Bio-Functionalization of Electrospun Nanofibre Scaffolds for Tissue Engineering Application

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***Abstract*—The number of patients suffering from chronic, non-healing skin wounds is steadily increasing, creating a significant social and economic burden on both individuals and healthcare systems. Severe skin damage remains a major clinical problem. A shortage of suitable skin donors, along with postoperative scarring and functional impairment of skin graft sites, further complicates treatment outcomes. Although, considerable efforts have been made worldwide to develop artificial human skin substitutes, progress is still limited by the difficulty in fully replicating the complex biological structure of native skin. Tissue engineering offers a promising strategy for repairing damaged tissues by seeding cells onto biocompatible and biodegradable porous scaffolds. These engineered skin scaffolds are designed not only to provide suitable mechanical and physical support but also to mimic the natural skin surface architecture and microenvironment, thereby enhancing cell attachment, growth, and differentiation.**

Currently, skin tissue engineering scaffolds are advancing toward clinical use, offering alternatives to traditional skin transplantation. They help overcome the limitations of donor availability, accelerate wound healing, and support effective regeneration of damaged skin. This makes them a valuable therapeutic option for treating skin injuries. This chapter focuses and fabrication techniques used in scaffold development. It also discusses key design factors for effective skin scaffolds, summarises existing scaffold types and clinically approved materials, and highlights major challenges that still need to be addressed in this field. Key words:

***Index Terms*—Fabrication, cell culture, skin scaffold.**

I. INTRODUCTION

The human skin is a vital, multifunctional organ protect internal tissues from physical, chemical, and biological. Because of its essential roles, any significant structural damage like large or deep wounds, can be problematic and requires prompt, effective treatment. Over recent decades, wound care has become a significant global public health concern, as inadequate treatment of skin injuries can be fatal. Consequently, extensive research has focused on developing advanced therapeutic methods and innovative dressing materials to enhance wound healing. to restore the damaged tissue.

Cell culture is the process by which prokaryotic, eukaryotic or plant cells are grown under controlled conditions. But in practice it refers to the culturing of cells derived from animal cells. Cell culture was first successfully undertaken by Ross Harrison in 1907. Roux in 1885 for the first time maintained embryonic chick cells in a cell culture.

II. MATERIALS AND METHODS

The materials used for the preparation of the PLA layer through electrospinning are PLA, HFIP, EKA, KCl, and pharma grade. The lanolin used was pure lanolin, from DAX, (GAG), chondroitin-6-sulfate, both collagen and GAG DMEM, prokaryotic, eukaryotic, plant cells, Adherent cells, GMEM, EMEM, DMEM trypsin, dipase, collagenase.

Experimental Methodology and Sample Preparation

Laminar cabinet-Vertical are preferable

Incubation facilities- Temperature of 25-30 C for insect & 37 C for mammalian cells, co2 2-5% & 95% air at 99% relative humidity. To prevent cell death incubators set to cut out at approx. 38.5 C

Refrigerators- Liquid media kept at 4 C, enzymes (e.g. trypsin) & media components (e.g. glutamine & serum) at -20 C

