

In vitro evaluation of biocompatibility and degradation of human blood-derived scaffolds by seeding of mouse embryo fibroblasts

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Guest Editor Juan Pablo Aguilar Alemán

Accepted by Editor David Brenner

Abstract

This study highlights the biocompatibility and biodegradability of a novel blood-derived scaffold for regenerative medicine. The scaffold supports cell integration, reduces immune rejection risks, and offers potential as a customizable solution for tissue repair and regeneration. As an autologous scaffold, it offers significant advantages, including reduced risk of immune rejection and improved integration with host tissues. These findings lay the foundation for further investigation of this scaffold as a safe and customizable solution for tissue repair and regeneration in clinical settings. The key objective was to evaluate the interaction of a novel blood-derived scaffold with mouse embryo fibroblasts for potential clinical use. Scaffolds prepared from human donor blood were seeded with fibroblasts. Cell survival and proliferation were assessed using the MTT assay, while morphological studies were conducted via light microscopy, scanning electron microscopy, and laser confocal scanning microscopy. Biodegradation was tested in the presence and absence of enzymes. We found that fibroblasts attached and penetrated the scaffold with a density of 1 cell per $10^3 \mu\text{m}^2$ ($\approx 1,000$ cells/ mm^2). Concentrations of interleukin-6 and transforming growth factor β were significantly higher in the experimental group ($P < 0.05$), whereas the levels of interleukin-1 and tumor necrosis factor-alpha did not differ significantly between the groups. Scaffolds degraded completely in the enzyme-treated group within 48 h, while controls remained intact. Thus, we found that scaffold demonstrated in vitro biocompatibility, cell adhesion, and controlled biodegradation, supporting its clinical application in tissue engineering.

Keywords: bioengineered scaffolds, autologous scaffolds, regenerative medicine, tissue growth

Significance Statement

This study reports the development and in vitro evaluation of a novel whole blood-derived scaffold for regenerative medicine. Unlike platelet-rich plasma-based formulations, the scaffold is generated from whole blood and retains a broader spectrum of cellular and plasma-derived components, resulting in a fibrillar architecture that supports cell-matrix interactions. The scaffold demonstrated biocompatibility, supported adhesion and penetration of mouse embryo fibroblasts, and exhibited controlled enzymatic biodegradation in vitro. As an autologous biomaterial, this approach offers potential advantages including reduced immune response and improved tissue integration. Although evaluated here using a murine fibroblast model as a proof of concept, these findings establish a foundation for future studies using human cells and for further translational development of whole blood-derived scaffolds for tissue repair and regeneration.

Competing Interest: This experiment was funded by Smartfem Pty Ltd. company. Smartfem Pty Ltd is the operational entity responsible for managing product development, regulatory approvals, manufacturing and product distribution, marketing & commercialization. The author, F.B.-W. is the director and sole shareholder of Smartfem Pty Ltd. company. All IP is held in Smartfem Medical Technology Pty Ltd. A license is granted by Smartfem Medical Technology Pty Ltd to all related entities in the group, so that each entity can carry out normal business conduct and operations without infringing Smartfem Medical Technology Pty Ltd. D.A. serves as an Associate Editor for PNAS Nexus.

Received: February 2, 2025. **Accepted:** February 10, 2026

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Introduction

The bioengineered scaffold loaded with autologous cells derived from the patient's tissue biopsy was introduced to replace damaged tissue or organs (1). The scaffold is a principal structure in tissue engineering and as a temporary matrix supports cell attachment, growth, proliferation, and differentiation into functional tissues. A variety of biomaterials have been used in regenerative medicine, among them, natural biomaterials are a desirable choice and derived from biological sources, and they mimic the extracellular matrix (ECM) of tissues.

Autologous, allogeneic, and xenogeneic natural scaffolds can be used in regenerative medicine. Unlike synthetic and nonautologous scaffolds, autologous natural scaffolds offer numerous advantages, including enhanced biocompatibility, minimizing the risk of immune rejection and foreign body responses, and reducing inflammatory reactions (2–4). They are derived from the patient's tissues, promoting improved integration with surrounding tissues. Additionally, autologous scaffolds can be customized to optimize, mechanical strength, degradation rate, and biochemical properties to support desired tissue regeneration (2–4). Clinically, they are applied in skin grafts, cartilage repair, bone regeneration, and wound healing, leading to improved outcomes, fewer complications, and enhanced patient satisfaction (1, 3).

