



Research Article

Biomaterial Characterization of Decellularized Human Amniotic Membrane Seeded with Fetal Human Cardiac Fibroblasts for Cardiac Tissue Engineering

Winda Azwani¹, Dini Aulia Cahya², Achmad Danny Gazali³, Amira Puti Karima¹, Galuh Oktavya⁴, Rahma Nur Istiqomah⁵, Arleni Bustami^{6,7}, Faiza Aisya Rizqy¹, Puspita Anggraini Katili⁸, Ujang Purnama⁹, Mulyadi M Djer^{10,*}

¹Division of Pediatric Cardiology, RSAB Harapan Kita National Women and Children's Health Center, Jakarta, 11420, Indonesia

²Faculty of Medicine, Universitas Negeri Surabaya, Surabaya, 60213, Indonesia

³Cell, Tissue Culture, and Bio-imaging Division, Integrated Laboratory, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, Indonesia

⁴Biobank Research Core Facilities, Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia

⁵Clinical Research Unit, RSAB Harapan Kita National Women and Children's Health Center, Jakarta, 11420, Indonesia

⁶Cell, Tissue Culture, and Bio-imaging Division, Integrated Laboratory, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, Indonesia

⁷Master's Programme in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, Indonesia

⁸Division of Biomedical Engineering, Department of Electrical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, 16424, Indonesia

⁹Healthcare Strategy Realization Office, Merck Healthcare, Darmstadt, 64293, Germany

¹⁰Division of Pediatric Cardiology, Department of Pediatric, Faculty of Medicine, Universitas Indonesia-Dr. Cipto Mangunkusumo Hospital, Jakarta, 10430, Indonesia

*Corresponding author: mdjer.mulyadi.md@gmail.com; Tel: +625668284; Fax: +62215601816

Abstract: The human amniotic membrane (hAM) is a promising biomaterial in cardiac tissue engineering known for excellent viability, anti-inflammatory properties, and ability to support cellular adhesion. Its potential as a biomaterial, particularly after decellularization, offers a novel approach for myocardial regeneration in conditions such as cardiomyopathy and heart failure. Therefore, this study aimed to characterize the ultrastructure of fetal human cardiac fibroblasts-decellularized hAM (fHCFs-dehAM) using scanning electron microscopy (SEM), identify the functional groups of dehAM through fourier transform infrared (FTIR) spectroscopy, assess vimentin expression in fHCFs-dehAM via immunocytochemistry, and evaluate fHCF cell proliferation to determine cell viability on dehAM. In the process, hAM was successfully decellularized using 0.2% (w/v) trypsin/0.25% (w/v) ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (PBS), confirmed by the removal of the native epithelial layer through hematoxylin-eosin (H&E) staining and ultrastructural analysis. The results showed that fHCFs adhered to the basement membrane of the dehAM and retained the phenotype, as evidenced by the expression of the

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intermediate filament marker vimentin via immunofluorescence staining. Furthermore, the viability of fibroblasts cultured on dehAM increased in a time-dependent manner, indicating enhanced proliferation. This is a novel study on the viability of hwith human cardiac resident cells. The result shows the potential of dehAM for biomaterial application in cardiac tissue engineering.

Keywords: Cardiac fibroblasts; Cardiac tissue engineering; Cell viability; Decellularization; Human amniotic membrane

1. Introduction

In pediatric patients, heart failure is predominantly caused by congenital heart disease and cardiomyopathy, both of which contribute to detrimental myocardial remodeling characterized by fibrosis (Gordon et al., 2022; Hsu, 2005). Cardiomyopathy is a major indication for heart transplantation, accounting for 35% of cases (Zhang et al., 2013). The chronic maladaptive process leading to left ventricular remodeling and heart failure in children often necessitates the use of ventricular assist devices as bridging therapy before heart transplants, with limited donor availability (Khosravimelal et al., 2020). This pathological remodeling requires innovative therapeutic strategies to restore myocardial function (González et al., 2011). A potential approach includes the use of biomaterials resembling the extracellular matrix (ECM) to create a supportive microenvironment for cardiomyocytes, cardiac fibroblasts, and epithelial cells (Pattar et al., 2019; Gattazzo et al., 2015; Whulanza et al., 2011). These myocardium resident cells play an important role in tissue remodeling and repair (Wang et al., 2025). However, optimizing biomaterials with good viability for cellular integration remains a challenge (Syuhada et al., 2018).