Microscope- An inverted microscope with 10x to 100x magnification

Tissue culture ware- Culture plastic ware treated by polystyrene

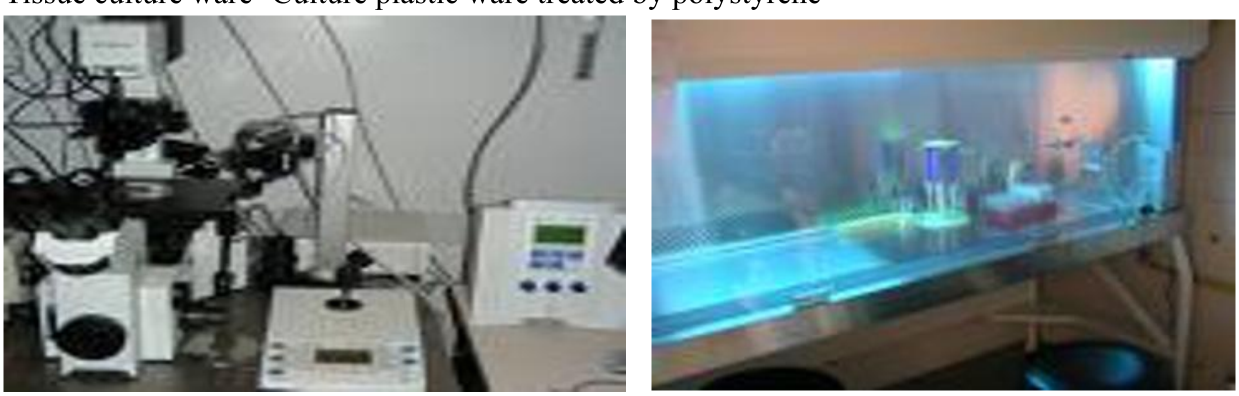


Figure :1 Tissue culture

In vitro cultivation of organs, tissues & cells at defined temperature using an incubator & supplemented with a medium containing cell nutrients & growth factor is collectively known as tissue culture

Different types of cells grown in culture includes connective tissue elements such as fibroblasts, skeletal tissue, cardiac, epithelial tissue (liver, breast, skin, kidney) and many different types of tumor cells.

Primary culture
Cells when surgically or enzymatically removed from an organism and placed in suitable culture environment will attach and grow are called as primary culture

- Primary cells have a finite life span
- Primary culture contains a very heterogeneous population of cells
- Sub culturing of primary cells leads to the generation of cell lines
- Cell lines have limited life span, they passage several times before they become senescent
- Cells such as macrophages and neurons do not divide in vitro so can be used as primary cultures
- Lineage of cells originating from the primary culture is called a cell strain
- Contionious cell line
- Most cell lines grow for a limited number of generations after which they cease
- Cell lines which either occur spontaneously or induced virally or chemically transformed into Continuous cell lines

Step 1: Scaffold Fabrication- PLA raw material, Fabrication methods, porous scaffold creation.
Step 2: In Vitro Testing- Morphological characterization (SEM), Mechanical testing (tensile, compression), Surface property analysis (contact angle), Cell culture studies (cell seeding, viability, proliferation), Cytotoxicity assessment, Differentiation assays
Step 3: In Vivo Testing- Animal model selection, Scaffold sterilization, Surgical implantation, Monitoring tissue integration and scaffold degradation, Histology and immunohistochemistry, Imaging (micro-CT, MRI), Functional and mechanical assessment of regenerated tissue.

Electrospinning Fabrication Setup
Electrospinning has a straightforward setup that allows continuous drawing of fibers directly from molten polymer, eliminating the need for solvents. However, melt spinning cannot produce fibers at the nanoscale, and it is not suitable for polymers like PL that may degrade under electrospinning conditions. During the process, the spinning temperature and the take-up roll speed were maintained at 110°C and 900 rpm, respectively. The fibers were cooled by air before being wound onto the take-up roll.
Melt electrospinning is an alternative to solution electrospinning, but it usually produces fibers with diameters in the range of tens of microns. Unlike solution electrospinning, which forms fibers through solvent evaporation, melt electrospinning relies on cooling the polymer jet to create fibers.