Recently more attention has been focused on using platelet-rich plasma (PRP) as a type of blood-derived biomaterial (5). They are innovative biomaterials used in dermatology, esthetic medicine, orthopedics, sports medicine, dental and oral surgery, cardiovascular tissue engineering, and nerve regeneration, in the form of PRP or platelet-rich fibrin (PRF) (6–11). It is a physiological reservoir of growth factors and cytokines that effectively affects wound and lesion healing (12). Although further clinical research is ongoing to optimize protocols and establish evidence-based guidelines. Different cell types such as fully differentiated adult cells and stem cells were used in combination with biomaterials for several purposes including the assessment of the biocompatibility, biodegradation, and bioactivity of constructing new engineered tissue (13–15). However, the scaffold composition and fine structure such as its porosity, have an important role in the process of cell attachment and penetration (16).

While PRP and PRF systems are widely used blood-derived biomaterials, the scaffold examined here is fundamentally different in composition and structure. Derived from whole blood rather than isolated platelets, it retains a broader range of cellular and plasma components, forming a fibrillar architecture that more closely resembles a native provisional ECM. To our knowledge, this is the first *in vitro* study to evaluate the biocompatibility and biodegradation of this specific whole blood-derived scaffold formulation.

Fibroblasts derived from the buccal membrane, vaginal mucosa, and foreskin are widely used in regenerative medicine studies (16–18). Recently, our team established a novel human blood-derived scaffold (hBDS) for improving the clinical symptoms associated with pelvic organ prolapse. In parallel with our preliminary studies that showed improved mechanical and physical properties of this scaffold, the present study was designed to investigate *in vitro* biodegradation and biocompatibility of this blood-derived scaffold. However, in this preliminary study, we evaluated the interaction of mouse embryo fibroblast (MEF) with the scaffold for further clinical usage.

Mouse fibroblasts are frequently employed as convenient and well-established models for preliminary experimental

investigations due to their ready availability, ease of culture, and well-documented genetic background (19, 20). These cells provide a robust platform for researchers to explore fundamental biological processes, including cellular behavior, responses to various treatments, and interactions with other cells or tissues. Utilizing mouse fibroblasts can serve as a proof of concept for broader applications, facilitating the translation of findings to human fibroblasts or other pertinent human cell types in subsequent studies. This type of fibroblast can differentiate into several cell types and has rapid proliferation capacity and is easy to access, therefore is an excellent tool for a wide range of experiments (19, 20).

Fibroblasts respond to various factors such as cytokines secreted by immune cells, mechanical stress from their surrounding environment, and the binding of their surface receptors to ligands that are present in the ECM. These interactions influence the processes of tissue repair and inflammation. Cytokine secretion by fibroblasts can modulate immune responses by recruiting immune cells to the site of injury (21, 22). Additionally, some of these cytokines act as growth factors, not only attracting and proliferating ECM-producing cells at the repair site but also stimulating protein synthesis and fibrosis during tissue regeneration and healing. Some of these factors are proinflammatory, while others are anti-inflammatory, and in the present study for the first time we evaluate the role of blood-derived scaffold on the *in vitro* secretion of inflammatory-related cytokines by fibroblast (23, 24).

Results

MTT assay results

The optical density (OD) ratios at 570 and 620 nm wavelengths were measured for both experimental and control groups at 24, 48, and 72 h. The experimental group showed OD ratios of 1.22 ± 0.36 , 0.93 ± 0.17 , and 0.84 ± 0.15 , while the control group ratios were 1 ± 0.11 , 1 ± 0.008 , and 1 ± 0.22 , respectively (Fig. 1). No significant differences were observed between groups or over time, indicating that the scaffold supports stable cell growth and is biocompatible.

Light microscopy observation

The scaffold exhibited an eosinophilic fibrillar structure with a smooth upper surface and irregular inner outline (Fig. 2A–D). The MEFs attached to the scaffold's periphery and penetrated its depth (Fig. 2E–G), with some areas showing multiple cell layers on the surface.

Scanning electron microscopy of recellularized hBDS with MEFs

Scanning electron microscopy (SEM) images (Fig. S1A–F) revealed a porous fibrillar scaffold structure. After 4 days of cell seeding, fibroblasts adhered to the scaffold via cytoplasmic processes, displaying star-shaped or spindle-shaped morphologies.

LSCM of recellularized hBDS with MEFs

2D and 3D laser confocal scanning microscopy images (Fig. S2) showed Hoechst-labeled MEFs localized on the scaffold's surface and depth, with a cell density of 1 cell per $10^3 \mu\text{m}^2$ ($\approx 1,000$ cells/ mm^2).