The human amniotic membrane (hAM) is a highly valued, naturally derived ECM known for remarkable biological and regenerative capabilities, making it a popular scaffold in tissue engineering and regenerative medicine (Svystonyuk et al., 2020). Its richness in multipotent stem cells, bioactive molecules, growth factors, and pro-regenerative cytokines promotes re-epithelialization and wound healing across various organs, while enhancing cell growth, angiogenesis, and vascularization of hAM patches (Hu et al., 2023; Solarte David et al., 2022; Moravvej et al., 2021). Beyond the benefits, the ECM offers anti-inflammatory, anti-fibrotic, and anti-bacterial properties, alongside excellent viability, low antigenicity, and strong cellular and tissue adhesion. The structural composition includes elastin fibers, collagen, laminin, hyaluronic acid, and glycosaminoglycans, effectively mimicking the natural ECM (Arrizabalaga and Nollert, 2018). These characteristics make hAM a promising candidate for biomaterial development in cardiac tissue regeneration (Mamede et al., 2012). Decellularized hAM (dehAM), produced by removing the amniotic epithelial cell layer to expose the basement membrane, is widely used in studies and clinical applications. It promotes the proliferation, expansion, and differentiation of human bone marrow mesenchymal stem cells (hBM-MSCs) into adipogenic and osteogenic lineages (Salah et al., 2018). The application of dehAM for cardiac tissue engineering needs to be elucidated before advancing into clinical translational purposes.

The collagen-based hydrogels for tissue engineering have been used in previous studies. Cell viability, viscosity, and syringeability of human bone marrow mesenchymal stem cells (HBM-MSCs) are enhanced on collagen–alginate hydrogels, suitable for injectable applications in tissue engineering (Ketabat et al., 2017). The biocompatibility of HBM-MSCs has been shown on Collagen/Hydroxy Apatite hydrogel with highly interconnected porosity, signifying strong potential for bone tissue engineering (Chen et al., 2017). Previous studies have developed biomaterial by combining collagen and alginate with a combination of PVA for bone regeneration purposes (Fajarani et al., 2024). Furthermore, a composite of collagen and alginate integrated with propolis as a therapeutic agent showed cardioprotective potential, suggesting its applicability in cardiac tissue engineering (Pangesty et al., 2025). Another study reported the potential of cardio-gel and collagen I for cardiac tissue engineering. This hydrogel has been shown to enhance human foreskin fibroblasts (hFFs) cell viability and retention (Khodayari et al., 2024). However, no

published studies have investigated the therapeutic application of dehAM seeded with resident cardiac cells for cardiac tissue engineering.

Cardiac fibroblasts, essential for myocardial function and structure, form a complex 3D network with cardiomyocytes (Hall et al., 2021). Specifically, fetal human cardiac fibroblasts (fHCFs) are crucial for maintaining cardiac tissue homeostasis and repair (Garate-Carrillo and Ramirez, 2018). The study uniquely assessed the interaction between fHCFs and dehAM, crucial for supporting cardiac tissue. It also characterized fHCFs-dehAM ultrastructure through SEM, identified dehAM functional groups using FTIR, assessed fHCF vimentin expression by immunocytochemistry, and evaluated fHCF proliferation on dehAM to determine viability.

2. Methods

2.1. Ethical Clearance

The hAM was procured from healthy pregnant women who were subjected to elective Caesarean surgery and tested negative for Hepatitis B, Hepatitis C, HIV, and COVID-19 (Moravvej et al., 2021). Informed consent was obtained from each participant who agreed to donate amniotic membrane in accordance with ethical approval granted by the Institutional Review Board of RSAB Harapan Kita National Women and Children's Health Center with Approval No. IRB/4712/12/ETIK/2022.

2.2. Preparation of DehAM

The procedure for hAM decellularization was performed using an aseptic technique to maintain stability in a class II Biosafety Cabinet (BSC). Fresh hAM was washed three times with phosphate-buffered saline (PBS) and cut into small pieces of 5 cm x 5 cm. The samples were separated into two groups for optimization purposes. The first group was incubated in 0.2% (w/v) trypsin/0.25% (w/v) EDTA (Gibco Cat. 15576-028, Carlsbad, USA) at 37°C for 30 minutes, while the second was incubated in 0.025% (w/v) EDTA at 37°C for 1 hour (Khosravimelal et al., 2020; Zhang et al., 2013). Following incubation, the 0.2% (w/v) trypsin/0.25% (w/v) EDTA group was neutralized with 10% fetal bovine serum (FBS) (Sigma-Aldrich, Cat. F9665, Chile) containing Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, Cat. 12100046), and scraped using a cell scraper to separate residual cells from the membranes. The morphology of the resulting dehAM was characterized by hematoxylin-eosin (H&E) staining. Finally, the remaining membrane sample was stored in 9:1(v/v) FBS: DMSO cryo medium at -80°C.

2.3. Culture of Fetal Human Cardiac Fibroblasts (fHCFs) Cells

The fHCFs were purchased from Cell Application Inc. (Cat. 306-05f, San Diego, USA) and cultured according to the manufacturer's recommendation with slight modification. The cells were maintained at density of more than 1×10^4 /cm² in cardiac fibroblast growth medium (CFGF, Cell Application Inc, San Diego, US) with additional supplementation of 15% pre-heated FBS and 1% (v/v) antibiotic-antimycotic (AbAm) cocktail (contains streptomycin, penicillin, and amphotericin) (Sigma-Aldrich, Cat. A5955). At a confluency of 70–80%, cells were passaged to proceed into a further experiment or cryo-preserved in 9:1(v/v) FBS–dimethyl sulfoxide (DMSO) (34943-1L, Sigma Aldrich) and stored in a liquid nitrogen tank.