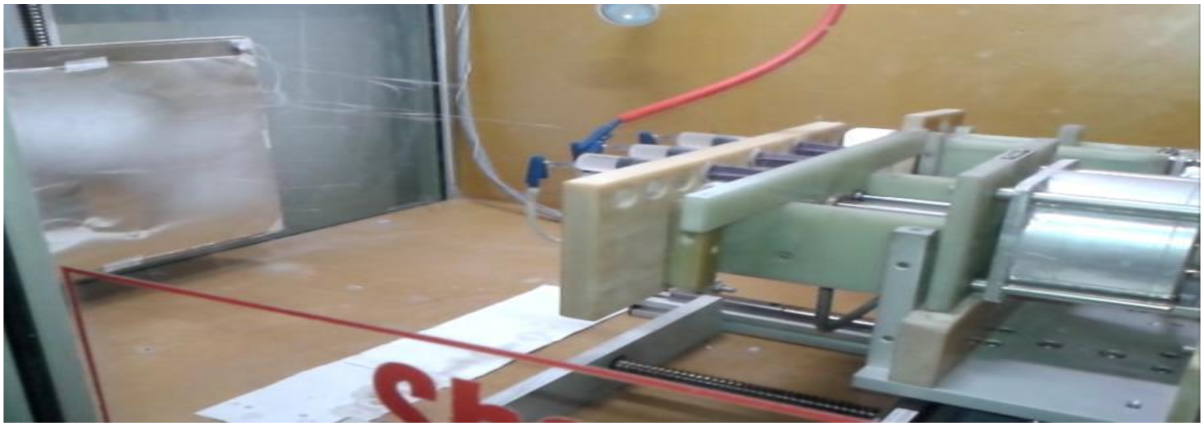


Figure :2 Methodology Dermal portion

The dermal layer of artificial skin is made from bovine hide collagen combined with chondroitin 6-sulfate, which helps control its physical, biochemical, and mechanical properties. This dermal part is sterilized by heating to 105°C and then soaking in a 0.05% glutaraldehyde solution. The epidermal layer is a uniform 0.1 mm thick sheet of medical-grade Silastic, which regulates water flow from the dermis to the skin. Liquid Silastic is applied to the sterile dermis, forming a strong bond as it cures, creating a sterile, intact bilayer artificial skin with both epidermal and dermal parts.

The sterile skin is stored in sealed polyethylene bags either as freeze-dried membranes or immersed in 70% isopropyl alcohol before use. For early clinical trials, the isopropyl alcohol packaging method is preferred. Polylactic acid (PLA) is commonly used as a synthetic polymer in biomedical applications.

Table 1: Samples weight

Sample	Total weight (g)	PLA weight (g)	Lanolin weight (g)	PLA %	Lanolin %
2 PLA 1 Lanolin	0,016	0,0032	0,0128	20	80
3 PLA 2 Lanolin	0,0238	0,0046	0,0192	19,3277	80,6723
4 PLA 3 Lanolin	0,0298	0,0063	0,0235	21,1409	78,8591
5 PLA 4 Lanolin	0,034	0,0074	0,0266	21,7647	78,2353
6 PLA 5 Lanolin	0,0393	0,0096	0,0297	24,4275	75,5725
7 PLA 6 Lanolin	0,0553	0,0125	0,0428	22,6040	77,3960
8 PLA 7 Lanolin	0,0631	0,0144	0,0487	22,8209	77,1791
8 PLA	0,0142	0,0142	0	100	0
8 PLA 1 Lanolin	0,0205	0,0121	0,0084	59,0244	40,9756
8 PLA 2 Lanolin	0,0303	0,0134	0,0169	44,2244	55,7756
8 PLA 3 Lanolin	0,0366	0,0142	0,0224	38,7978	61,2022

Zeta Potential

Zeta potential measurements were carried out for all prepared samples. To ensure accuracy and reproducibility, each sample was analyzed three times in both directions. For the asymmetric samples, the lanolin layer was positioned on the right side of the measurement cell.

Wettability of solid surfaces was mainly assessed through contact angle measurement. The contact angle reflects. It is defined as the angle between the tangent to the droplet at the point where the liquid, solid, and air meet, and the solid surface..

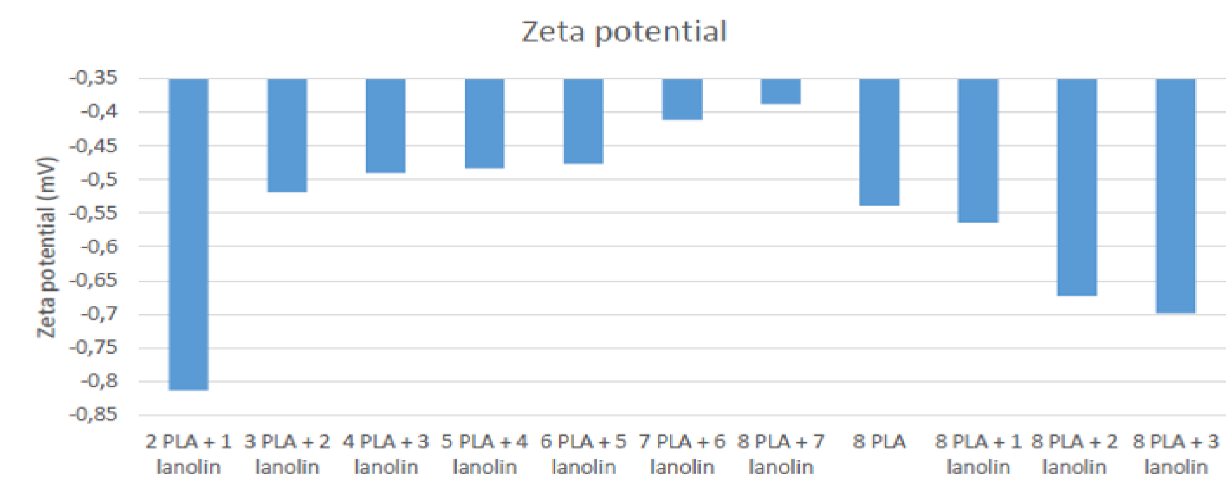


Figure :3 Graphic representation of the zeta potential values.

