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BIOMATERIAL INFUSED TILAPIA SKIN FOR WOUND INJURIES

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Abstract
The current trend of regenerative medicine is Focusing on altered skin which can be made with the combination of scaffold and Bio molecules. At present there is no ideal substitute in the market that provides an effective and scar free wound healing. As a change we are using animal skin as a substitute because it has high collagen content and also disease transmission to humans is eliminated, due to their biocompatibility, biodegradability and collagen nature that is mainly used for skin repair. This product could avoid the complication due to potential disease transmission, so like other animal products fish skin also provides the body's own cell and also grow around the affected tissue to form a healthy tissue and close the wound. Thus the reconstructive marine biomaterial will be available at minimal cost for open wound injury.

Keywords— *regenerative medicine, fish skin, collagen*

INTRODUCTION

Skin is the largest organ on human body that covers entire body and protects the internal organs against infection, injury and harmful sun rays.[1] the skin is made up of three layers, the epidermis, dermis, and the fat layer, also called the hypodermis . the epidermis is the outer layer of skin that keeps vital fluids in and harmful bacteria out of the body, the dermis is the inner layer of the skin that contains blood vessels, nerves, hair follicles, oil, and sweat glands.
When skin is critically damaged due to some problems there are many substitutes to reconstruct our skin back it is by two methods one is by our own skin that is a natural biomaterial and another one is synthetic biomaterial. The biological skin substitute have a more intact and native ECM structure which may allow the construction of a more natural dermis .they also allow excellent re-epithelialisation due to the presence of a basement membrane. However, natural constructs can exhibit problems with slow vascularisation of the material. The most widely used biological substitute are cadaveric skin allograft, porcine skin xenograft and amnion .

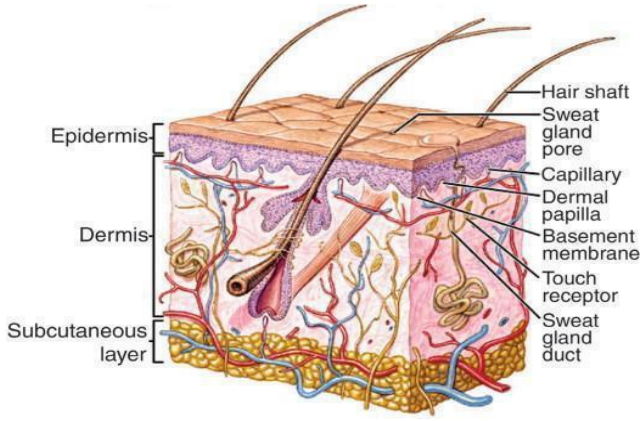


Figure 1: Skin layers

HOW ARE YOU SOLVING

The focus on alternative skin which can be transplanted with combination of naturally existing material, scaffold and bio molecules .the matrix material serves as a support structure for the ingrowth of cells and vessels it hits the elastin components of fatty acids are to the improve the stability and regenerative tissue.

OPEN WOUND HEALING

In recent times advancement in the clinical for wounds and their path physiology has significant biomedical innovations in the treatment of acute, chronic, and other types of wounds. The time span of wound healing varies from few days for a sutured clean wound(first intention healing) to weeks or months for an open wound containing necrotic tissue (second intention healing). The healing processes are identical in each case, but more scarring and wound contraction occurs in second intention healing. Wound infection often follows inappropriate primary repair of lacerations and is usually result of errors of inadequate debridement. An open wound is an injury involving an external or internal break in body tissue, usually involve in the skin. By means of falls, accidents with sharp objects or tools and car accidents are the most common causes of open wounds Exploration of open wounds identifies nerve division. Primary nerve repair with fine epineuralsutures (8-0 or 10-0), often using micro vascular techniques, is indicated for a clean, sharply lacerated nerve. There are four types of open wounds which are classified depending on their cause 1.ABARASION 2.LACERATION 3. PUNCTURE.