Cytokine analysis results

The levels of interleukin-1 (IL-1), interleukin-6 (IL-6), transforming growth factor β (TGF β), and tumor necrosis factor-alpha (TNF α) in

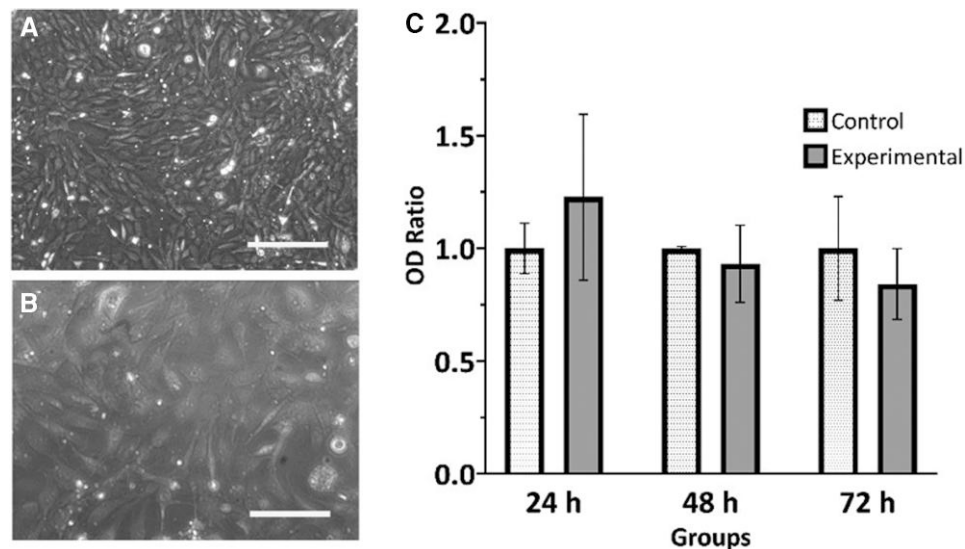


Fig. 1. The morphology of MEFs under an inverted microscope at both low (A) and high (B) magnifications. C) The results of the MTT assay conducted after 24–72 h of cultivation on the blood-derived scaffold. Statistical analysis indicated no significant differences between groups or across different time points. Scale bars: A; 50 μ m, and B; 20 μ m. The OD ratio is defined as the OD of the sample divided by the OD of the reference control for normalization.

supernatants from cells seeded on scaffolds were 7.91 pg/mL, 5 pg/mL, 126.55 ng/L, and 6.62 pg/mL, respectively. In the control group, the corresponding cytokine levels were 6.99 pg/mL, 1.96 pg/mL, 82.96 ng/L, and 7.30 pg/mL. The concentrations of IL-6 and TGF β were significantly higher in the experimental group ($P < 0.05$), whereas the levels of IL-1 and TNF α did not differ significantly between the groups. The summary of these results is presented in Fig. 3.

Biodegradation test results

The enzyme-treated scaffold fragments degraded completely by 48 h, while control group scaffolds remained intact up to 72 h (Fig. 4).

Discussion

In tissue engineering, one of the most important characteristics of scaffolds is their biocompatibility and biodegradability (1, 4). In the present study, our observations showed that the blood-derived scaffold used possessed these two properties. Our morphological observations using light microscopy (LM) and SEM demonstrated that the seeded fibroblasts attached to the scaffold with their cytoplasmic projections spread across its surface. Several factors are important for in vitro cell adhesion, and homing within the scaffold. One crucial factor is the topography of the scaffold surface, which must be suitable for cell attachment. Also, the cell adhesion molecules should be preserved during scaffold design and preparation (1, 4, 21). The scaffold used in the present study, derived from the patient's blood, contains various adhesion proteins, including fibronectin, which can act as a key molecule in cell attachment (9). Fibronectin interacts with its cell surface receptors such as integrins and forms focal adhesion complexes that stabilize cell attachment (25, 26).

In addition, our in vivo transplantation study (27), in which this scaffold was implanted into murine subdermal and skeletal muscle tissues, confirmed active interactions between host fibroblasts and the transplanted scaffold. Migratory fibroblasts

initially formed a peripheral boundary layer around the scaffold and subsequently infiltrated and penetrated the scaffold matrix.