2.4. Seeding fHCFs on DehAM

The dehAM was washed three times in PBS and excised into the size of an 8-well chamber (Thermo Fisher, USA), approximately 10.9 x 8.9 mm, ensuring complete coverage. The fibroblasts (8×10^4 cells/well) were seeded onto an 8-well chamber in CFGF-15%FBS as the control group and onto dehAM forming fHCFs-dehAM. Finally, the cultures were incubated at 37°C, with 5% CO₂ for 4 ± 2.1 days targeting 70–80% confluency, and were refreshed every three days.

2.5. Characterization Studies

2.5.1. Scanning Electron Microscopy (SEM)

Biomaterial of fHCFs-dehAM was fixed in 2.5% (v/v) glutaraldehyde prepared in deionized water for 1 hour at room temperature. After fixation, samples were rinsed three times in PBS before

being subjected to sequential dehydration in a graded ethanol series of 40%, 50%, 70%, 90%, and 2 × 100% ethanol (v/v) in deionized water, with each step lasting 10 minutes. Scaffolds were left inside the fume cupboard for 1 hour to complete drying, then stored in a desiccator until use. A Palladium-coated scaffold was assessed by scanning electron microscopy (SEM Evo LS15 Variable Pressure Scanning Electron Microscope, Jena, Germany). Secondary electrons were used for imaging purposes at an operating voltage of 5 keV, while compositional analyses were conducted at 15 keV (Careta et al., 2021; Savić et al., 2021).

2.5.2. Fourier Transform InfraRed (FTIR)

The chemical structures or functional groups of hAM were characterized using FTIR spectroscopy. The concept works on radiation interference between two rays to produce an interferogram. Infrared spectroscopy rays produce atomic vibrations of a molecule, while the spectra were obtained by passing radiation through a sample and determining the fraction of radiation absorbed at a given energy. At each peak in the absorption spectrum, the energy appeared according to the vibration frequency of a part of the sample molecule (Stuart 2005). The FTIR spectra of the hydrogels were recorded using a Thermo Scientific Nicolet iN 10 FTIR Microscope (Thermo Scientific Nicolet iS50, USA). The spectra were recorded from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and 128 scans per sample.

2.5.3. Immunocytochemistry

The fHCFs-dehAM was fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS for 10 minutes. The nonspecific target was blocked with 1% (w/v) bovine serum albumin (Sigma-Aldrich, Cat. A9647, Saint Louis, USA) in PBS for 1 hour. The constructs were then incubated with 10 $\mu\text{g}/\text{mL}$ mouse anti-human vimentin primary antibody for 1 hour (MA5-11883, Thermo Fisher, USA) and 2 $\mu\text{g}/\text{mL}$ goat anti-mouse IgG (H+L) secondary antibody for 45 minutes in a dark setup (A21050, Thermo Fisher, USA). Nuclear counterstaining was performed with 10 $\mu\text{g}/\text{mL}$ (v/v) DAPI (Sigma-Aldrich, Cat. D9542, Jerusalem, Israel) for 10 minutes. The procedure was conducted at room temperature with two PBS washes between each step (Svystonyuk et al., 2020). The experiment was based on three independent biological replicates, and images were captured using a confocal laser scanning microscope (CLSM 700, Jena, Germany) with Z-stack acquisition through Zen 2010 software. Excitation was performed at 405 nm for DAPI and 639 nm for Alexa Fluor 633 (AF633), with emission collected in Long Pass mode. Data analysis for 2D reconstruction and vimentin-positive fHCFs was performed on ImageJ version 1.54g (Wayne Rasband and contributors National Institutes of Health, USA) software using the Fiji (Wayne Rasband and contributors National Institutes of Health, USA) plugin and Cell Counter module, respectively.

2.5.4. MTS Assay

This study further assessed the viability of fHCF cells on the dehAM matrix. A series of cells was grown independently in a flat-bottomed 96-well plate at a density of 1.9×10^4 cells/well in cardiac fibroblast growth medium (CFGF, Cell Application Inc, Cat. 316-500, San Diego, USA) following culture for 1, 3, and 7 days at 37°C with 5% CO_2 . Cell control was maintained without dehAM, while the negative control was to keep only CFGF. After incubation, 20 μL of MTS mix solution (CellTiter 96, Promega, Cat. G5421, Madison, USA) was added to each well. Plates were incubated for 2.5 hours in dark conditions at 37°C with 5% CO_2 . Finally, live cell absorbance was measured at 490 nm using a microplate reader (Multiskan GO, Thermo Fisher, Ratastie, Finland).

2.5.5. Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 10.4.1 for MacOS, GraphPad Software, San Diego, SA, USA. The Shapiro-Wilk test was adopted to determine data distribution, and two variable data with normal distribution was tested by unpaired Student's T-test, or Mann-Whitney U test. Meanwhile, multiple comparisons from more than two variables were analyzed by two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Data were presented as a standard error of the mean. Statistically significant value denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$,

and **** $p < 0.0001$, implied significant, very significant, highly significant, and extremely significant results, respectively.