III. RESULT AND DISCUSSION

3.1 Cell culture for development of skin scaffold

Cell cultivation is the technique of growing prokaryotic, eukaryotic, or plant cells under carefully controlled laboratory conditions. However, in practical use, the term most commonly refers to the in vitro cultivation of cells obtained from animal tissues. Was first successfully undertaken by Ross Harrison in 1907, Roux in 1885 for the first time maintained embryonic chick cells in a cell culture.

Based on morphology and functional characteristics, cells are generally classified into three main types. Epithelial-like cells adhere to a substrate and typically exhibit a flattened, polygonal appearance. Lymphoblast-like cells do not attach to surfaces; instead, they remain in suspension and are usually spherical in shape. Fibroblast-like cells attach to a substrate and are characterized by an elongated, spindle-shaped, or bipolar morphology. PLA, PCL, and PLGA are commonly used biodegradable polymers in biomedical applications.

Culture Media

Choice of media depends on the type of cell being cultured. Commonly used Medium are GMEM, EMEM, DMEM, etc. Media is supplemented with antibiotics, viz., penicillin, streptomycin, etc. Prepared media is filtered.

- Sub Culturing

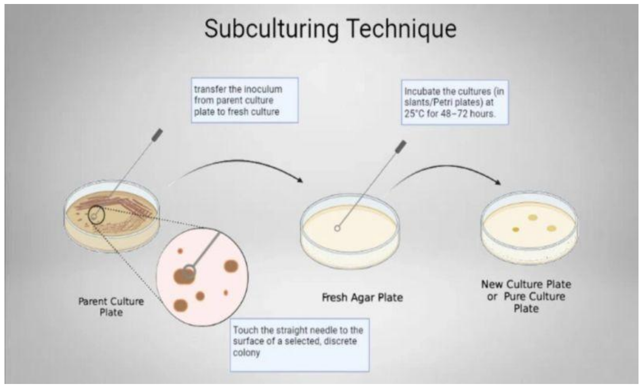


Figure :4 In Vitro and In Vivo Workflow of PLA-Based Scaffold Development Types of Cells

Cells to be kept in healthy & in growing state have to be sub-cultured or passaged. It's the passage of cells when they reach to 80-90% confluency in flask/dishes/plates Enzyme such as trypsin, dipase, collagenase in combination with EDTA breaks the cellular glue that attached the cells to the surface

- Adherent Cells

Cells which are anchorage dependent Cells are washed with PBS phospate buffer saline (free of ca & mg) solution. Add enough trypsin/EDTA to cover the monolayer Incubate the plate at 37 C for 1-2 mts. Tap the vessel from the sides to dislodge the cells add complete medium to dissociate and dislodge the cells with the help of pipette which are remained to be adherent Add complete medium depends on the subculture requirement either to 75 cm or 175 cm flask, DEAE-dextran, FBS.

3.2. Suspension Cells

Easier to passage as no need to detach them as the suspension cells reach to confluency Aseptically remove $1/3^{rd}$ of medium Replaced with the same amount of pre-warmed medium.

3.3. Transfection Methods

Calcium phosphate precipitation DEAE-dextran (dimethyl aminoethyl-dextran) Lipid mediated lipofection Electroporation Retroviral Infection Microinjection Working with cryopreserved cells Vial from liquid nitrogen is placed into the vial is thawed in a 37 °C water bath with gentle shaking until the medium is fully melted, followed by centrifugation at 1000 rpm for 10 minutes at room temperature. After wiping the vial with 70% ethanol and removing the supernatant, the cell pellet is resuspended in 1 mL of complete medium containing 20% fetal bovine serum (FBS). The suspension is then transferred to a labeled culture plate, incubated, checked after 24 hours for cell attachment, and the medium is changed whenever its color indicates depletion, maintaining 20% FBS supplementation.