Moreover, the bioscaffold should have suitable porosity and space to support the movement, and proliferation of the seeded cells. The micrographs showed some fibroblasts were localized within the thickness of the scaffold, and laser scanning confocal microscopy (LSCM) observations revealed that they penetrated and distributed homogeneously within it. This observation confirmed that the applied scaffold provides enough space and routes for cell homing and division. Our ultrastructural observations demonstrated that the scaffold possesses a good fibrillar network structure with suitable porosity that facilitated cell movement and homing. In this regard, Skala et al. (16), evaluated the fibroblast cell attachment to several types of mesh with different sizes and they showed that pore size is very important for cell attachment.

In addition, it suggested that the presence of growth factors or cytokines released by platelets or other types of blood cells trapped in the scaffold could direct fibroblast movement and migration within the scaffold such as platelet-derived growth factors (9, 28). In agreement with our results and suggestion Gorlero et al., applied the PRF matrix for the treatment of pelvic floor prolapse and the results showed prolapse repair with good functional outcomes. They concluded these results are associated with the component and mechanical properties of PRF matrix (29). We acknowledge that this study does not include a direct quantitative compositional comparison with PRP or PRF formulations. Rather, the present work was designed as a proof-of-concept evaluation of scaffold biocompatibility, cellular interaction, and biodegradation. Future studies will focus on systematic compositional and structural comparisons between whole blood-derived scaffolds and PRP-based matrices, including platelet concentration, fibrin architecture, and growth factor retention.

To further support this suggestion, another part of our study investigated the effects of the blood-derived scaffold and the potential growth factors—released from blood cells and retained within the scaffold—on the secretion of key inflammatory cytokines by cultured fibroblasts. Samples were collected at peak secretion times for each cytokine. The results demonstrated that fibroblasts cultured in the presence of the scaffold secreted significantly

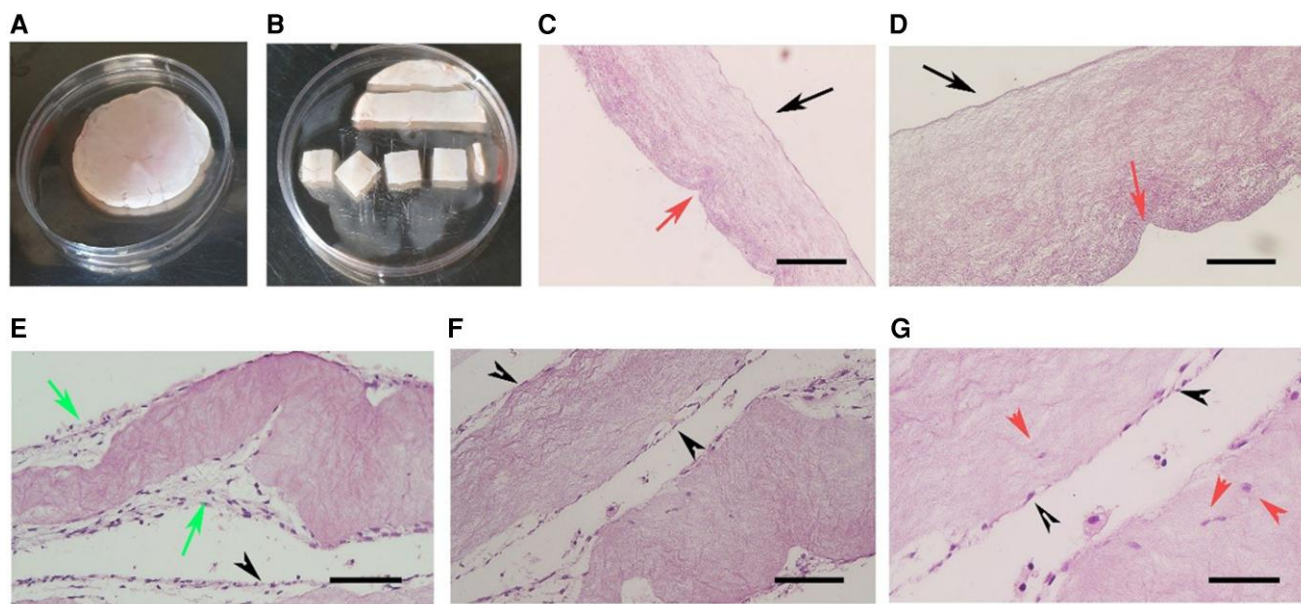


Fig. 2. The gross morphology of the scaffold after preparation (A) and subsequent dissection (B), followed by LM observations of the scaffold postpreparation stained with hematoxylin and eosin, depicted at low (C) and high (D) magnifications. Additionally, it explores the morphology of the cell-seeded scaffold after 4 days of in vitro culture. Fibroblasts at the periphery and depth of the scaffold are indicated by black and red arrows, respectively, while a multilayer of fibroblasts on the scaffold surface is denoted by the green arrow. Scale bars: C, E, F; 100 μ m, and D, G; 50 μ m.