3. Results and Discussion

3.1. Decellularization hAM

The placentas were laid on a sterile surgical tray and thoroughly washed with 0.9% NaCl saline until all blood clots were rinsed down and the membrane showed a light pink color. The amniotic membrane layer was separated manually from the chorionic membrane, as presented in Figure 1A. Subsequently, it was washed three times with a PBS (MP Biomedicals, Germany) containing 10% antibiotic-antimycotic cocktail (Streptomycin 50 $\mu\text{g/mL}$, Penicillin 50 $\mu\text{g/mL}$, and Amphotericin 2.5 $\mu\text{g/mL}$) and rinsed three times with sterile PBS, respectively, as shown in Figure 1B. The amniotic membrane is divided into squares of approximately 10 x 10 cm^2 , as detailed in Figure 1C. Finally, it was transferred to the laboratory for further proceedings using PBS-contained transport media.



Figure 1 Laboratory workflow on hAM processing from a Caesarean section patient. (A) The separation between the amniotic membrane and chorionic membrane procedure was manually operated in the operating ward. (B) Amniotic membrane pre-treatment with 10% (v/v) AbAm cocktail and rinsed with PBS in a biosafety cabinet, adopting aseptic techniques. (C) Procedure of amniotic membrane decellularization with 0.2% (w/v) trypsin/0.25% (w/v) EDTA incorporated with cell scraping. AM, amniotic membrane; CM, chorionic membrane.

3.2. Evaluation of Decellularization and fHCFs Seeding on dehAM

fHCFs were cultured on 6-well plates using a growth medium formulated according to the Sigma-Aldrich (Merck) protocol, supplemented with 15% modified FBS. Cell morphology and growth were examined using an inverted phase microscope. The subculture process was ready to be used for scaffold testing at the third to eight cell passages.

To determine the cell attachment of fHCFs cultured on dehAM, several tests, including HE staining, SEM, and immunocytochemistry were performed. HE staining confirmed the successful adhesion of fHCFs to the surface of the dehAM, featuring spindle-shaped cells with elongated cytoplasm uniformly distributed across the scaffold, as detailed in Figure 2A-C. The decellularization process effectively removed epithelial cells from the peripheral basement membrane, as evident when comparing native hAM and dehAM.

Decellularization of the epithelial layer of hAM aimed to suppress immunomodulation, membrane-associated antigens, and soluble proteins, thereby preventing the initiation of a cell-mediated or humoral immune response (Badyalak et al., 2011; Crapo et al., 2011; Wilshaw et al., 2008). Techniques such as enzymatic, chemical, and physical methods significantly impacted the structural

integrity and bioactivity of the scaffold (Sarvari et al., 2022). A study showed that hAM decellularization affected only the epithelial layer, and no observable difference was detected in the ultrastructural characteristics of the compact basement membrane of dehAM. Furthermore, bundles of ECM proteins and scattered elastic fibers remained unaffected (Salah et al., 2018). This study showed successful decellularization of hAM with 0.2% (w/v) trypsin and 0.25% (w/v) EDTA.

3.3. Scanning Electron Microscopy (SEM)

An ultrastructure test of the fHCFs-dehAM was conducted to further confirm the topology and integrity of the basement membrane and allogeneic fibroblast reintroduction attachment using SEM analysis. The mosaic of native dendritic-shaped stromal cells was visible in fresh hAM (Li et al., 2008) with no lesion on the basement membrane. Spindle-shaped morphology on the surface of the basement membrane of dehAM signified fibroblast characteristics of fHCFs, as shown in Figure 3A-C. This result reflected the viability of fHCFs in dehAM, but there was no evaluation of the remaining basement membrane and the ECM components. Further study should be conducted focusing on the characterization of dehAM, predominantly the structural properties, and whether the decellularization process impaired mechanical characteristics.

