

Emerging Trends in Electrospun Polymeric Scaffolds for Tissue Engineering and Regenerative Medicine

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Abstract : Skin is the largest organ of the human body and serves as the primary protective barrier against physical, chemical, and microbial damage. Extensive skin injuries caused by burns, trauma, chronic wounds, or diabetic ulcers often exceed the body's natural healing capacity, necessitating the development of advanced therapeutic strategies. Skin tissue engineering has emerged as a promising approach for the regeneration of damaged skin by integrating biomaterials, cells, and bioactive molecules to create functional skin substitutes. Among various biomaterials, biodegradable polymeric scaffolds have gained significant attention due to their excellent biocompatibility, controlled biodegradation, and ability to mimic the native extracellular matrix (ECM).

Electrospun nanofibrous scaffolds are particularly attractive for skin tissue engineering because of their high surface area, interconnected porous structure, and morphological similarity to the ECM, which promote cell adhesion, proliferation, migration, and tissue regeneration. Biodegradable polymers such as poly(lactic acid) (PLA), polycaprolactone (PCL), and poly(lactic-co-glycolic acid) (PLGA) are widely used for scaffold fabrication owing to their favourable mechanical properties and biodegradability. Furthermore, the incorporation of natural bioactive compounds, such as lanolin, collagen, or growth factors, can enhance the biological performance of these scaffolds by improving cell attachment, moisture retention, and wound healing.

An ideal skin scaffold should possess excellent biocompatibility, suitable mechanical strength, high porosity, controlled degradation, and the ability to support nutrient transport and vascularization while maintaining structural integrity during tissue regeneration. Recent advances in electrospinning technology have enabled the fabrication of multifunctional nanofibrous scaffolds capable of delivering therapeutic agents and supporting the regeneration of both the epidermal and dermal layers of skin.

I. Introduction

For skin regeneration, a scaffold acts as a temporary artificial extracellular matrix that supports the attachment and growth of **keratinocytes** and **fibroblasts**, promotes collagen deposition, encourages angiogenesis, and accelerates wound healing. Electrospun nanofibrous scaffolds are particularly suitable because they closely mimic the fibrous architecture of the native skin. Severe skin injuries resulting from burns, trauma, surgical excision, chronic ulcers, diabetic wounds, infections, or congenital disorders often exceed the natural regenerative capacity of the skin. Conventional treatment methods such as autografts, allografts, xenografts, and synthetic wound dressings have been widely employed; however, these approaches are associated with several limitations, including donor site morbidity, limited tissue availability, immune rejection, disease transmission, scarring, prolonged healing time, and high treatment costs. These challenges have stimulated extensive research into alternative regenerative therapies.

Skin tissue engineering has emerged as an advanced interdisciplinary field that integrates principles of biology, materials science, engineering, and medicine to develop functional skin substitutes capable of restoring the structure and function of damaged skin. The fundamental concept of tissue engineering is based on the combination of three essential components: **biomaterial scaffolds, living cells, and bioactive molecules**. Among these, the scaffold plays a critical role by providing a three-dimensional (3D) architecture that mimics the native extracellular matrix (ECM), thereby facilitating cell attachment, proliferation, migration, differentiation, and extracellular matrix deposition.

An ideal scaffold for skin tissue engineering should exhibit excellent biocompatibility, biodegradability, non-toxicity, appropriate mechanical strength, high porosity, interconnected pore structure, and suitable degradation kinetics that match the rate of new tissue formation. Additionally, the scaffold should support oxygen and nutrient diffusion, promote angiogenesis, prevent microbial infection, and provide a favourable microenvironment for cellular growth. These characteristics are essential for the successful regeneration of functional skin tissue.

Biodegradable polymeric materials have become the most widely investigated scaffold materials because they gradually degrade into non-toxic by-products while new tissue is formed, eliminating the need for surgical removal. Both natural polymers, such as collagen, gelatine, chitosan, alginate, hyaluronic acid, and silk fibroin, and synthetic polymers, including **poly(lactic acid) (PLA)**, **polycaprolactone (PCL)**, **poly(glycolic acid) (PGA)**, and **poly(lactic-co-glycolic acid) (PLGA)**, have been extensively explored for skin tissue engineering applications. Synthetic polymers offer excellent mechanical properties, reproducibility, and tunable degradation rates, whereas natural polymers provide superior biological recognition and cell affinity. Hybrid scaffolds combining natural and synthetic polymers can therefore provide enhanced biological and mechanical performance.