higher levels of IL-6 and TGF- β . IL-6, a proinflammatory cytokine, plays a critical role in promoting angiogenesis, fibrosis, and the clearance of dead cells. It also stimulates the secretion of additional cytokines and growth factors, while facilitating repair and granulation tissue formation by suppressing TNF- α activity and reducing inflammation. Here, TGF- β , as a multifunctional growth factor, enhances fibroblast and keratinocyte proliferation and migration to the site of injury. Moreover, it promotes the synthesis of structural ECM proteins such as fibronectin and collagen, thereby accelerating tissue remodeling and repair (22, 23). The observed elevation of IL-6 and TGF- β is consistent with scaffold-induced fibroblast activation rather than a nonspecific inflammatory response. Interaction between fibroblasts and the fibrillar scaffold matrix likely triggers mechanotransductive and integrin-mediated signaling pathways, promoting cytokine secretion associated with early tissue repair. Here, IL-6 plays a dual role by supporting angiogenesis and granulation tissue formation while modulating excessive inflammation, whereas TGF- β is a central regulator of fibroblast proliferation, migration, and ECM deposition. Together, these cytokines suggest activation of regenerative rather than pathological pathways.

In contrast, no significant increase was observed in the levels of TNF- α and IL-1 in fibroblasts cultured in the presence of the scaffold compared to the control group. Nonetheless, even baseline levels of these cytokines can contribute to the healing process. Both TNF- α and IL-1 stimulate the proliferation and migration of fibroblasts and keratinocytes to the repair site. Additionally, IL-1 enhances collagen synthesis, playing a key role in accelerating tissue regeneration. However, excessive production of these cytokines during the healing process can disrupt the balance between pro- and anti-inflammatory factors, potentially leading to delayed or impaired tissue repair (22, 23).

In some parts of the scaffold surfaces the fibroblasts were observed as multilayer structures, which is promising for the formation of developing tissue or organ structures; however, further study is needed. From another point of view, we used MEFs due to their role in synthesizing ECM, wound healing, and

differentiation into several cell types. Therefore, the construction created by loading fibroblasts with various sources onto a blood-derived scaffold could be a viable option to deliver functional cells to support ECM biosynthesis, especially in diseases caused by the destruction of connective tissue integrity. For example, the main pathophysiology of pelvic floor disorders, which affects the mechanical properties of pelvic tissues, involves disturbances in fibroblast function and impairments in collagen biosynthesis and metabolism (30–32). Although MEFs provide a robust and reproducible model for preliminary evaluation of scaffold biocompatibility and cellular interaction, we acknowledge that they do not fully recapitulate human physiological responses. Accordingly, future studies will investigate scaffold interactions with human fibroblasts and other clinically relevant human cell types to further validate translational applicability.

In a similar study, Medel et al. evaluated the healing potential of human vaginal fibroblasts loaded onto two types of vaginal meshes—absorbable and nonabsorbable—that were coated with PRP. After in vitro culture, they observed attachment to implant materials when treated with PRP (14). However, the in vitro studies confirmed the similar potential of vaginal fibroblasts for cell attachment into the scaffold (16–18, 33). In another study by Mangera et al. (17), the isolated autologous fibroblast from the buccal membrane and foreskin were seeded on several synthetic meshes for the repair of urinary incontinence and pelvic organ prolapse. Roman et al. (18), compared the properties of oral fibroblast and adipose-derived stem cells for attachment to the scaffold and ECM production and showed both cells are suitable candidates for the treatment of pelvic organ prolapse. These reports encourage the clinical usage of autologous fibroblast-loaded constructs in urogynecological surgeries, such as pelvic floor disorders (14, 16, 18). Collectively, our current findings and prior in vivo study (27) demonstrate the scaffold's capacity to promote native fibroblast attachment after implantation.

