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The Effect of Umbilical Cord Blood Serum and Platelet-Rich Plasma Coatings on the Characteristics of Poly(ε-caprolactone) Scaffolds for Skin Tissue Engineering Applications

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Abstract. The need for effective artificial skin as a substitute for damaged skin in chronic wound therapy is recently growing. Poly(ε-caprolactone) (PCL) has been identified as a potential material for artificial skin scaffolds due to its exceptional mechanical properties and biocompatibility. However, PCL lacks sufficient bioactivity, necessitating the introduction of bioactive molecules to scaffolds. Human umbilical cord blood serum (UCBS) and platelet-rich plasma (PRP), rich in bioactive molecules, are promising coating materials for PCL-based scaffolds. Therefore, this research aimed to investigate the effect of UCBS and PRP coatings on the mechanical properties, cytotoxicity, and cell attachment ability of PCL scaffolds. Scaffolds prepared through glutaraldehyde-mediated cross-linking of 20% (w/v) PCL followed by freeze-drying were immersed with UCBS or PRP overnight. Coating scaffolds with UCBS generated a significantly lower Young's modulus (0.20 MPa) compared to non-coated counterparts (0.27 MPa), while PRP-coated scaffolds showed no substantial change (0.24 MPa). Both UCBS and PRP coatings significantly increased ($p < 0.05$) the viability and attachment of primary human fibroblast cells on scaffolds, showing the potential to enhance PCL cytocompatibility for artificial skin.

Keywords: Artificial skin; Fibroblasts; Human umbilical cord blood serum; Platelet-rich plasma; Poly(ε-caprolactone)

1. Introduction

Natural A chronic wound is a significant healthcare challenge, affecting a large population globally, with an estimated occurrence ranging from 1.51 to 2.21 cases per 1,000

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individuals (Martinengo *et al.,* 2019). This is commonly managed with skin graft therapy, but the process presents various limitations, such as donor shortages, risk of disease transmission, immunogenicity, and high medical costs (Chandika *et al.,* 2021). In response, the development of artificial skin has become a promising alternative treatment.

Artificial skin, comprising cells, scaffolds, and bioactive molecules, holds great potential for tissue regeneration and wound healing (Chung *et al.,* 2020). While cells and bioactive molecules are widely available, current research focuses on identifying biologically and physicochemically compatible scaffolds to create effective artificial skin constructs.

Poly(ε-caprolactone) (PCL), a hydrophobic semi-crystalline polyester synthesized by ring-opening polymerization of ε-caprolactone (Homaeigohar dan Boccaccini, 2022), possesses desirable properties for scaffolds applications in tissue engineering (Vach-Agocsova *et al.,* 2023; Gao *et al.,* 2018; Siddiqui *et al.,* 2018), including artificial skin. Despite its biocompatibility, mechanical strength, low melting point (60°C), and versatility in producing various shapes and porous structures, PCL has relatively low inherent bioactivity (Petretta *et al.,* 2021). This limitation necessitates the incorporation of additional bioactive molecules on the scaffold's surface.

Surface properties, including surface topography, hydrophilicity, and chemical composition, often influence cell-substrate interactions (Dewi *et al.,* 2020). Previous research explored the application of collagen in coatings for promoting cell adhesion (Sharif *et al.,* 2017) and the modification of growth factors such as vascular endothelial growth factors (VEGF) and bone morphogenic protein-2 (BMP-2) on PCL-based scaffolds (Qin *et al.,* 2022; Suárez-González *et al.,* 2012). However, the limited availability, high cost, and immune-stimulating potential of these factors, specifically those from non-human sources, pose significant challenges (Mariani *et al.,* 2019). Umbilical cord blood serum (UCBS) and platelet-rich plasma (PRP) are promising alternative sources rich in bioactive molecules that can enhance PCL scaffold bioactivity. UCBS contains cytokines and extracellular matrix proteins (Maharajan *et al.,* 2021), along with growth factors produced by Umbilical Cord Mesenchymal Stem Cells (Nurhayati *et al.,* 2021a). Meanwhile, PRP comprises platelets, growth factors, and fibrinogen crucial for cell attachment and migration (Maharajan *et al.,* 2021; Nurhayati *et al.,* 2021a; Devereaux *et al.,* 2020; Pavlovic *et al.,* 2016).