In recent years, electrospun biodegradable polymer scaffolds based on **PLA, PCL, and PLGA** have shown promising results for the regeneration of damaged skin because of their favorable physicochemical properties, controlled biodegradation, and excellent processability. Furthermore, the incorporation of natural additives such as **lanolin** can improve scaffold flexibility, moisture retention, surface wettability, and cellular compatibility, making these scaffolds highly suitable for skin tissue engineering and wound healing applications.

The continuous advancement of biomaterials, scaffold fabrication technologies, stem cell biology, growth factor delivery, and regenerative medicine has significantly expanded the potential of engineered skin substitutes. These innovations aim to develop biomimetic scaffolds capable of restoring both the structural integrity and physiological functions of native skin. Consequently, biodegradable electrospun polymeric scaffolds represent one of the most promising strategies for the development of next-generation skin substitutes for clinical applications in wound healing, burn management, and regenerative medicine.

I. RESEARCH METHODOLOGY

The collected tissue was transferred into 10% Betadine solution. After 2 minutes, it was rinsed in fresh 0.01% Penicillin–Streptomycin solution for 1 minute. The skin was then placed in a 150 mm Petri dish with the dermal side facing upward, identified by the yellowish fatty layer with traces of blood. Holding the tissue with forceps, the adipose tissue and muscle were carefully scraped away using a sterile scalpel blade. The tissue was cut into four equal pieces, each approximately $2 \times 2 \text{ cm}^2$, and finally rinsed in 0.1 M PBS.

Isolation of Keratinocytes using the Double Enzyme Method from the Prepared Tissue Sample

Sterile scalpels, sterile gloves, a sterile scalpel blade holder, 150 mm and 60 mm sterile Petri plates, 10% Betadine solution, 0.1 M Phosphate Buffered Saline (PBS), and 0.01% Penicillin–Streptomycin solution were used for the processing of the excised skin tissue.

Method

Following cell counting and viability assessment, the isolated keratinocytes were seeded onto collagen-coated electrospun PLA nanofiber scaffolds and incubated at 37°C in a

IV. RESULTS AND DISCUSSION

Table 4.1: Descriptive Statics

Variable	Minimum	Maximum	Mean	Std. Deviation	Jarque-Bera test	Sig
KSE-100 Index	-0.11	0.14	0.020	0.047	5.558	0.062
Inflation	-0.01	0.02	0.007	0.008	1.345	0.510
Exchange rate	-0.07	0.04	0.003	0.013	1.517	0.467
Oil Prices	-0.24	0.11	0.041	0.060	2.474	0.290
Interest rate	-0.13	0.05	0.047	0.029	1.745	0.418

Table 4.1 displayed mean, standard deviation, maximum minimum and jarque-bera test and its p value of the macroeconomic variables of the study. The descriptive statistics indicated that the mean values of variables (index, INF, EX, OilP and INT) were 0.020, 0.007, 0.003, 0.041 and 0.047 respectively. The maximum values of the variables between the study periods were 0.14, 0.02, 0.04, 0.41, 0.11 and 0.05 for the KSE- 100 Index, inflation, exchange rate, oil prices and interest rate.

The standard deviations for each variable indicated that data were widely spread around their respective means.

Column 6 in table 4.1 shows jarque bera test which is used to check the normality of data. The hypotheses of the normal distribution are given;

H_0 : The data is normally distributed.

H_1 : The data is not normally distributed.

Table 4.1 shows that at 5 % level of confidence, the null hypothesis of normality cannot be rejected. KSE-100 index and macroeconomic variables inflation, exchange rate, oil prices and interest rate are normally distributed.