The healing of wounds is a complex process that involves the activation and synchronization of intracellular, intercellular and extracellular elements, including coagulator and inflammatory events, fibrous tissue accretion, deposition of collagen, epithelialisation, wound contraction, tissue granulation and remodelling . This process occurs via activation of local, systemic cells to restore tissue through regeneration and scar formation, and often these involves in satisfactory repair of damaged sites. Disruptions caused by tissue loss, inadequate blood flow, and co morbid disease states can lead to chronic wounds that are difficult to manage [2].

IV.WOUND DRESSINGS AND SKIN GRAFT

Materials used to cover wounds and burns are also called artificial skin, as they fulfil the functions of normal skin within areas with wounds and partly destroyed skin. Wound and burn covering materials are classified as follows

Traditional dressing

Biomaterial-based dressings

Artificial dressings

Wound dressing primary function ready to serve as a physical barrier to prevent infection and to promote moisture absorption and blood coagulation. Woven cotton gauze has been used traditionally as a wound dressing because of its moisture absorption and blood clotting ability. However since it is composed of short staple fibres of cellulose , it adheres to the wound and causes

MATERIALS & METHODS

COLLAGEN

Collagen is the most prevalent protein in the connective tissue of animals and constitutes approximately 25% of total body protein in the vertebrates. The molecular sub unit of collagen called tropocollagen is a rigid rod with a molecular weight ~300kDa . Tropocollagen self assembles into larger structural units is called fibril or fibres, while there are several types of collagen, these larger units or fibres, all share the characteristic triple helical structure, but have variations in the length of non helical and helical sections. There are four main types of collagen are type I collagen is the type commonly found in skin, bone, tendons, while type II is common in cartilage. Type III collagen is prevalent in the vasculature structure in small amounts in skin. Type IV collagen is unique in that it is largely non helical and does not form fibrils. Collagen type IV is found in the basement membrane that separates epithelial and mesodermal tissues. Collagen matrices are commonly sterilised by gamma irradiation, ethylene oxide treatment or electron beam

irradiation.The prevalence of hydroxyl, amine, and carboxylic acid groups allows simple cross-linking between and within collagen units. Collagen dressings stimulate new tissue growth and encourage the deposition and organization of newly formed collagen fibres and granulation tissue in the wound bed. At the end of this process, fibrillary type 1 collagen is responsible for resulting strength, integrity, and functionality. It is fairly rapid healing process with deposition of type 1 collage that allows the individual to recover from tissue damage in short order and return to biological function. As the major structural protein in the body, collagen is responsible for most natural tissue design and matrix organization. That wound dressings containing collagen give matrix metalloproteinases an alternative collagen source, leaving the body's natural collagen available for normal wound healing.

VITAMIN C

Vitamin C is important to the synthesis of collagen and the growth of new blood vessels and also replaces the damaged tissue. This element also has a strong anti- oxidizing effect that enhances the immune system and, in effect, protects against **wound infection**. Another perk is that vitamin C helps the body better absorb iron, which works to supply the wound bed with oxygen and energy for more efficient cellular development. Vitamin C, the most plentiful antioxidant in human skin, forms a part of the complex group of enzymatic and non-enzymatic antioxidants that co-exist to protect the skin from reactive oxygen species (ROS). As Vitamin C is water soluble, it functions in the aqueous compartments of the cell . When the skin is exposed to UV light, ROS such as the superoxide ion, peroxide and singlet oxygen are generated. Vitamin C protects the skin from oxidative stress by sequentially donating electrons to neutralize the free radicals. The oxidised forms of Vitamin C are relatively non-reactive Vitamin C is essential for collagen biosynthesis. It has been proposed that Vitamin. C influences quantitative collagen synthesis in addition to stimulating qualitative changes in the collagen molecule. [3] Vitamin C has a potential anti-inflammatory activity and can be used in conditions and also. It can promote wound healing and prevent post-inflammatory hyper pigmentation. **Vitamin C** helps the body make collagen and is **essential** to wound healing because it helps the body form new tissue. Lower dose if diarrhoea develops. **Vitamin C supplements** may interact with other medications, including chemotherapy drugs, warfarin (Coumadin), and others.