Coating PCL scaffolds with UCBS/PRP allows bioactive molecules to interact with seeded cells, promoting attachment, proliferation, and functional behavior (Francavilla and O'Brien, 2022; Wheeler and Yarden, 2015). Moreover, the immunomodulatory properties of UCBS and PRP can modulate the immune response and mitigate inflammation, reducing rejection of the artificial skin constructs (Sriram *et al.,* 2023; Lotfinejad *et al.,* 2021). Using UCBS/PRP as coating materials presents a cost-effective and easily accessible solution compared to commercial growth factor supplements. properties.

Despite the advantages of UCBS and PRP, their applications as coating materials have not been explored. Therefore, this research aimed to investigate the effect of UCBS and PRP coatings on the characteristics of PCL scaffolds for skin tissue engineering, as well as evaluate the impact exerted on the viability and adhesion of primary human fibroblast cells (Figure 1).

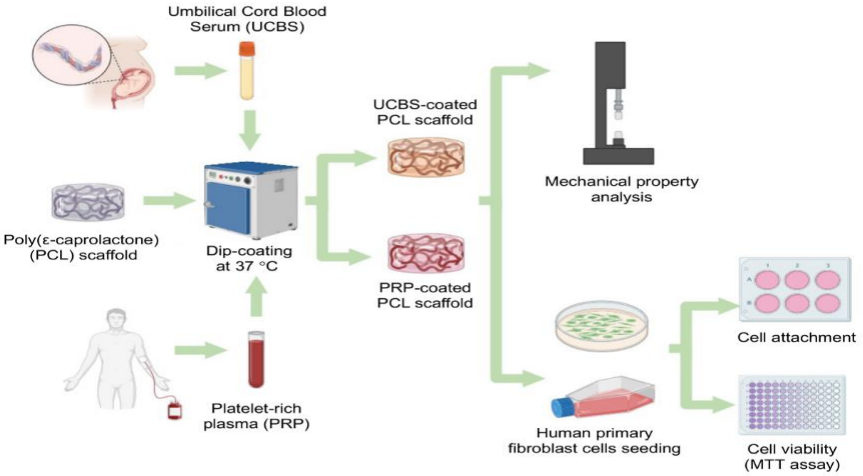

Figure 1 Conceptual scheme of this research (created with BioRender.com)

2. Methods

2.1. Ethical Clearance

Ethical clearance with Approval No. KET-1003/UN2.F1/ETIK/PPM.00.02/2021 was obtained from the Ethical Committee for Medical Research of the Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo General Hospital.

2.2. Materials

The materials used in this research included PCL (Mn=80,000), acetic acid, glutaraldehyde, and calcium chloride ($CaCl_2$), purchased from Sigma-Aldrich (St. Louis, MO, USA). Additionally, outdated human PRP was obtained from the Indonesian Red Cross (Jakarta, Indonesia). Dimethylsulfoxide (DMSO) was procured from Molecular Probes (Eugene, OR, USA). Dulbecco's Modified Eagle Medium low glucose (DMEM; glucose concentration 1 g/L), fetal bovine serum (FBS), penicillin/streptomycin, phosphate-buffered saline (PBS; pH 7.4), and trypan blue were acquired from Gibco (New York, NY, USA). The 2,5-diphenyl-2H-tetrazolium bromide (MTT) solution was purchased from Invitrogen (Carlsbad, CA, USA).

2.3. Scaffolds Fabrication and Coating

A 20% (w/v) PCL solution was prepared by dissolving PCL in glacial acetic acid at 60°C for 1 h. This was then transferred into a 24-well plate, frozen at -20°C overnight, and treated with 5% (v/v) glutaraldehyde for 30 min. The resulting frozen scaffolds were freeze-dried for 5 h at 0.5 atm and -120°C. For coating, UCBS and PRP solution were mixed with 0.5 mM $CaCl_2$ at a ratio of 9:1. The freeze-dried PCL scaffolds were washed with PBS and immersed in UCBS and PRP solution at 37°C overnight, then stored at -20℃ for subsequent analysis.