The descriptive statistics from Table 4.1 showed that the values were normally distributed about their mean and variance. This indicated that aggregate stock prices on the KSE and the macroeconomic factors, inflation rate, oil prices, exchange rate, and interest rate are all not too much sensitive to periodic changes and speculation. To interpret, this study found that an individual investor could not earn higher rate of profit from the KSE. Additionally, individual investors and corporations could not earn higher profits and interest rates from the economy and foreign companies could not earn considerably higher returns in terms of exchange rate. The investor could only earn a normal profit from KSE.

humidified atmosphere containing 5% CO₂. The porous nanofibrous scaffold supported rapid cell attachment and uniform cell distribution.

Materials

Liquid nitrogen, isopentane, aluminum foil, blunt-end forceps, liquid nitrogen cylinder, -80°C deep freezer, poly-L-lysine coated slides, Leica cryostat model CM3050S, acetone, 1% BSA, 0.1 M PBS, tissue paper, humidified chamber, primary and secondary antibodies as mention in Table 7.2, fluorescent mounting medium (Dako Cytomation, USA), cover slips, and fluorescent microscope (Leica).

Table 2 Primary and Secondary antibodies

Primary antibody	Secondary antibody
Rabbit monoclonal Cytokeratin-1 (ABCAM)	Anti-rabbit IgG–TRITC (Sigma Aldrich)
Mouse monoclonal Cytokeratin-14 (ABCAM)	Anti-mouse IgG–FITC (Sigma Aldrich)
Rabbit polyclonal Involucrin (ABCAM)	Anti-rabbit IgG–TRITC (Sigma Aldrich)

Method

The excised skin tissue from mouse cadavers was snap-frozen in liquid nitrogen-cooled isopentane and stored overnight in liquid nitrogen. The sample was subsequently stored at -80°C. Cryosections (7 µm thick) were prepared using a Leica CM3050S cryostat. Sections were mounted on poly-L-lysine-coated glass slides, air-dried, and stored at -80°C until use. On the day of the experiment, the slides were removed from the freezer and allowed to thaw at room temperature for approximately 30 minutes before immunostaining.

The sections were fixed in cold acetone (at -20°C). The slides were washed three times with PBS for 5 minutes each. After washing, the slides were gently wiped around each section.

A drop of 1.0% Bovine serum Albumin (BSA) solution was added to cover each section completely and incubated at room temperature for 10 minutes. After incubation, the BSA solution was removed, and the slides were gently wiped around each section. A drop of primary antibody was added to cover the section completely, and the slides were incubated for 1 hr in a humidified chamber. Following incubation, the slides were washed three times with PBS for 5 minutes each and incubated with the corresponding secondary antibody for 1 hour in a humidified chamber. After incubation, the slides were again washed three times with PBS for 5 minutes each. Finally, the sections were mounted with coverslips using fluorescent mounting medium and observed under a fluorescent microscope.

Zeta Potential analysis of PLA/ Lanolin

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. Figure 7.10 presents the Zeta potential of various samples of PLA + Lanolin formulations.

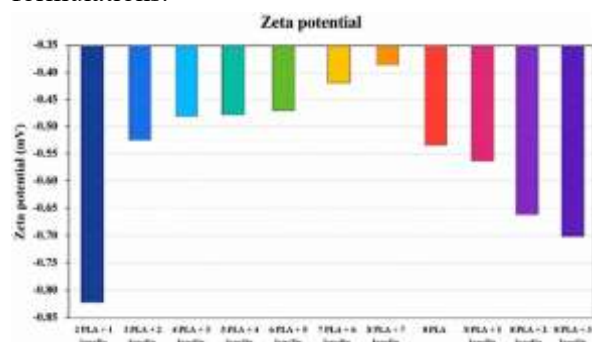


Figure1 Graphical representation of the zeta potential values.

Cell Sheet Retrieval from Coated Culture Plate

Materials

PVDF (Polyvinylidene Fluoride) membrane (sterile), blunt-end forceps, DMEM + 10% FBS, TCPS-coated plate (35 mm).