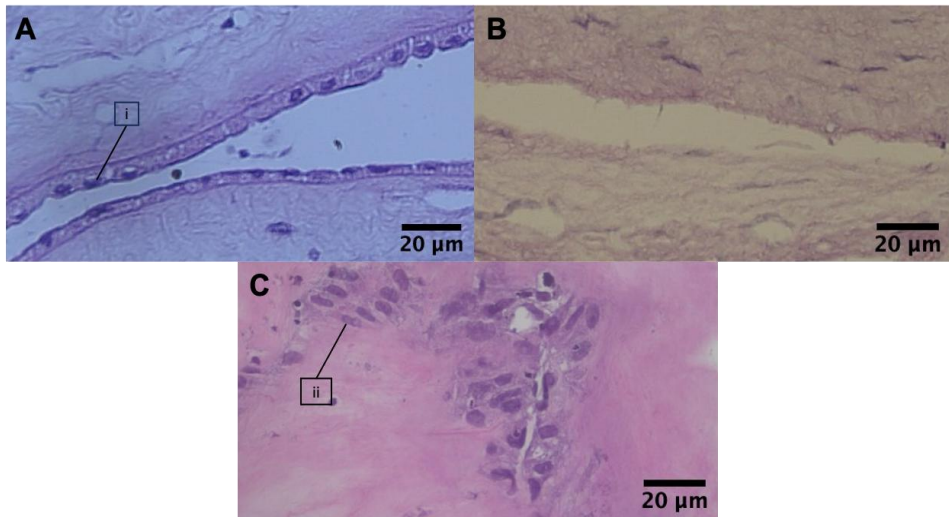


Figure 2 The biocompatibility of the dehAM biomaterial was characterized by fHCFs attachment to the basement membrane. (A) The control group showed the presence of epithelial cells on native hAM (B) and dehAM, with no visible epithelial cells with an intact basement membrane, reflecting a successful decellularization. (C) fHCFs seeded on dehAM biomaterial showed cell attachment with fibroblast characteristics on the surface of the basement membrane. (i) epithelial cells, (ii) fHCFs cells. Magnification, scale bar: x40, 20μm

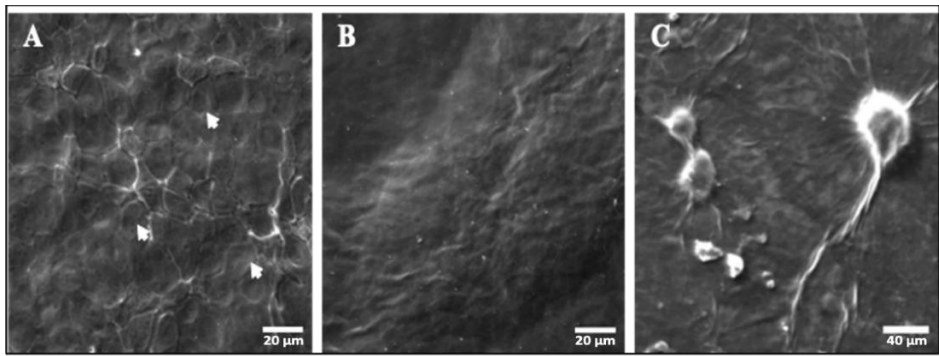


Figure 3 Morphology of dehAM as a biomaterial for fHCFs adherence using SEM analysis. (A) Fresh hAM showed a mosaic arrangement of native epithelial cells (arrowhead); meanwhile, (B) trypsin-treated dehAM successfully denuded the epithelial layer with a compact structure of the basement

membrane of dehAM. Magnification 100 $\times$; scale bar 20 μ m. (C) fHCFs cultured on dehAM biomaterial exhibited spindle-shaped cells, indicating fibroblast adherence on the basement membrane. Magnification 500 $\times$; scale bar 40 μ m

3.4. FTIR Molecular Characterization

The FTIR transmittance spectrum of pretreated, native hAM showed the characteristic bands at 3298 cm^{-1} , 1631 cm^{-1} (Skopinska-Wisniewska et al., 2023), and 1079 cm^{-1} (Khalili et al., 2025). The peaks at approximately 3300–3500 cm^{-1} were attributed to N-H/O-H stretching, suggesting the presence of hydroxyl groups of amide (Ji et al., 2020). Those between 1600–1640 cm^{-1} were assigned to the group of amide I and were predominantly attributed to the C=O stretching. The peaks at approximately 1070–1080 cm^{-1} were attributed to PO_2^- stretching, signifying the presence of nucleic acids, phospholipids, and glycolipids (Khalili et al., 2025).

After the decellularization process, the FTIR transmittance spectrum of dehAM retained the characteristic amide A (3300 cm^{-1}) and amide I (1630 cm^{-1}) groups (Figure 4). The spectrum of pretreated, native hAM at 3306.18 cm^{-1} had no significant difference with dehAM at 3310 cm^{-1} (Sripriya and Kumar, 2016). Both amide A and I were assigned to collagen's hydrogen bonding and structural integrity (Skopinska-Wisniewska et al., 2023). The minimal shift in the peak position of amide A from 3298 cm^{-1} to 3300 cm^{-1} and amide I from 1631 cm^{-1} to 1630 cm^{-1} , suggested that the decellularization process preserved the collagen structure and maintained the extracellular matrix.

The absence of the phosphodiester group following decellularization suggested effective removal of cellular components. Based on the result, the decellularization method did not change the chemical properties of the amniotic membrane, which was decisive in designing the scaffold.