2.4. Mechanical Property Measurement

The mechanical properties of scaffolds were evaluated using an Instron Universal Testing Machine (UTM, 6800, Instron, Norwood, MA, USA). Scaffolds with a 1 cm diameter were horizontally placed between UTM pressure plates and subjected to a compressive force at a controlled rate of 50 mm/min, reaching a maximum force of 500 N until complete crushing occurred. Young's modulus was determined based on the stress-strain curve in the range of 0% — 1.2% strain (Figure 2a).

2.5. Isolation and Culture of Human Primary Fibroblast Cells

Human primary fibroblast cells were isolated from discarded skin following C-section surgery using an explant method (Nurhayati *et al.,* 2019). The skin tissue was sterilized with

0.5% (v/v) povidone-iodine in PBS, cut into small pieces (~5 mm), and placed in a 24-well plate. Approximately 200 µL of culture medium containing DMEM, 1% (v/v) penicillin/streptomycin, and 10% (v/v) FBS was added to the well to prevent tissue floatation and ensure complete coverage. Fibroblast cells originating from the explanted tissue were collected through trypsinization and subsequently cultured in a humidified incubator at 37℃ with 5% CO_2.

2.6. Isolation of Umbilical Cord Blood Serum

Human UCBS was isolated from umbilical cord blood obtained post-C-section surgery using a gradient density centrifugation method (Nurhayati *et al.,* 2021b). The blood was pipetted onto the Ficoll solution, centrifuged at 400 × g for 10 min, and the top layer containing UCBS was carefully transferred to a new tube and filtered with a 0.2 µm membrane filter.

2.7. Cell Viability Assay

Cell viability was assessed using an MTT assay, where the prepared scaffolds were initially immersed in a 1.5 mL culture medium. At 24, 48, and 72 h, 500 µL medium was collected and stored at 4°C. Fibroblast cells were seeded in a 96-well plate at a density of 7.0×10^3 cells/well. After reaching 80% confluence, the culture medium was replaced with a 100 µL size for immersion at the respective time points. After 24 h, cells were incubated with the culture medium mixed with MTT solution at a ratio of 9:1. Following a 4 h period, DMSO was added to stop the reaction, and the absorbance at 570 nm was measured using a microplate reader (Varioskan Lux, Thermofisher Scientific, Waltham, MA, USA), then cell viability was calculated with Formula (1):

$$\%_{cell\ viability} = \frac{OD_s - OD_m}{OD_c - OD_m} \times 100\% \quad (1)$$

Where ODs shows absorbance of the sample (cells exposed to medium incubated with scaffolds), ODc signifies absorbance of cultured cells, and ODm represents absorbance of culture medium as blank.

2.8. Cell Attachment Test

Scaffolds were individually placed on an ultra-low attachment 6-well plate supplied with 3 mL culture medium to ensure complete submersion and fibroblast cells were seeded at a density of 1.0×10^5 cells/well. After 2, 4, and 6 h of culture, non-attached cells were counted using a hemocytometer based on a dye exclusion method, and cell attachment was calculated using Formula (2):

$$\%_{cell\ attachment} = \frac{n_0 - n_f}{n_0} \times 100\% \quad (2)$$

Where n_0 indicates the initial cell number during seeding and nf denotes the number of non-attached cells in the culture medium.

2.9. Statistical Analysis

All experiments were performed three times (n = 3), and data were expressed as mean ± standard deviation (S.D.). Statistical analyses were conducted with GraphPad Prism 9 (GraphPad Software Inc., Boston, MA, USA), using one-way ANOVA for Young's modulus data and two-way ANOVA for cell viability and attachment data. A post-hoc t-test was carried out using Tukey HSD, considering results with $p < 0.05$ as statistically significant.

3. Results and Discussion

3.1. Mechanical Properties

The influence of UCBS and PRP coatings was examined on the PCL scaffold's mechanical properties. The mechanical properties of the scaffold are a critical factor in tissue engineering as they govern the cell's behavior (Mubarok, Elvitigala, and Sakai 2022; Mubarok, Qu, and Sakai, 2021; Nadhif *et al.,* 2020). Herein, the mechanical properties were evaluated by measuring Young's modulus based on the stress-strain curve (Figure 2a), and the result was depicted in Figure 2b. Coating PCL scaffolds with PRP caused no substantial changes ($p > 0.05$, Tukey HSD), while UCBS-coated PCL showed a significantly lower Young's modulus ($p < 0.05$, Tukey HSD) compared to the non-coated counterparts.