However, other critical properties of the bioscaffold include the nontoxicity of both its composition and its byproducts during scaffold degradation. Our results confirmed that this scaffold

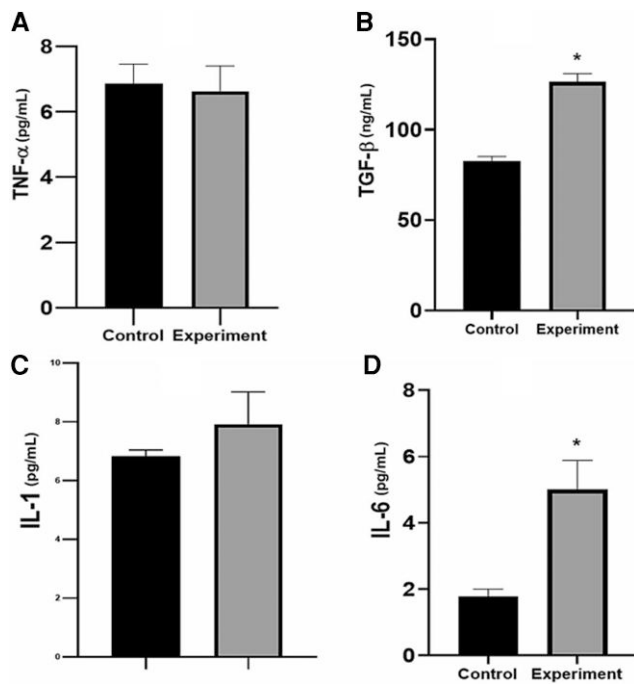


Fig. 3. The comparison of inflammatory cytokine secretion by cultured fibroblast in the presence (experimental) and absence (control) of blood-derived scaffold. *Showed significant difference with control group ($P < 0.05$).

degraded very quickly in the presence of an enzyme, and none of the released products were toxic to cell survival during 72 h of cultivation, as confirmed by the MTT assay. These results were predictable, given that the scaffold was natural and derived from blood, which is one of its significant advantages.

In conclusion, our results confirmed the *in vitro* biocompatibility and biodegradation of a blood-derived scaffold in terms of cell survival, adhesion, and penetration, and these experiments provide insights for using this autologous scaffold in clinical applications.

Materials and methods

Research design

All materials and reagents were provided by Sigma Aldrich (London, UK) except where mentioned in the text. This study assessed cell survival, proliferation, attachment, and penetration into a hBDS. Scaffolds were divided into segments and seeded with MEFs. Cell survival and proliferation were evaluated using an MTT assay, while morphological studies were conducted using LM with hematoxylin and eosin (H&E) staining, SEM, and LCSM. The levels of IL-1, IL-6, TGFβ, and TNFα in supernatants collected at multiple time points from cells seeded on scaffolds and without scaffolds were analyzed using enzyme-linked immunosorbent assay (ELISA). Additionally, an *in vitro* biodegradation test was performed both in the presence and absence of enzymes.

Scaffold preparation

Blood-derived scaffolds were prepared following Smartfem Medical Technology Pty Ltd's protocol (WO 2023/028651 A1). Blood samples (16 mL) were mixed with coagulant activator and centrifuged. Scaffolds were washed with phosphate-buffered saline (PBS), dissected, and used for studies.

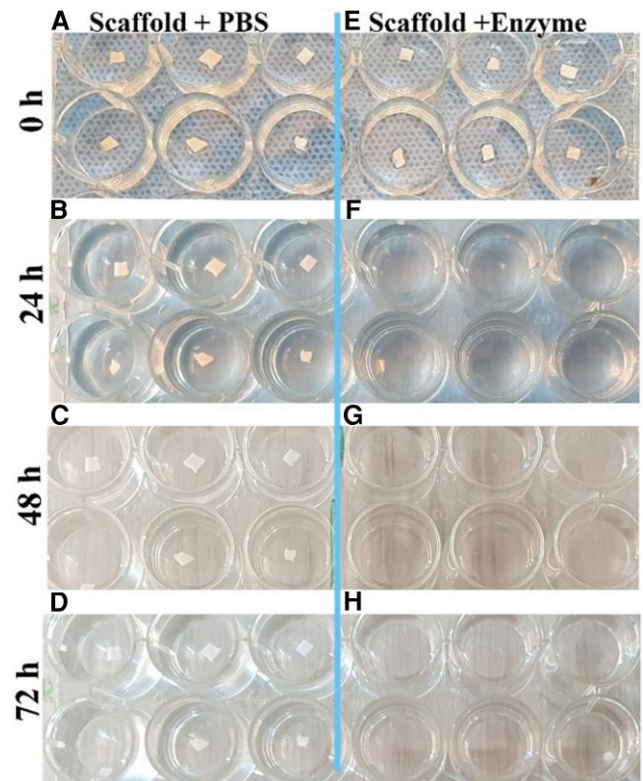


Fig. 4. The biodegradation test of the hBDS is presented with representative figures of samples from both the nontreated or control group (A–D) and the experimental or enzyme-treated group (E–H). These figures show the progression of degradation at different time points: beginning, 24, 48, and 72 h of incubation, arranged sequentially from the first to the fourth row. Here, PBS served as the control medium.