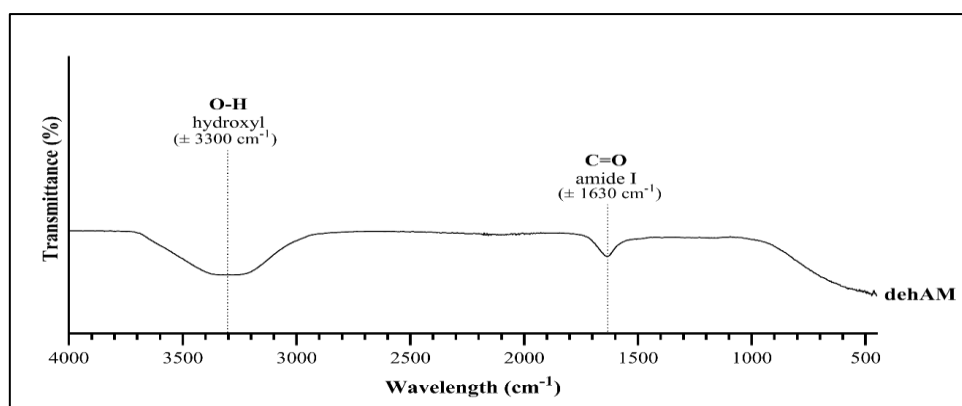


Figure 4 FTIR result showing the functional group present of O-H and C=O stretching

3.5. Immunocytochemistry

Immunostaining of cells and tissues is widely used as a standard method for the detection of specific targeted proteins, through antibody binding alongside fluorescence labels (Figure 5A). In this study, fHCFs in either group had a stereoscopically clear plasma membrane with a long spindle shape, juxtapositioned vimentin organization, and round to oval nuclei as previously described (Camelliti et al., 2005). Based on observation, vimentin-positive cells in the fHCFs-dehAM biomaterial complex group at $96.90 \pm 3.2\%$ had slightly higher numbers than the control at $94.38 \pm 6.0\%$ but not statistically significant, as presented in Figure 5B.

In this study, cells maintained vimentin expression on both the well plate and the dehAM matrix. The vimentin expression in fHCFs was approximately 80%, while fibroblasts cultured on dehAM showed an increased trend to approximately 90%, although not significant. The number of vimentin-positive cells showed no significant difference in either group (Figure 5B). A slightly higher proportion in fHCFs-dehAM, suggested that dehAM could support and maintain the intracellular matrix of the fibroblasts. Significant differences in vimentin expression were observed across the cytoplasm, with intensity measurements reflecting subtle variations between groups.

Fibroblast cells cultured on biomaterial could maintain proliferation activity due to collagen presence, which provides adhesive and tensile strength (Taghiabadi et al., 2015). Vimentin played an important role in cellular mechanical integrity and facilitating migration (Camelliti et al., 2005). Immunocytochemistry was performed to observe its expression as a filament marker of cardiac fibroblasts. Vimentin is also a filamentous protein expressed by mesenchymal cells (Chen and Frangogiannis, 2013). A study showed that fibroblasts seeded on dehAM maintained the morphology, with positive expression of vimentin and the proliferation marker Ki-67 over a four week cultured period (Wilshaw et al., 2008).

Studies have shown that dehAM retains bioactive components capable of enhancing fibroblast proliferation and viability, essential for effective cardiac tissue regeneration (Bahrami et al., 2023). The presence of collagen and laminin supports cell adhesion and survival, ensuring prolonged cellular activity on the scaffold (Hasmad et al., 2022). In vitro studies have shown that hAM extract maintained the original fibroblastic phenotype and reversed differentiated myofibroblasts into fibroblasts (Hu et al., 2023). Another investigation of Amnio-M affirmed anti-fibrotic effects by downregulating TGF- β 3 and its receptor, as well as suppressing TGF- β transcription and signalling (Elkhenany et al., 2022). The use of dehAM in regenerative medicine became more beneficial because there is no risk of tumorigenicity, decreased inflammation, lower infection, and reduced scar formation (Hu et al., 2023; Elkhenany et al., 2022).