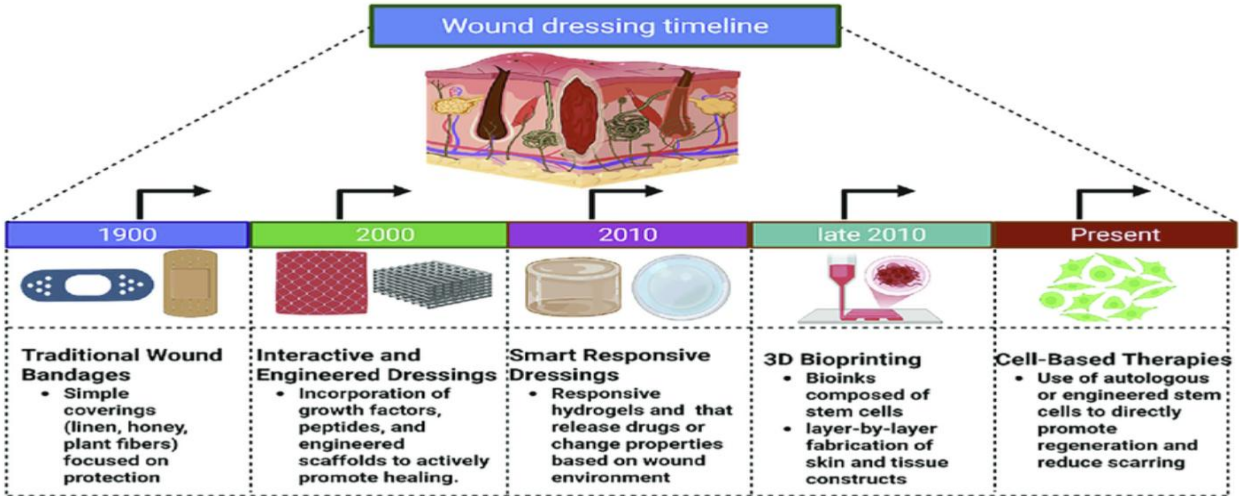


Figure 5 wound dressing timeline

Freezing Cells for Storage

Figure: 6 Freezing Cells for Storage

Remove the growth medium and PBS. Detach cells using trypsin-versine, then dilute them medium. transfer cell 15 mL tube and centrifuge at 200 g for 5 minutes at room temperature. Discard the supernatant and resuspend the pellet in 1–2 mL of freezing medium. Aliquot the suspension into cryovials, store at −80 °C overnight, and then move to liquid nitrogen for long-term storage.

Cell Viability

Cell viability = how many skin cells remain alive and functioning under certain conditions.
In biomedical applications involving skin (such as tissue engineering, wound healing, skin grafts, cosmetics testing, or biomaterials), cell viability refers to the percentage or proportion of living, healthy cells present in a skin sample or cultured tissue after treatment, testing, or exposure to a material.
cell viability can be measured Common laboratory tests include:
MTT assay, Live/Dead staining, Trypan blue exclusion, Alamar Blue assay
Cell Viability (%) =No. of Cells \Total number of cells Number of living cells×10

Contaminants of Cell Culture
Cell culture contaminants of two types: Chemical, difficult to detect, caused by endotoxins, plasticizers, metal ions or traces of disinfectants that are invisible. Biological, cause visible effects on the culture they are mycoplasma, yeast, bacteria or fungus or also from cross-contamination of cells from other cell lines.