MEF preparation

MEFs were isolated from day 13 mouse embryos and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), penicillin, and streptomycin. Cells were cultured to confluency, trypsinized, and used for seeding.

MTT assay

Cell proliferation and survival were assessed with the MTT assay. MEFs were seeded in 96-well plates with scaffold fragments for 24, 48, and 72 h. OD values at 570 and 620 nm were measured. Control wells without scaffolds served as controls. Experiments were repeated three times.

Cell seeding and morphological studies

Scaffolds seeded with MEFs (10^6 cells/well) were cultured for 4 days in DMEM. Cell attachment and penetration were analyzed using LM, SEM, and LCSM. The LM involved H&E staining of scaffold sections, while SEM revealed scaffold porosity and cell morphology. The LCSM visualized cell penetration, and cell distribution was determined using Hoechst-stained MEFs.

Biodegradation tests

Scaffold fragments were divided into control (PBS) and experimental groups (trypsin/EDTA solution). Samples were incubated at 37 °C and observed at 24, 48, and 72 h.

Statistical analysis

Statistical differences in MTT assay data were analyzed using *t* tests and Mann–Whitney tests, with significance set at $P < 0.05$. Results were expressed as mean $\pm$ SD.

Cytokine detection

To investigate the effect of blood-derived scaffolds on the secretion of inflammatory-related cytokines in MEFs, cell-free supernatant samples were collected at multiple time points after in vitro culture of MEFs on the scaffolds (experimental groups). Supernatants from MEFs cultured without scaffolds served as controls and were analyzed using ELISA. TNF α was detected in the media after 12 h of culture, following the instructions of the ZellBio GmbH (Germany) kit. For the detection of IL-1, IL-6, and TGF β , samples were collected at 23, 48, and 72 h, respectively, and cytokine levels were measured using ELISA kits from R&D systems (USA), according to the manufacturer's instructions.

Scaffold preparation

Informed consent was obtained from healthy men ($n = 2$) and women ($n = 2$) under the guidelines of the Ethics Committee of the Medical Faculty of Tarbiat Modares University (ethics reference code: IR.MODARES.AEC.1402.012). The blood-derived scaffold was prepared according to the protocol of Smartfem Medical Technology Pty Ltd patent (International Publication Number: WO 2023/028651 Al) (34). Briefly, whole blood was processed to generate a scaffold, which was subsequently washed, sectioned, and used for in vitro experiments.

MEFs preparation

The MEFs were prepared and cultured based on a previous method (32). Female pregnant Naval Medical Research Institute mice were purchased and housed at the university animal facility under controlled conditions: a 12-h light/dark cycle at 22 ± 2 °C and 40–50% humidity. On the 13th day of pregnancy, embryos were extracted, and their heads and internal organs were removed. The remaining tissues were shredded into small pieces with scissors and treated with 2 mg/mL collagenase type I in PBS for 60 min. After washing with media and centrifuging, the isolated cells were cultured in DMEM (Gibco, Thermo Fisher Scientific) containing 10% FBS (Gibco, UK), 100 IU/mL penicillin, and 75 μ g/mL streptomycin. The cells were cultured for 48 h at 37 °C with 5% CO₂ until reaching confluency. The cultured cells were then trypsinized and used for cell seeding and the MTT assay.

Cell proliferation test by MTT assay

The cell proliferation and survival in the presence of hBDS fragments (1 mm² in size) were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MEFs (2,000 cells/mL) were seeded into 96-well plates then one fragment of hBDS was put on each well and left them for 24, 48, and 72 h in 5% CO₂ at 37 °C. At each interval, the scaffolds and medium were removed, and the remaining cells in each well were treated with 100 μ L of MTT solution (1 mg/mL of MTT in PBS) and incubated for 4 h. The solution was then replaced with dimethyl sulfoxide in the dark for 20 min, after which their OD values were read at 570 and 620 nm using a microplate reader (Biochrom, Germany). The same culture conditions without exposure to the scaffold served as a control group. This experiment was repeated three times.

Cell seeding on the scaffold with MEFs

Scaffolds of 5 $\times$ 5 mm size were placed into the floor of each well of a 4-well plate, then MEFs (10⁶ cells per well) were added to the scaffold, and then they were cultured in DMEM medium containing 10% FBS, 100 IU/mL penicillin, and 75 μ g/mL streptomycin for 4 days in 5% CO₂ at 37 °C. The media were replaced with fresh ones every other day. After 4 days of cultivation, cell attachment and penetration within the scaffolds were studied using LM, SEM, and LCSM as follows.