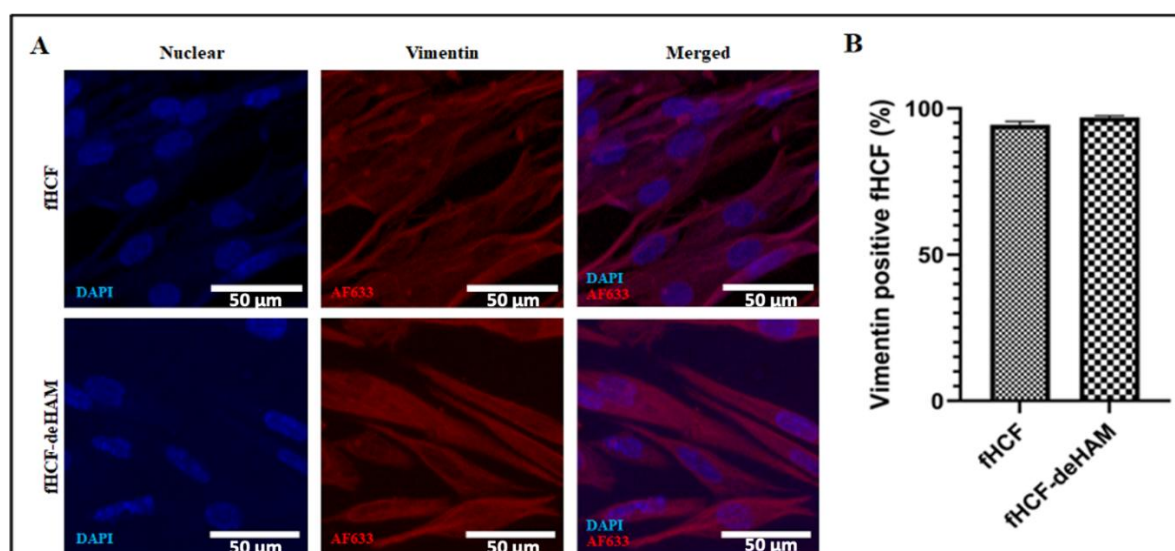


Figure 5 Expression of vimentin in the fHCFs-dehAM was slightly higher but not significantly different. (A) Immunocytochemistry observation using Zeiss confocal laser scanning microscopy (CLSM) 700; fHCFs seeded onto 8-well chamber plasticware as the control group (top panel), and fHCFs-dehAM (bottom panel). Indirect immunofluorescence staining was conducted using anti-vimentin (IgG1) (AF633, red, middle) and nuclear counterstaining (DAPI, blue, left). Magnification, scale bar: x20, 50 μ m. (B) Quantification of the percentage of vimentin-positive fHCFs between the two groups was compared with the Mann-Whitney U test.

The cytoskeleton structure of the vimentin was characterized by filamentous distribution expanded throughout the cytoplasm with an interconnected, curvilinear, and smooth structure. Studies on fibroblast cells from various ages and passage stages showed that vimentin expression, measured by intensity, increased with age and passage. Higher expression levels are particularly evident in cells from older donors and are associated with changes in stiffness and potential glaucoma link (Sliogeryte and Gavara, 2019; Nishio et al., 2001). Vimentin, through its physical properties and signalling pathways, accelerates cell migration and attachment to collagen by regulating the formation of cell extensions through connective tissues. Based on observation,

activated vimentin is important in ECM synthesis and remodelling for cellular mechano-protection (Ostrowska-Podhorodecka et al., 2022).

3.6. MTS Assay

The MTS assay was conducted initially with fHCFs seeded on dehAM at 24 hours, 72 hours, and 168 hours. A control group containing fHCFs medium cultured from a well plate was included for comparison. The relative cell viability of fibroblasts cultured on dehAM was recorded at $132.70 \pm 12.01\%$, $121.15 \pm 5.77\%$, and $72.78 \pm 3.30\%$ after 24, 72, and 168 hours, respectively. Higher viability values at 24 and 72 hours signify that fHCFs could adhere to and grow on dehAM. However, at 168 hours, the growth decreased, possibly due to the inability to spread further on the surface of the membrane (Solecki et al., 2024). The result of the MTS assay is presented in Figure 6. Across all time points, fHCFs-dehAM maintained relative viability above 70%, which is the threshold for non-cytotoxicity as defined by ISO 10993-5:2009 (Faravelli et al., 2021). Therefore, it could be considered non-toxic and safe for cell growth (Rahyussalim et al., 2021).

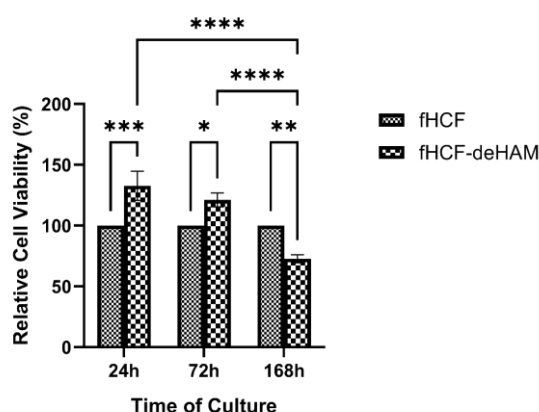


Figure 6 Cell viability of fHCFs on dehAM using the MTS Assay. The analysis was performed using Two-way ANOVA with Tukey's multiple comparisons ($n = 5$ for each group), * ($p < 0.05$) statistically significant, ** ($p < 0.01$) very significant, *** ($p < 0.001$) highly significant, **** ($p < 0.0001$) extremely significant.

The potency of amniotic membrane as an alternative biomaterial has been assessed in various studies by MTS assay. The results showed that dehAM supported cell viability in rat brain cells by more than 90% in the first 24 hours of incubation (Susilo et al., 2021). In a study of fibroblasts derived from human neonatal foreskin, tissues were immersed in a trypsin-EDTA solution at 4°C overnight. The viability and proliferative ability of the fibroblasts seeded on dehAM were evaluated with an MTT assay for 48h, reflecting an excellent result. The cell viability in the sample group (amniotic membrane seeded with fibroblast cells) compared to the control groups (fibroblast cells) showed a statistically significant increase until 500% ($P < 0.05$). Furthermore, the metabolism activity of viable cells was influenced by growth factors supported by the amniotic membrane (Moravvej et al., 2021). An in vitro investigation about PVA/gelatin hydrogel loaded with propolis has evaluated cytotoxicity using an MTT assay for human embryonic kidney (HEK) 293 cells (Pangesty et al., 2024). The study of stem cells using Wharton's jelly mesenchymal stem cells showed a good differentiation capacity into adipocytes, chondrocytes, and osteocytes for tissue engineering (Rizal et al., 2020). Another in vitro study presented that biomaterials derived from umbilical cord blood serum and platelet-rich plasma, when used as coating agents for poly(ϵ -caprolactone), facilitated high viability and attachment of human primary fibroblast cells (Nurhayati et al., 2023).