CHITOSAN

Chitin (β-(1-4)-poly-*N*-acetyl-d-glucosamine) is widely distributed in nature and it is the second most abundant polysaccharide after cellulose . Chitin is the major structural component in the exoskeletons of crab and shrimp shells and the cell walls of fungi and yeast. Chitin and Chitosan are biocompatible, biodegradable, and non-toxic. and also antimicrobial and hydrating agents .Chitin and Chitosan are also used in various types of biomedical applications such as drug and gene delivery, wound healing, tissue engineering, and stem cell technology [4].Chitosan also stimulates haemostasis and accelerate the regeneration of tissues, therefore it found to be useful for wound healing . For a material to be used for biomedical research, a natural product is preferred because these materials are more biocompatible than synthetic materials.. Chitosan is a potent **antimicrobial** agent. An antimicrobial is an agent that kills microorganisms or inhibits their growth. The substance or treatment that reduces inflammation is known as anti-inflammatory. Chitosan is considered as a new innovative material in, wound healing, antibacterial, fat binder, haemostatic agent, and effect as indicated by the large number of studies over the last few years. Chitosan is formulated for wound management may induce analgesia by providing a cool, pleasant and soothing effect when applied to an open wound, burns, skin abrasions, skin ulcers and skin grafted areas .

GELATINE

Gelatin is made of collagen that is acquired from various animal by-products. It is a brittle, colourless, translucent, and flavourless substance. That doesn't sound particularly interesting, but it is an essential part of many gel-like substances, including jellies, ice creams, yogurts, gummy candies, marshmallows, certain gelatin desserts, and various dips. In non-food applications, it is even used in photography, pharmaceuticals, and various cosmetic products. You can get gelatin in sheets, granules, or powdered form for home use. You can also take gelatin supplements to get all of the health benefits in a concentrated form gelatin is a combination of proteins and peptides, making it a wonderful source of amino acids that are essential for a variety of body processes. In terms of the nutritional composition of gelatin, it is a good source of numerous vitamins, minerals, and organic compounds, including copper, selenium, and phosphorous, along with being an excellent source of proteins. By dry weight, gelatin is 98-99% protein, but it does not contain all of the necessary amino acids for humans. Proteins are an essential part of wound healing, and gelatin contains a specific amino acid called glycine, which is directly

connected to reducing inflammation. This means that a wound can move from the inflammation stage to the healing stage much faster,and the additional amino acids and proteins help to develop new skin and scar tissues. healing any tears or issues in the digestive tract, gelatine is able to heal allergic problems and make our body open to handle a variety of food.

AGAR

Agar, which looks similar to Jelly, is derived from red/purple algae. It utilizes two polysaccharides (sugars) called **agarose** and **agar pectin** found in the cell walls of certain algae. The red algae that are used to produce the agar are from Pacific coastal regions of Asia and California. The specific algae are from the **Gelidium** and **Gracilaria** genera. Agar can essentially be found anywhere from kitchens to laboratories. Agar is commonly used in the laboratory to help feed and grow bacteria and other microorganisms. It acts as a culture that provides nutrients and a place for these items to grow, but since it is indigestible to the microorganisms, they cannot eat and destroy it. There are various types of agar, some of which include MacConkey Agar, Chocolate Agar, Tryptic Soy Agar, Triple Sugar Iron Agar, Helton Agar, and Mannitol Salt Agar. Agar is insoluble in cold water; it absorbs as much as 20 times its own weight. It dissolves readily in boiling water; a dilute solution is still liquid at 42 °C (108 °F) but solidifies at 37 °C (99 °F) into a firm gel. In its natural state, agar occurs as a complex cell-wall constituent containing the polysaccharide agarose with sulphate and calcium. Here we use agar for the gel preparation along with the vitamin c, chitosan, gelatine and collagen that will heal the wound faster.