Method

In order to study the thermoresponsive property of the TCPS-coated culture plates, cell sheet retrieval was performed using a PVDF membrane. L929 cells were cultured on TCPS- Coated plates until confluent. The culture medium was removed, and a PVDF membrane preconditioned with medium was placed flat on the cell surface. The plates were incubated at 10°C for 1 minute, followed by 3 minutes at 25°C . After incubation, the cells were observed under a microscope. Once cell rounding was observed, the PVDF membrane was floated by adding excess medium and transferred to a fresh culture plate with the cell side in direct contact with the culture surface. The plate was incubated at 37°C for 1 hour. The PVDF membrane was then floated again by adding excess medium and removed. One milliliter of medium was added to the fresh plate, and the cells were observed under a microscope. The fresh plate was subsequently incubated at 37°C in 5% CO_2 .

RESULTS AND DISCUSSIONS

Keratinocyte Culture on Electrospun PLA Scaffold

The collagen-coated electrospun PLA nanofiber scaffold provided an excellent substrate for adult murine keratinocyte attachment and proliferation. The interconnected porous structure of the scaffold closely resembled the extracellular matrix of native skin, enabling uniform cell attachment within a few hours of seeding. Progressive colony formation and cobblestone morphology were observed during culture, confirming maintenance of the epithelial phenotype. The scaffold exhibited good biocompatibility without any evidence

Isolation and Culture of Adult Murine Keratinocytes

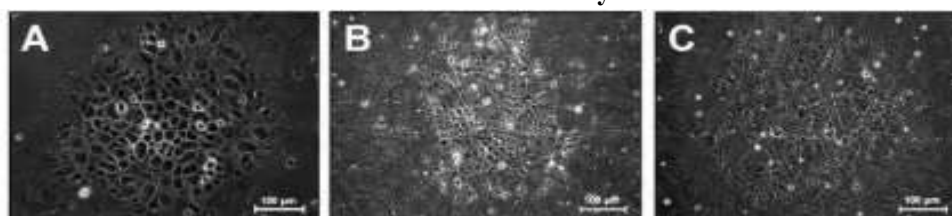


Figure 2 Primary Keratinocyte Culture. A, B and C show cultures of primary keratinocytes on day 4, day 8 and day 13.

Cells grew in the form of colonies and exhibited characteristic cobblestone morphology. Figure 12 A and 12 B show the morphology of keratinocytes on the 8th day and 13th day of culture in different magnifications.

cobble stone morphology.

Trypsin Treatment

The 8-day keratinocyte culture was given trypsin treatment for 1 min. This favoured the migration of cells from patches as shown in Figure7

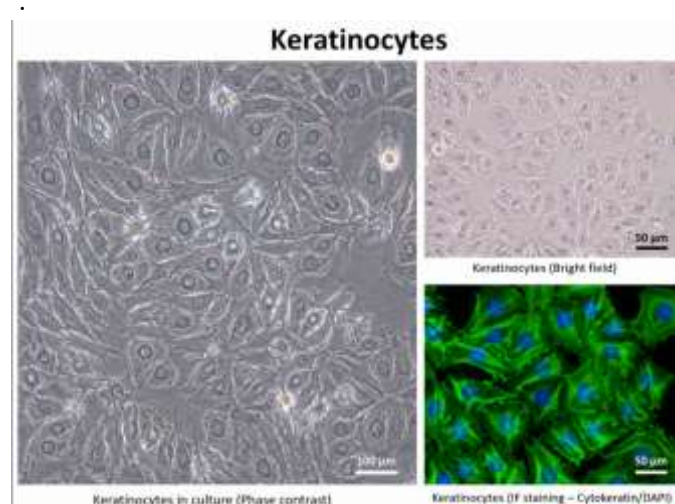


Figure 3 Keratinocyte isolation using single enzyme method

A & B – Warm trypsinization 24 hr and 2 days respectively; C & D – Cold trypsinization 24 hr and 2 days respectively.