The ECM of freeze-thawed hAM consistently suppressed TGF- β and its receptor at both transcriptional and protein levels in various fibroblast cell types from ocular tissues, even in the presence of exogenous TGF- β (Hu et al., 2023). This suppressive effect was evidenced by the observed downregulation of α -smooth muscle actin transcript expression, a key myofibroblast

differentiation marker, within the initial 24 hours of fibroblast culture on hAM, persisting significantly until day 7 in the presence of FBS (Craig et al., 2024). Direct contact between fibroblasts and the hAM's basement membrane ECM effectively suppressed TGF- β isoform expression and the co-regulation of α -smooth muscle actin, thereby reducing the potential for scarring in clinical hAM transplantation (Bray et al., 2012). The freeze-thawed hAM matrix shows significant promise in attenuating scarring in future translational study, offering compelling implications for clinical applications (Nagpal et al., 2016).

The limitation of this study is that the viability assay only showed indirect metabolic activity, without including DNA analysis of the epithelial cells in the amniotic membrane. However, hAM remains a promising biomaterial for cardiac tissue engineering due to its biocompatibility and regenerative potential. Future investigations could focus on utilizing hAM-derived mesenchymal cells from the placenta to explore myocardial injury in both in vivo and in vitro models (Maleki et al., 2019) or incorporate human-induced pluripotent stem cells (Zhou et al., 2019). Additionally, combining dehAM with hydrogel-based synthetic polymers to fabricate composite scaffolds may enhance outcomes in cardiac tissue engineering (Wang et al., 2021). Incorporating bioreactor-based cell culture systems may also provide advantages, offering automated and remotely controlled 3D culture environments through multichamber designs that allow the inflow of fresh liquid or output for sample collection (Irsyad et al., 2022).

4. Conclusions

In conclusion, this study showed that dehAM is a non-toxic and effective bioscaffold for cardiac tissue engineering. SEM results affirmed an ultrastructure morphology with spindle-shaped fibroblast characteristics on the surface of the membrane. FTIR results showed that physical cross-links provided a stable matrix for the bioscaffold, reflecting the efficacy of the decellularization method. Based on immunocytochemistry analysis, dehAM could maintain filament markers of vimentin, affirming the stability and functionality while embedded in the bioscaffold. Additionally, the MTS assay confirmed high cell viability across multiple time points, supporting the biocompatibility of dehAM. As a natural and sustainable material derived from the human placenta, dehAM presents significant potential in cardiac tissue engineering. The ability to support cell attachment, proliferation, and tissue regeneration makes it a strong candidate for applications such as cardiac patches and injectable scaffolds. However, long-term in vivo studies remained essential to assess the safety, efficacy, and immunological compatibility of dehAM. Continuous investigation and development are essential to optimize dehAM scaffold innovations for specific cardiac tissue engineering applications. Optimizing the mechanical properties through characterization methods such as FTIR and SEM is crucial for advancing the application. These efforts contribute to developing more effective and biomimetic scaffolds for heart repair. Advancements in fabrication techniques, including electrospinning and melt electrowriting, enable the creation of dehAM scaffolds with controlled mechanical properties and microstructures. Incorporating bioactive molecules or cells further enhances functionality. The integration of fHCFs into dehAM scaffolds facilitates the development of functional cardiac tissue constructs. FHCfs were directed toward a reparative phenotype, promoting tissue regeneration and vascularization. Future studies should focus on optimizing scaffold designs and developing strategies to enhance cell retention and survival post-implantation, thereby advancing effective cardiac tissue engineering solutions.

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Author Contributions

W.A.: conceptualization, methodology, software, formal analysis, resources, data curation, writing – original draft preparation, writing – review & editing. D.A.C.: methodology, validation, formal analysis,

investigation, data curation, writing – original draft preparation. A.D.G.: methodology, validation, formal analysis, writing – original draft preparation. A.P.K.: methodology, validation, investigation, writing – original draft preparation. G.O.: methodology, writing – original draft preparation, visualization. R.N.I.: methodology, writing – original draft preparation. A.B.: writing – review & editing. F.A.R.: writing – review & editing. P.A.K.: software, writing, review & editing, visualization, supervision. U.P.: writing – review & editing, supervision. M.M.D.: resources, supervision, project administration, funding acquisition.

Conflict of Interest

The authors declare no conflicts of interest.

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