LM study

After 4 days of in vitro culture of the MEFs on hBDS fragments ($n = 4$ from separate blood donors), the scaffolds were fixed in 10% formalin and dehydrated in ascending concentrations of ethanol (70, 90, and 100%). Following clearing in xylene, the scaffolds were impregnated and embedded in paraffin wax, then sectioned at a thickness of 5 microns. The tissue sections were deparaffinized, hydrated, and stained with hematoxylin and eosin solution. Finally, the stained scaffold sections were dehydrated, cleared, and mounted with resin on glass slides for visualization under the LM, and images were captured. Additionally, some scaffold fragments without any cell seeding were prepared, fixed, sectioned, and stained as described above for the LM study.

SEM study

Several fragments of hBDS from three individual blood donors ($n = 3$) and some of the cell-seeded scaffolds after 4 days of cultivation ($n = 3$) were collected and fixed in 2.5% glutaraldehyde. They were then dehydrated in ascending concentrations of ethanol (50, 60, 70, 80, 90, 96, and 100%) for SEM study. The dehydration process was completed using hexamethyldisilazane overnight. The samples were coated with a thin layer of gold and observed under a scanning electron microscope (VEGA/TESCAN-XMU, Czech Republic), and their micrographs at different magnifications were captured.

Laser confocal scanning microscope study

We used a laser confocal scanning microscope (Zeiss LSM800, Japan) to study the penetration of cells within different thicknesses of the scaffold. Before this part of the study, the MEFs were labeled by incubation in a solution of Hoechst 34580 (0.002% dye in DMEM/Ham's F-12) for 1 h and then washed with media. Another set of cell seeding was performed using these labeled cells as described previously. On day 4 of in vitro culture, the scaffold fragments were examined under LCSM, and the labeled MEFs were detected using the blue channel (400–550 nm wavelength). 2D and 3D micrographs were captured from various parts of each sample, and the proportion of penetrated MEFs in these scaffolds was calculated.

In vitro biodegradation tests

After the preparation of hBDS ($n = 2$), the scaffolds were segmented into several parts (< 24). The hBDS fragments were then dehydrated using ascending grades of ethanol (50, 70, and 100%), dried overnight, and divided into experimental and control groups ($n = 12$ fragments in each group). The samples in the control group were placed in PBS, while the scaffolds in the experimental group were placed in a solution containing 0.025% trypsin/EDTA (Invitrogen, England) in PBS. All samples in both groups were incubated at 37 °C. After 24, 48, and 72 h, the samples were observed, and the presence of scaffolds was evaluated.

Statistical analysis

The statistical analysis for the MTT assay and cytokine analyses were carried out using parametric (t test) and nonparametric (Mann–Whitney) tests to determine the significant differences between control and experimental groups at different time points (24, 48, and 72 h). The hypothesis being tested with the P-value in this study is whether the blood-derived scaffold (hBDS) has a significant impact on MEF cell survival and proliferation over time, specifically at 24-, 48-, and 72-h postseeding. Statistical significance was indicated by $P < 0.05$, and the results were presented as mean $\pm$ SD.

Further materials and methods

See the supplementary “[Supporting information](#).”

Acknowledgments

The authors gratefully acknowledge the technical and infrastructure support provided by the Discipline of Biomedical Engineering, The University of Adelaide, and the animal research facility of Tarbiat Modares University. The authors also thank laboratory and technical staff for assistance with scaffold preparation, cell culture, microscopy, and histological processing.

Supplementary Material

Supplementary material is available at [PNAS Nexus](#) online.

Funding

Support from Smartfem Pty Ltd. and Australian Research Council DP240103404 and FL240100217 is gratefully acknowledged.

Author Contributions

Fariba Behnia-Willison (Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing—review & editing), Pouria Aryan (Data curation, Formal analysis, Writing—original draft, Writing—review & editing), Mojdeh Salehnia (Investigation, Supervision, Writing—original draft, Writing—review & editing), Nadia Willison (Formal analysis, Writing—original draft), Tran Nguyen (Supervision, Writing—review & editing), Nelson Tansu (Supervision, Writing—review & editing), and Derek Abbott (Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—review & editing)

Data Availability

All data supporting this study are either included in this report or in the [Supplementary material](#).

Declaration of Generative AI and AI-assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Ethics Approval

The Ethics Committee of the Medical Faculty of Tarbiat Modares University approved all procedures of this study (ethics reference code: IR.MODARES.AEC.1402.012).

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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