VI. FISH SKIN PREPARATION

Fish skin was obtained from villivakkam fish market . The fish skin was obtained from tilapia fish. It was placed in bag full of ice. At the lab, the skin was cleaned from the attached muscle and scales using sharp knife and scissors. Only 29 gram of skin was acquired. After cleaning the skin, it was chopped into fine small pieces (0.5*0.5 cm). Then it was placed in 500ml flask. In order to maintain the skin at 4C temperature, the flask was placed in a container full of ice.

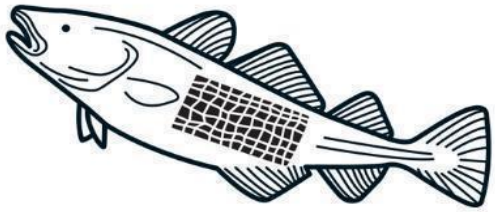


Figure 2: Fish skin preparation

VII. REMOVING NON COLLAGENOUS TISSUES

After the skin was prepared and in order to remove non collagenous substance and to make the skin very loose, the skin was treated with 50 ml of 0.1M of NaOH (PH 12) for 24 hours, which was obtained by mixing 2g of sold NaOH with 50 ml of distal water. Then, the solution was placed on the skin and gently stirred for 5 minutes.

After that, the resulting solution was placed in the fridge for 24 hours. After 24 hours, the solution was removed by using filter paper (size 125mm). Then the skin was washed thoroughly with cold distal water until its PH become neutral. In this step, the skin was washed thoroughly four times. After each wash, PH measurement was taken. In each wash, 300ml of cold distal water was used. The total of the cold distal water was 1.2 litres. After washing the skin thoroughly, it was treated with 10% butyl alcohol for 48 hours with solid to solvent ratio 1:10. The main reason for this treatment was to remove fat tissue from the skin. The solution was obtained by adding 50 ml of butyl alcohol to 450ml distal water. Then, only 250ml of the solution was mixed with the skin. The solution was placed in the fridge. After 24 hours, the solution was removed using filter paper then a new 250ml of 10% butyl alcohol was added. Then, the solution was placed in the fridge for another 24 hours and collagen was produced [20].

VIII. ISOLATION OF MARINE FISH-DERIVED COLLAGEN

The acid-soluble collagen (ASC) method, 0.5 M acetic acid is used to digest the fish skin in sufficient time, whereas 10% w/v pepsin is used for the pepsin-soluble collagen (PSC) method.. It is observed that the PSC method leads to higher amounts of collagen as compared to the ASC method. This implies that pepsin in the PSC method is more efficient in digesting skin or bone tissues as compared to acid solution in the ASC method. But here we are using ASC method, after 2 days of butanol suspension the sample is washed with the cold distilled water using a filter paper for 3 to 4 times until the PH becomes neutral. Then the Figure 3 shows the common procedures for isolating collagen from the skin and bones of marine fish Acid solubilisation and pepsin solubilisation are major methods for isolating collagen from various parts of fish species (e.g., skin, bones, and scales). After 3 days the acetic acid suspension is removed using a filter paper. Here it solubilise the fibril collagen into tropocollagen which is the submit of collagen fibril.

CENTRIFUGING & PRECIPITATING COLLAGEN

After 3 days of acetic acid suspension solution is filtered using double filter paper and only 140 ml of solution is obtained , initially we checked the solution for precipitation process by taking 10ml of supernatant and centrifuge it at 5000rpm for 10 minutes. After that add 0.526gram of Nacl to that solution and stir, check for the precipitation , the precipitation will occur here and refrigerate the solution and do the same for the remaining supernatant solution, 140 ml of distilled water + with 6.9gram of NaCl is added precipitation is occurred. Now remove the precipitate by centrifuge it at 5000 rpm for 10 minutes then add 10% ethanol into the supernatant(5ml distilled water + 5 ml distilled water).

FINAL PROCESS

The precipitated supernatant is pour on the petri dish equally and kept in the hot air oven at 50 degC for 2 days here the water content in the solution will ruin out and protein will settle down in the dry state that will be taken as collagen powder.