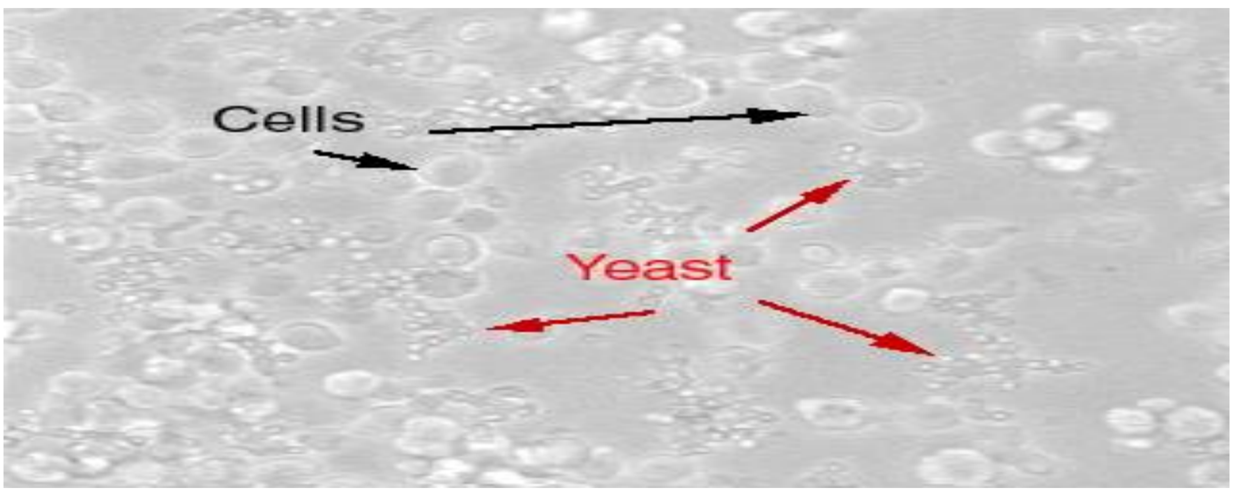


Figure :7 Contaminants of Cell Culture

Detection of contaminants
Mycoplasma detected by direct DNA staining with intercalating fluorescent substances e.g. Hoechst 33258 Mycoplasma also detected by enzyme immunoassay by specific antisera or monoclonal abs or by PCR amplification of mycoplasmal RNA. The best and the oldest way to eliminate contamination is to discard the infected cell lines directly.

- Cell Culture Rules and Conditions

Never use contaminated material within a sterile area. Use the correct sequence when working with more than one cell lines. Diploid cells (Primary cultures, lines for the production of vaccines etc.) Diploid cells (Laboratory lines) continuous, slow growing line continuous, rapidly growing lines which may be contaminated Virus producing lines Swab all bottle tops & necks with 70%

ethanol Flame all bottle necks & pipette by passing very quickly through the hottest part of the flame avoiding placing caps & pipettes down on the bench.

3.4. Prepared sample Scaffold Material

To understand the structure of the samples prepared with 8 layers of PLA, a scheme is shown below, where the first one in the left represents the 8 PLA and 1 Lanolin; the one in the middle, 8 PLA and 2 Lanolin; and the one on the right, 8 PLA and 3 Lanolin. The blueish parts represent the PLA sheets and the yellowish parts, the lanolin cap

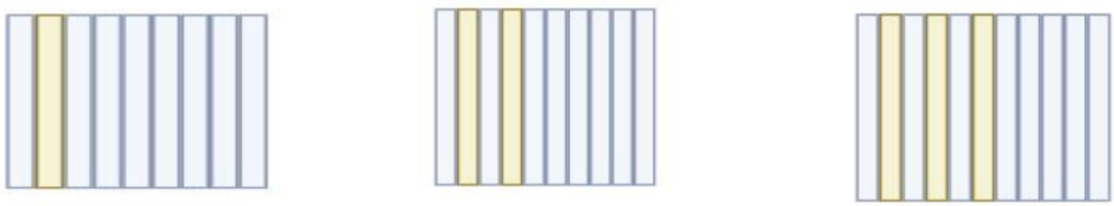


Figure 8: Schematic representation of the structure of the samples made of 8 sheets of PLA. **a) 8** PLA + 1 Lanolin, b) 8 PLA + 2 Lanolin, c) 8 PLA + 3 Lanoli

In scaffold development, these polymers provide suitable mechanical strength while gradually degrading into non-toxic by-products that can be naturally eliminated from the body. Among them, PLGA offers tunable degradation rates depending on the lactic-to-glycolic acid ratio, while PCL is known for its slower degradation and flexibility. PLA-based polymers provide good structural integrity, making them suitable for long-term support in tissue regeneration