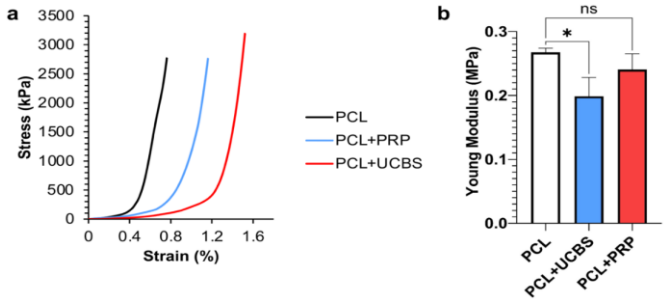

Figure 2 Influence of UCBS and PRP coatings on the mechanical properties of PCL scaffolds. (a) Stress-strain curve and (b) Young's modulus of scaffolds. Error bars represent S.D. (n = 3). * $p < 0.05$, ns: no significant difference ($p > 0.05$), determined by Tukey HSD analysis

The differences among scaffolds could be attributed to the inherent components of UCBS and PRP (Table 1). UCBS, known for high esterase content (Welzing *et al.,* 2011), potentially degraded the ester linkage in PCL affecting the mechanical properties of scaffolds. Additionally, it might infiltrate PCL, altering the polymer network and weakening scaffolds. A similar effect was reported in recent research concerning PCL surface modification through hydrolysis with NaOH and aminolysis using hexamethylenediamine/isopropanol, leading to decreased mechanical properties due to interconnected network disruption (Yaseri *et al.,* 2023). Meanwhile, PRP might contain smaller or no esterase, resulting in minimal interference with the PCL network. Despite these alterations, all PCL-based scaffolds maintained Young's modulus values between 0.20—0.27 MPa, close to the range observed in human skin (0.135 MPa — 0.169 MPa) (Nokoorani *et al.,* 2021), ensuring a suitable physical environment for skin cells.

Table 1 Summary of UCBS and PRP components and the functions

Class	Components	UCBS	PRP	Functions	References
Enzyme	Esterase	✓	×	Degrades ester bonds in PCL	(Welzing *et al.,* 2011)
0Antioxidants	Superoxidase dismutase	✓	✓	Neutralize reactive oxygen system (ROS)	(Shetty, Bharucha, and Tanavde, 2007)
	Catalase	✓	✓		
	Glutathione	✓	✓		
Growth Factors	Vascular endothelial growth factor (VEGF)	✓	✓	Promote cell attachment	(Maharajan *et al.,* 2021; Pavlovic *et al.,* 2016; Montero,Santos, and Fernández, 2015)
	Epidermal growth factor (EGF)	✓	✓		
	Platelet-derived growth factor (PDGF)	✓	✓		
	Fibroblast growth factor (FGF)	✓	✓		
	Transforming growth factor (TGF)	✓	✓		
	Insulin-like growth factor (IGF)	✓	✓		
Extracellular matrix	Fibronectin	✓	×	Promote cell attachment	(Devereaux *et al.,* 2020; Morshed *et al.,* 2019)
	Fibrinogen	×	✓		

3.2. Cell Viability Assay

The viability of human primary fibroblast cells exposed to the culture medium pre-incubated with scaffolds for 24 to 72 h was evaluated using an MTT assay capable of assessing metabolic activity. The results showed that coating PCL scaffolds with UCBS and PRP significantly improved cell viability ($p < 0.05$, two-way ANOVA) (Figure 3).