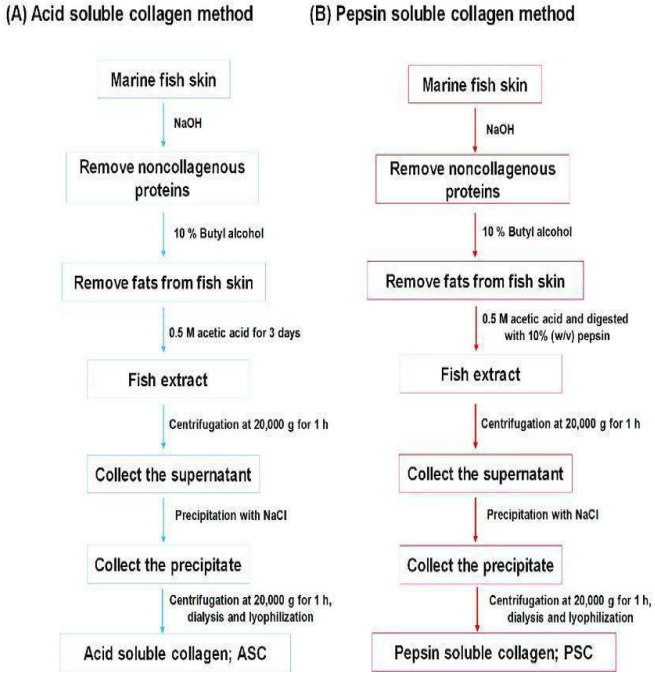


Figure 3: Collagen extraction from fish skin by ASC and PSC method.

SOLUBILIZING COLLAGEN

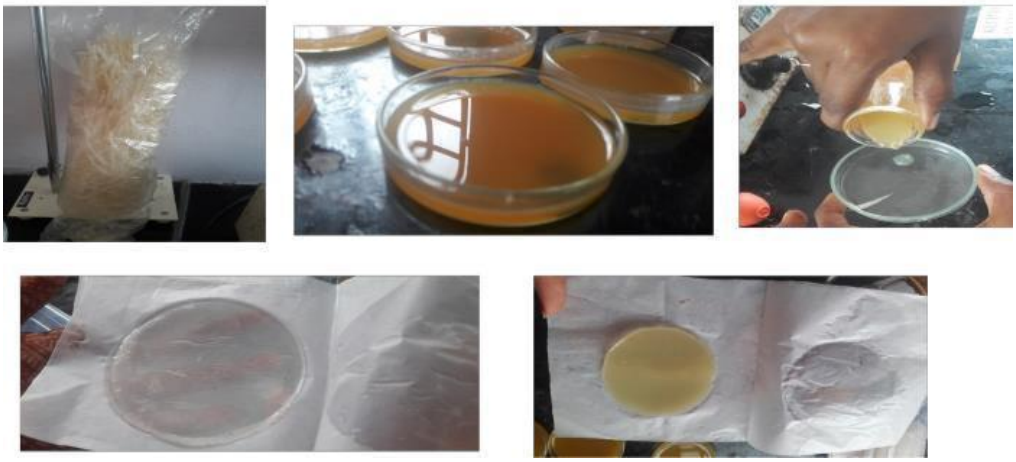
Here we are adding 0.5m acetic acid4.5g is added with 150 ml of distilled water into the fish sample and kept the solution at 4c in fridge, check the solution periodically in between these 3 days,

GEL PREPARATION PROCESS

In this gel preparation here we used vitamin c, chitosan, gelatine, collagen powder, and agar, in this gel preparation initially we had taken 1 tablet of vitamin C which contains 150 mg that is crushed and added with 10ml of distilled water then kept in the stirrer, by the side add 0.03g of chitosan and 0.9g of gelatine in 20 ml of water and heat the solution in the heater at 50 deg C until the solution dissolved with the gelatine and chitosan in the Luke warm water add vitamin C and also add 0.12g of collagen powder. Then keep the solution in the stirrer for 5 min and pour the solution in the petri dish then refrigerate it for a day.