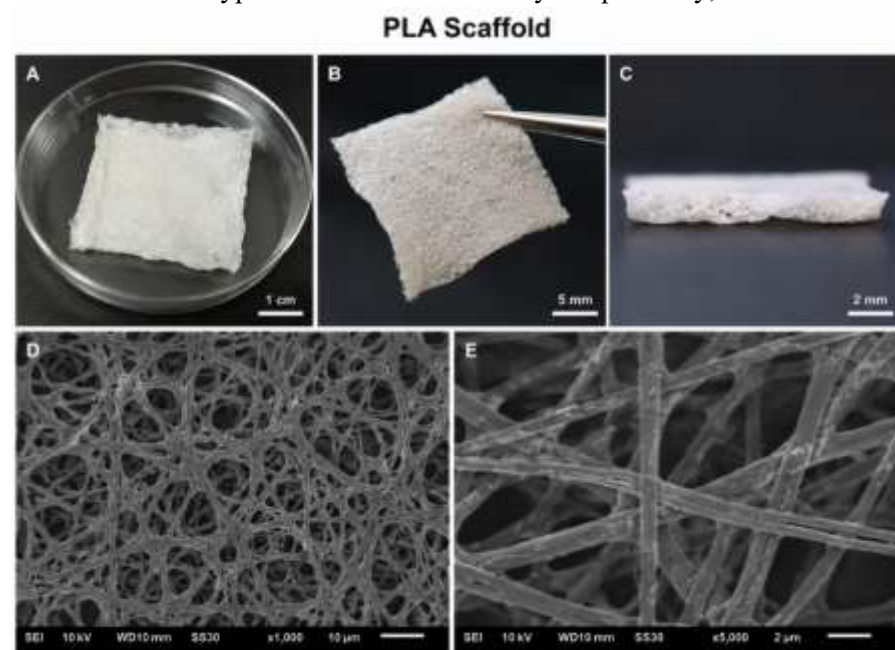


Figure 4 representative Images of the Fabricated Electrospun PLA Scaffold and Its Surface Morphology.

The summary of the fabrication of engineered cells is presented as follows:

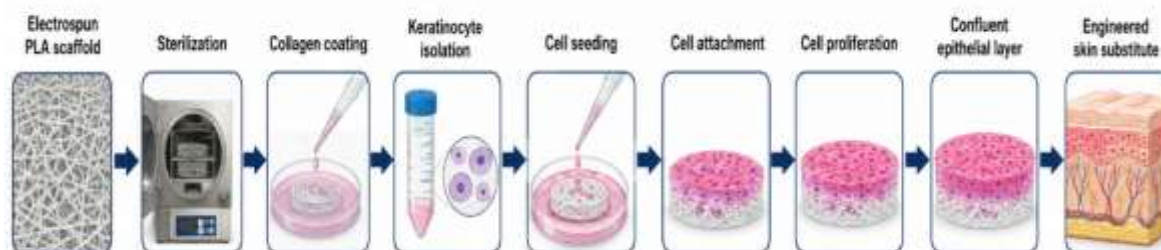


Figure 5 Workflow for the development of an engineered skin substitute using an electrospun PLA scaffold.

CONCLUSIONS

The present study successfully demonstrated the bio-functionalization of electrospun PLA nanofiber scaffolds for skin tissue engineering applications. The optimized electrospun PLA scaffold provided a highly porous, extracellular matrix (ECM)-like structure that supported efficient attachment, proliferation, and maintenance of adult murine keratinocytes. Sterilization, collagen coating, and the optimized double enzyme isolation protocol collectively enhanced cell adhesion and growth, resulting in the formation of a confluent epithelial layer with characteristic cobblestone morphology. These findings confirm the excellent biocompatibility of the scaffold and its suitability as a substrate for skin cell culture.

The incorporation of Lanolin further improved the surface characteristics of the PLA scaffold by modifying its surface charge and enhancing its bioactivity, as demonstrated through zeta potential analysis. The improved surface properties are expected to promote better cell–material interactions and contribute to enhanced wound healing performance. Overall, the developed electrospun PLA–Lanolin scaffold exhibited favourable physicochemical and biological properties required for engineered skin substitutes.

The results indicate that the optimized PLA-based scaffold has significant potential as a biodegradable and biocompatible wound dressing and skin tissue engineering scaffold. This approach provides a promising platform for future development of functional skin substitutes and advanced regenerative therapies, with potential applications in the treatment of burns, chronic wounds, and other skin defects.

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