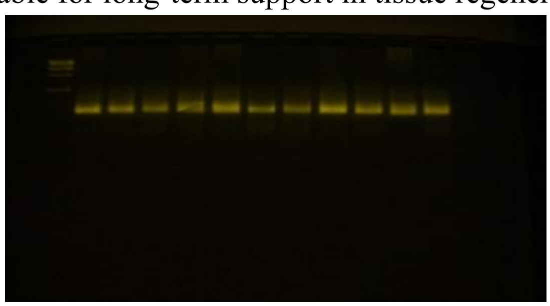


Figure :9: Sample 1

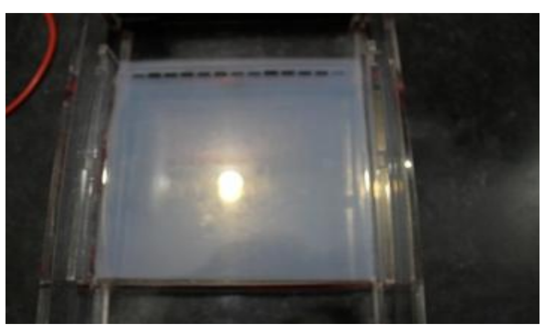


Figure:10 Sample 2

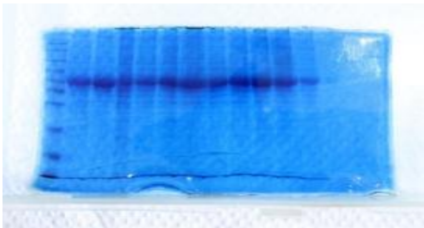
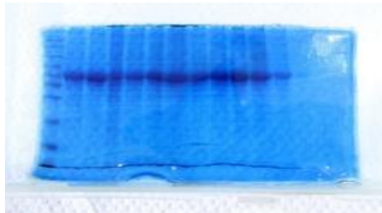
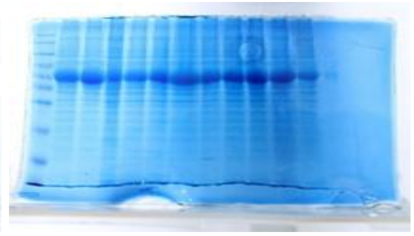




Figure: 11 Sample 3 Sample 4 Sample 5

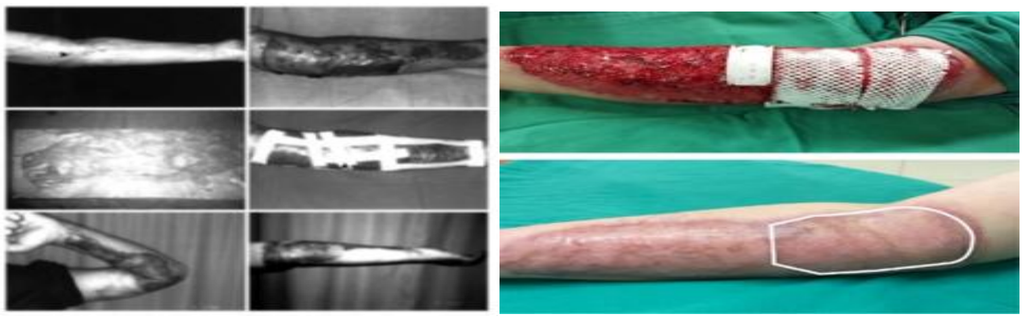
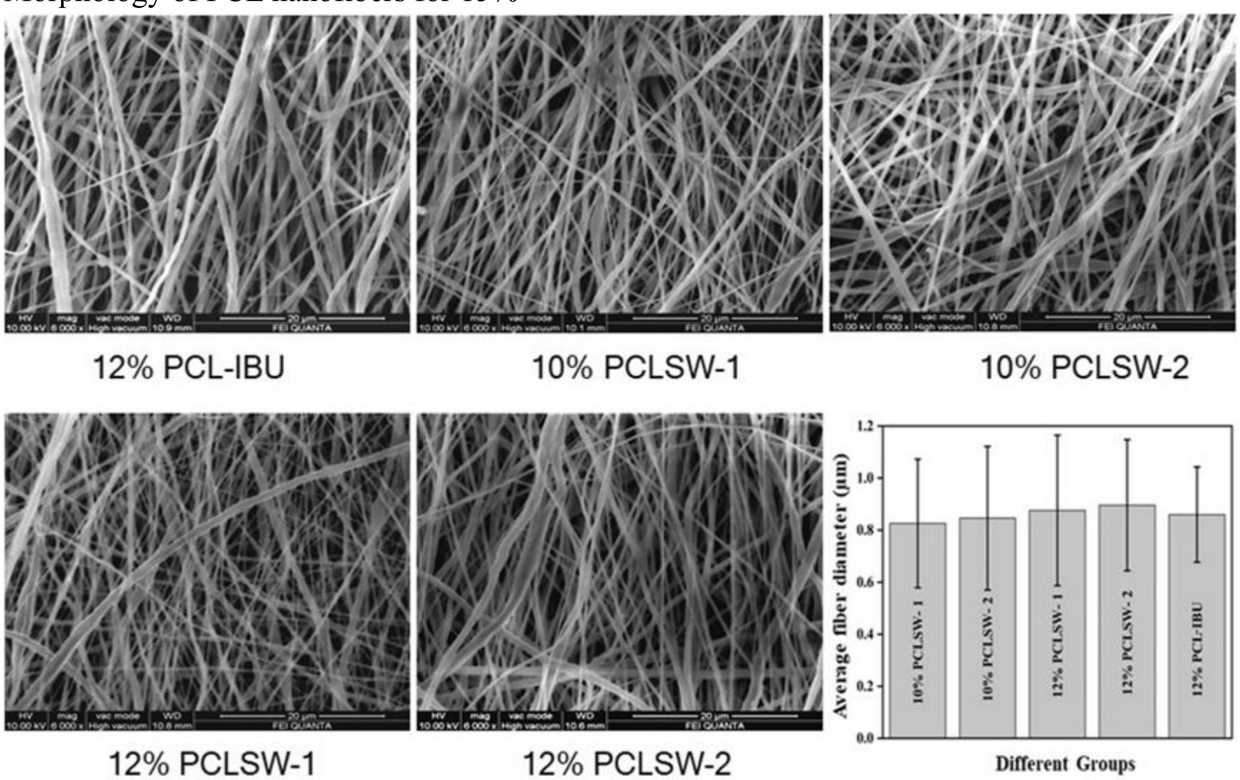