The next day the gel solution was prepared by the same procedure and kept the solution in the petridish at 4 deg C, After some time the gel was kept in the normal room temperature the gel became soluble so again gelatine was added (220ml mixture + 6.5g gelatine) and kept in the rerfridegerator for a day Then the next day it seems to be same while kept in the room temperature so we added agar into the solution (1.5g agar strips + 100ml of distilled water) the mixture is taken to a heater because agar will not dissolve in cold water , then that agar solution is added into the mixture , according to the mixture the agar has to be added but for the good add as muchless agar because it may lead to loss the dissolving of other molecules into the wound when it is kept at the wound site.

GEL FOR BANDAID PREPARATION



IX. WHY FISH IS USING FOR COLLAGEN EXTRACTION

Tissue-engineered skin substitutes serve as a promising therapeutic agent in replacing the skin lost in wounds such as burns and open wounds by providing cells, bioactive compounds, bioactive polymers, and proper microenvironments, thereby it will initiate the wound healing process Currently, a main source of collagen is bovine skin as well as porcine skin, which has some drawbacks such as transmission of prions. Therefore, marine organism-derived materials have become initiators of hundreds of tissue-engineered skin substitutes Many studies based on marine organism-derived collagen substitute is used for skin tissue regeneration havedemonstrated a high potential in clinical applications..

In this regard, we have used here tilapia fish skin which has abundant content of type I collagen that will initiate the wound healing process and also it has widely increased the applications of collagen-based scaffolds for tissue engineering [5] .

PREPARATION SKIN SUBSTITUTE

The skin substitute is obtained by treating the collagen extracted from the tilapia fish by the collagen extraction process from the marine resources which are naturally available , then vitamin c is additionally added up to a particular rate then check the pH as neutral then add Chitosan which is an anti oxidant agent that will promote the wound healing.

Additionally we are adding the agar and gelatine which is a hydro gel that will maintain a jelly nature over the substitute and also it will maintain the moisture content in the wound site and that will also prevent the bacterial invasion towards the wound site.

Thus this preparation is not processed on the fish skin, instead the needed collagen is extracted and processed separately made it as a skin substitute for wound healing why because if we are processing on the fish skin means

it will sustain for so long it may get spoiled instead we are separately processing everything thus it will make it as a non tear able, anti microbial , cost efficient and faster wound healing skin substitute.

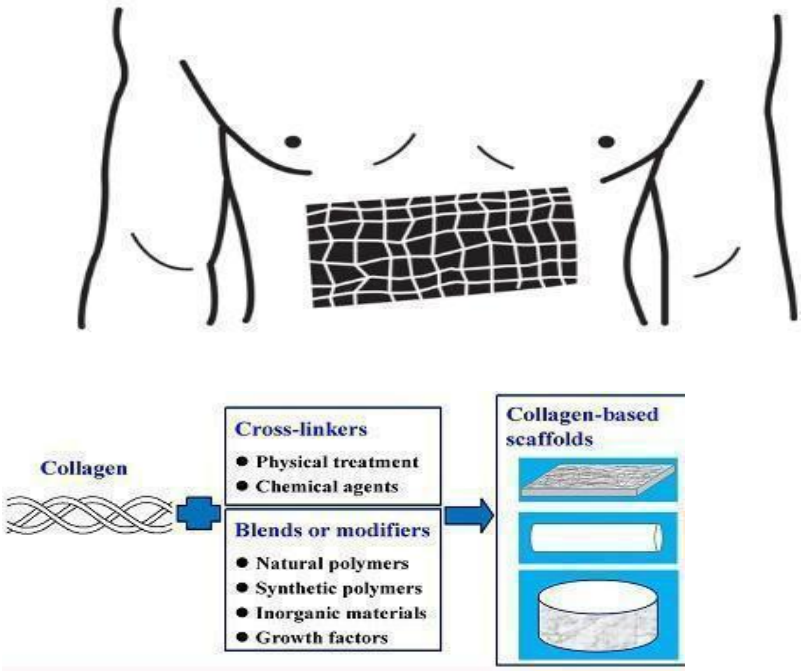


Figure 4: Preparation of skin substitute using collagen and other polymers.