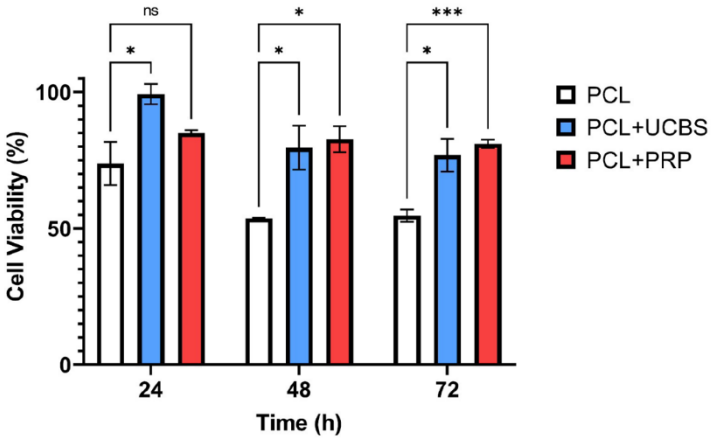

Figure 3 Viability of human primary fibroblast cells exposed to culture medium incubated with PCL, PCL+UCBS, and PCL+PRP scaffolds. Data are represented as mean ± S.D. (n = 3). *$p < 0.05$, ***$p < 0.001$, ns: no significant difference ($p > 0.05$), determined by Tukey HSD analysis

The augmented cell viability observed in UCBS- and PRP-coated scaffolds was due to the presence of growth factors and cytokines stimulating the survival pathways (Chen *et al.,* 2022). These coatings might prevent PCL release or degradation, preserving cell viability. Antioxidants found in both UCBS and PRP, such as superoxide dismutase, catalase, and glutathione (Shetty *et al.,* 2007), neutralize reactive oxidative stress (ROS) and free radicals, thereby protecting cells from oxidative damage and enhancing viability (Sharif *et al.,* 2017).

3.3. Cell Attachment Test

The attachment of human primary fibroblast cells on scaffolds was evaluated at 2, 4, and 6 h post-seeding. According to Figure 4, the lowest percentage of cell attachment was observed at all time points. UCBS and PRP coatings containing growth factors, such as VEGF, EGF, PDGF, FGF, TGF, and IGF, significantly increased cell attachment ($p < 0.05$, two-way ANOVA) by stimulating adhesion (Maharajan *et al.,* 2021; Pavlovic *et al.,* 2016; Montero, Santos, and Fernández, 2015). The interaction between these growth factors and the fibroblast cell receptor played a crucial role in enhancing attachment. Both UCBS and PRP contained extracellular matrix components, supporting increased cell attachment. UCBS, with fibronectin serving as a ligand for α5β1 integrin expressed by fibroblasts, facilitated attachment to the extracellular matrix (Morshed *et al.,* 2019). On the other hand, PRP comprised fibrinogen, which was converted into a fibrin matrix during the fibrin polymerization process aided by $CaCl_2$ to function as a ligand for cell adhesion receptors (Devereaux *et al.,* 2020). The interaction between fibrin with αvβ3 and α5β1 integrins on fibroblast cells further promoted attachment. Additionally, the cytokine IL-6 found in UCBS upregulated integrin expression on the cell surface, increasing the adhesion and stability of attachment to scaffolds (Romanov *et al.,* 2019).

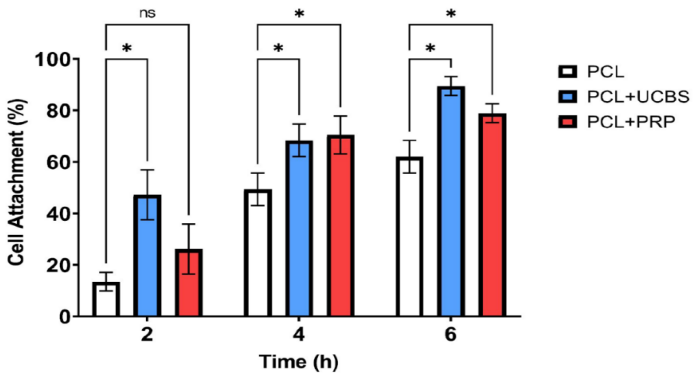

Figure 4 The attachment of human primary fibroblast cells on PCL, PCL+UCBS, and PCL+PRP scaffolds. Data are represented as mean ± S.D. (n = 3). *p < 0.05, ns: no significant difference (p > 0.05), determined by Tukey HSD analysis

4. Conclusions

In conclusion, the incorporation of UCBS and PRP as coating materials distinctly impacted the mechanical and biological properties of PCL scaffolds. UCBS moderately reduced (~25%) the stiffness of PCL scaffolds meanwhile PRP caused no significant alteration in stiffness. Both UCBS and PRP coatings significantly improved (p < 0.05) the viability and attachment of human primary fibroblast cells. These coatings could be considered a promising method for the development of artificial skin in tissue engineering applications.

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