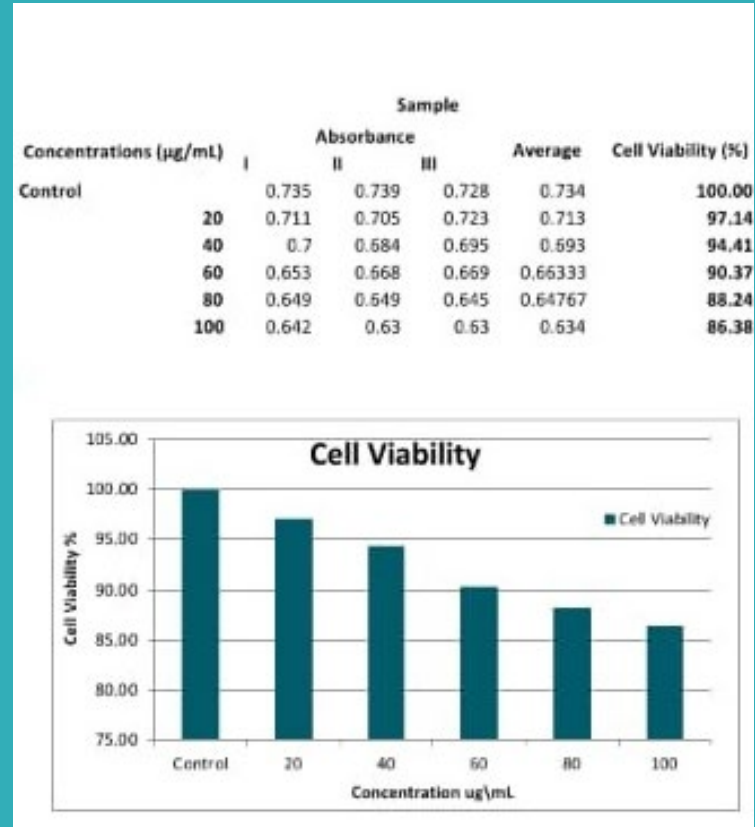


AFTER 48HRS-



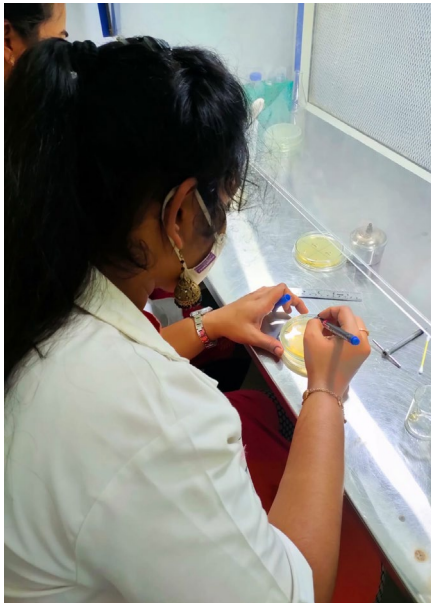
CYTOTOXICITY –

- The results show that the Avg % cell viability to be 91% .
- Hence , the product is not toxic to the cells.



ANTIMICROBIAL ACTIVITY :

- The nutrient agar medium was prepared and autoclaved.
- The medium was poured in petriplates and pathogens were added.
- The wells were created and samples were loaded.
- The antimicrobial activity was viewed after 24hrs.



OUTCOMES:

BIOMEDICAL APPLICATIONS :

- ☐ The product is confirmed to have antimicrobial and wound healing qualities .
- ☐ The types of wounds which can be healed are -
 - i. Diabetic wounds
 - ii. Burns
 - iii. Scratches / cuts.



LETTER OF ACCEPTANCE :



AviGen BioTech Pvt Ltd.
Experience Research; Transform Life

LETTER OF ACCEPTANCE

This is to certify that the following students of B.Tech. Biotechnology, Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, has been accepted to carry out their Interdisciplinary project work at AviGen BioTech Pvt Ltd., Chennai, for a period of one month.

1. Y. Sheril Tabitha (39230042)
2. A. Shobana (39230043)
3. Tiviya Thangaswamy (39230053)
4. V. Pooja (39230031)

The proposed title is 'Extraction of collagen and its application as wound healing gel loaded with biosynthesized metal nanoparticles for tissue engineering.'

Date : 03.03.2022

Place : Chennai


Authorized Signatory
Director



No. 7, First Floor, 1st Main Road, New Colony,
Chromepet, Chennai 600044, Tamil Nadu.

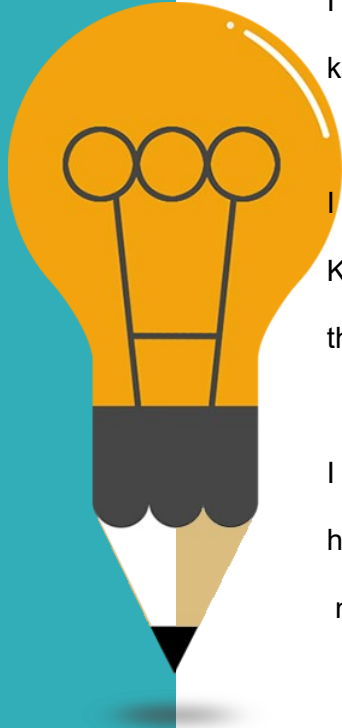


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Thank you