XI.OUTPUT

This skin substitute will promote the wound healing faster, and it is mainly used for people who have wound along with flu, Diabetic patients and other open wounds. this will reduce the pain at wound site and avoid bacteria and fungus to invade inside the wound. This skin substitute is biodegradable on after the recovery of the original skin the artificial skin substitute can be peeled out.

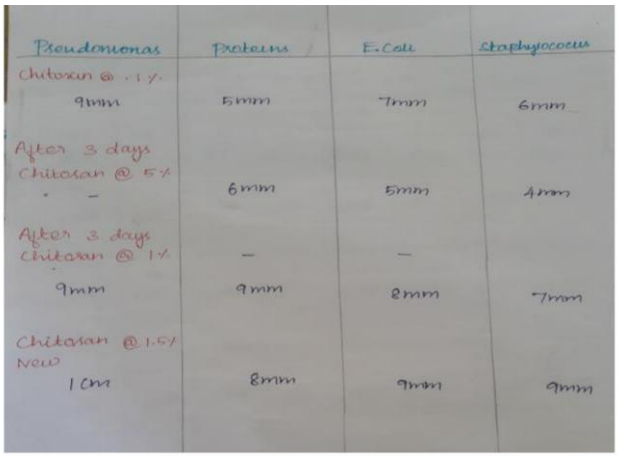


Figure 5: Skin substitute placed on the wound.

XII. DRUG DIFFUSION STUDY

standard values had been taken using standard vitamin c tablets[7] .

CHITOSAN

Estimation Of Chitosan Using Methyl Red Dye

Chitosan estimation test was done by methyl red dye, Naoh solution, acetic acid is used here to dissolve the Chitosan (standard value) and Hcl buffer to prevent protonation in which the reagents were calculated and taken in a beaker along with that sample has been added then incubate the final concentration and observe the results using colorimetric method, before using samples standard values had been taken using standard Chitosan powder. At the absorbance rate of 560nm [8].

XIII. ANTIMICROBIAL ACTIVITY

The drug diffusion study is to check the sample whether it is diffusing the properties when kept under ph 7 which is our skin ph range for that process the gel and fish sample at three different concentration was taken and dip it in a 10ml distilled water after each 24 hrs, 48hrs, 72hrs 1ml of sample was taken from the existing sample in a separate testubes.

The readings was taken under each hours to check how long the properties emitting out. This study reveals that the gels diffusion level over the skin in each day at different concentration

PROTEIN

Estimation of Protein Using Bradford Reagent

Protein estimation test was done by using Bradford reagent which includes reagents such as coomaise blue and orthophosphoric acid in which the reagents were calculated and taken in a beaker along with that sample has been added then incubate the final concentration and take the results using colorimetric method [6]

VITAMIN C

Estimation of Vitamin C Using Ammonium Molybdate

Vitamin c estimation test was done by using ammonium Molybdate and sulphuric acid in which the reagents were calculated and taken in a beaker along with that sample has been added then incubate the final concentration and take the results using colorimetric method, before using samples

This is the important test in this project to check the anti bacterial property of this prepared gel and fish sample , here we are using Chitosan as the antibacterial agent this test is to check how long this property will withstand in the gel kept in a bacteria filled broth in the incubator.

The disc diffusion study is also done to check the diffusion rate of the sample under the bacterial condition. Here we are using 4 bacterial organisms that are

- ***Pseudomonas aeruginosa***.
- ***Proteus vulgaris***.
- ***Escherichia coli*** .
- ***Staphylococcus aureus***

This test is giving good results for our gel and fish skin thus here we are concluding that this biomaterial as a good anti-bacterial effect.