Figure 11. Wound dressing scaffold

Morphology:

Morphology of PCL nanofibers for 15%



- SEM image of a randomly oriented 15% PLA nanofiber deposition. Electrospun 15% PCL nanofiber diameter was 220 to 445 nm. Scale bar = 1000x

- SEM image of Aligned 15% PLA nanofiber deposition. Electrospun 15% PLGA, nanofiber Scale bar = 5000x
- Polarizing microscope image of 15% PLA nanofibers

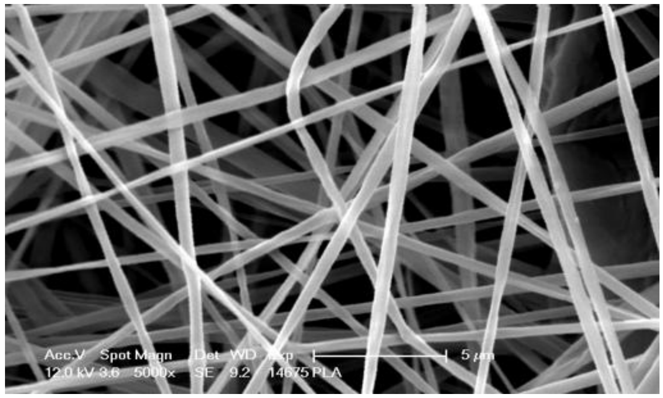


Figure :12 PLA nanofibers for 15%.

Studies also report that a 15/85 ratio (PCL/PLA) raises osteogenic potential, while a 1:1 or 50/50 blend balances mechanical and biological properties well for cell adhesion and proliferation. Scaffolds with around 60/40 or 50/50 PLA/PCL ratios have shown improved hydrophilicity and cell attachment compared to pure PCL, which is more hydrophobic.

IV. CONCLUSION

The skin is essential for protecting the body, and damage from burns, wounds, or diseases raises risks like infection and dehydration. While traditional and modern wound dressings help, severe cases often require skin grafts. The standard grafts, typically from cadaver skin, are limited by the availability of healthy donor skin and risks of infection or rejection. To address these challenges, bioengineered skin grafts made from advanced biomaterials are being developed as promising alternatives for more effective and safer skin repair. In patients suffering from major burns, artificial dermis enables early wound closure with a success rate comparable to that of allografts. When subsequently covered with an epidermal graft, it provides a durable and permanent skin cover that is satisfactory when compared with conventional skin grafting methods. Additionally, it allows the use of thinner donor grafts, resulting in donor sites that heal more quickly, while also offering a cost-effective treatment approach.

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