XIV.RESULTS

From the above test we conclude that this gel contains collagen, vitamin c and Chitosan content at particular level in each concentration. This will be a good and cost effective product that will promote the wound healing faster when compared to the normal healing time because it contains type I collagen which is highly useful in wound healing, more than that vitamin c is also a good anti-oxidant as well having good wound healing ability, and also Chitosan is a good anti-bacterial agent.

There is no report that evaluates the collagen property and components for gel-treated wounds up to 6 weeks. It was performed to determine fish skin for treating without any transmission and quick recovery with help of natural components for making this wound skin replacement this is the main unique factor of our project and speed healing. At endures for clinical, homecare and pharmacy in the form adhesive bandage and packed skin substitute.

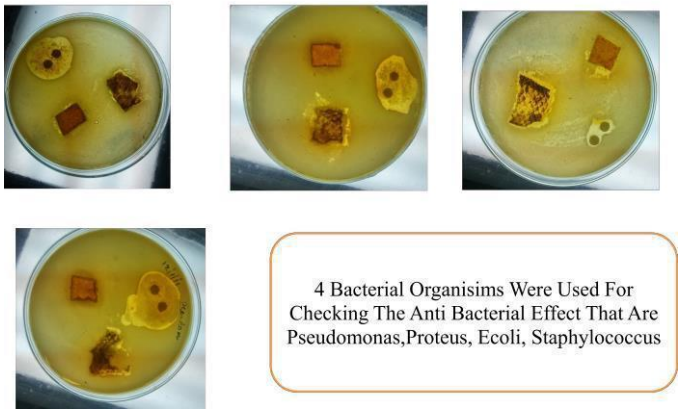


Figure 6: Results of Disc Diffusion Study

XV. FUTURE WORK

Now this substitute is used for open wounds, our future work to use this for treating skin burns and regenerating the original skin which in cheap, because there is no ideal substitute for treating burn wounds completely. Treating trauma and diabetic wounds will be an additional work in future towards making a skin substitute and recover the original skin back to those people generally do plastic surgery and remove their legs. It will overcome by disease transmission and availability.

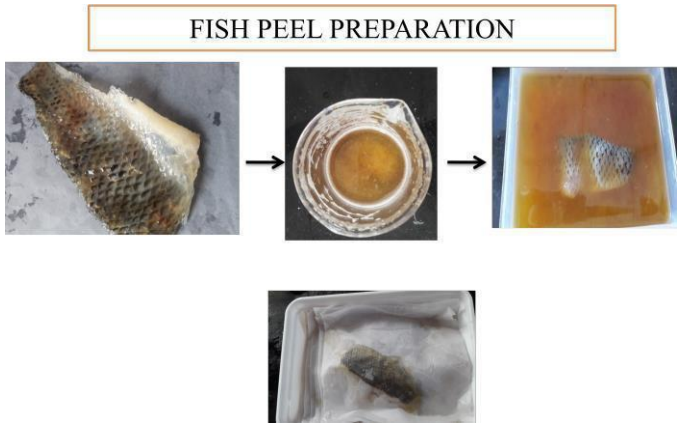
XVI. ADVANTAGES OF SKIN SUBSTITUTE FOR WOUND HEALING.

- Fast implanting speed to avoid bacterial infection to patients
- Cost effective.
- Widely available.
- Able to prevent water loss
- Lower chance of rejection after implantation
- Can be used for patients with wound area great than 50% of their bodies. Life-saving approach towards patients with severe injuries
- Not require a donor site
- Avoids disease transmission.
- Long shelf life and easy to store.
- Maintains the moisture at the wound site.

- Vitamin c and collagen in the fish will heal the wound faster.

XVII. SIMILAR WORK

We have prepared fish skin peel from 7 tilapia fish and washed 14 skin peel that was treated initially with NAOH to remove the fat tissue for 24 hrs later that peel was dipped into the solution which consist of chitosan, vitamin c, and gelatine which was as similar as the composition of the gel preparation, then it was refrigerate at 4degC. Then that was cut into small square pieces which are ready to place at the wound site.



This fish skin peel also giving good anti-bacterial and diffusion results.

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