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RESEARCH ARTICLE

Physical Characterization of Micro-Porosity in Decellularized Gambier Leaf for Potential Use as a Plant-Based Scaffold

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ABSTRACT

Background: *Scaffolds represent biomaterials designed to provide structural support for cellular adhesion and growth factor sequestration, emulating the extracellular matrix (ECM) to promote tissue regeneration. Plant-based tissues have garnered attention as viable scaffold alternatives owing to their architectural homology with human extracellular structures. Gambier leaves (Uncaria gambir) stand out for their inherent porous, trabecular morphology, where microporosity is pivotal in facilitating cell attachment, proliferation, and differentiation.* ***Objective:*** *This study aims to elucidate the microporous characteristics of decellularized gambier leaves via scanning electron microscopy (SEM).* ***Method:*** *Fresh leaves were meticulously cleaned, cryopreserved at −20°C, and fashioned into five circular discs employing a biopsy punch. Decellularization entailed submersion in 10% sodium dodecyl sulfate (SDS) for five days, succeeded by distilled water lavage. Subsequent cyclic treatment with Tween-20 and NaClO solutions, applied every 24 hours, continued until optical translucency was achieved. Processed tissues underwent thorough washing, overnight fixation, serial ethanol dehydration, hexamethyldisilazane (HMDS) treatment, 50 nm gold sputter-coating, and SEM evaluation across three magnifications.* ***Results:*** *Microscopy revealed surface wrinkling and partial architectural collapse in multiple specimens, likely due to dehydration-induced artifacts. Conversely, a single intact sample exhibited pronounced microporosity, as evidenced by pore diameters of 0.689 µm and 0.5512 µm.* ***Conclusion:*** *These observations affirm the microporous potential of decellularized gambier leaves for cellular anchorage and nutrient permeation, bolstering their candidacy as plant-derived scaffolds in tissue engineering. Nonetheless, inter-sample variability underscores the need for refined decellularization/dehydration methods and expanded quantitative assessments to ensure reproducible structural integrity.*

Keywords: *Decellularization, gambier leaf, microporosity, plant-based scaffold, SEM*

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INTRODUCTION

Pathological bone damage arises from an imbalance between osteoblast and osteoclast activities, disrupting the equilibrium between bone formation and resorption. This imbalance ultimately leads to structural deterioration of bone tissue.[1] Advances in technology and medical science have enabled the development of Bone Tissue Engineering (BTE), which integrates knowledge of bone structure, biomechanics, and regenerative processes to facilitate functional tissue repair.[2] BTE relies on three essential components: growth factors, cells, and biomaterial scaffolds.[3]

Scaffolds serve as three-dimensional templates that support cell attachment, proliferation, and differentiation while mimicking the role of the Extracellular Matrix (ECM) in maintaining and restoring tissue function.[4] Based on their source, scaffolds can be broadly categorized into synthetic and natural materials. Natural scaffolds derived from animal tissues typically contain key ECM components, such as collagen and elastin. These ECM proteins exhibit specialized properties tailored to specific tissues. For example, scaffolds for partially damaged liver tissue are rich in laminin, fibronectin, and collagen type IV.[5,6]

In recent years, researchers have explored using plant tissues as alternative three-dimensional acellular scaffolds. Plant-derived scaffolds retain their structural, chemical, and mechanical characteristics even after cell removal through decellularization.[7] This process removes cellular components while preserving an ECM-like framework to support cell attachment and tissue regeneration.[8] The remaining matrix functions as a biological substrate that influences cellular metabolism, including proliferation, morphogenesis, and differentiation.[9] Previous studies by Adamski et al. (2018) and Harris et al. (2021) highlighted the differences between detergent-based and detergent-free decellularization approaches.[10,11] Detergent-based methods have become the gold standard because they effectively remove cells while preserving tissue architecture across plant species with varying structures and compositions. Plant-derived scaffolds also provide a wide range of natural vascular architectures, making them adaptable to specific regenerative applications.[10] One local plant with significant regional availability in South Sumatra is gambir.[12]

The gambir plant (*Uncaria gambir* Roxb.) contains a variety of bioactive compounds widely used in traditional and modern medicine.[12,13] Including catechu tannic acid (tannin), catechin, pyrocatechol, fluorine, waxes, and oils.[14,15] Catechins, a major constituent of gambir leaves, have demonstrated potential to stimulate osteoblastogenesis and promote osteoclast apoptosis, thereby reducing osteoclastogenesis and limiting bone resorption. Structurally, gambir leaves possess sponge-like mesophyll layers and interconnected air spaces.[16] This architecture resembles porous scaffolds and trabecular bone, facilitating vascularization, nutrient exchange, cell adhesion, and tissue ingrowth.[16,17]

Effective scaffold function requires materials with key attributes, including biocompatibility, biodegradability, adequate mechanical strength, and suitable structural characteristics.[8] Porous scaffolds are especially important in bone regeneration because their interconnected pores and large surface area support osteogenic processes.[18] Microporosity enhances the specific surface area, improves permeability, promotes osteogenic protein binding, and accelerates the release of degradation products, all of which facilitate cell adhesion, proliferation, differentiation, and biomineralization.[19] Microporosity can be evaluated using Scanning Electron Microscopy (SEM).[20] Generally, pores ≥100 µm with interconnected networks are considered optimal for cell proliferation, vascularization, and tissue integration.[2]

To the best of our knowledge, no research has specifically investigated the

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utilization of gambir leaves as a plant-based scaffold for bone regeneration. This gap in the literature highlights the need to explore the microstructural characteristics of gambir leaves following decellularization, particularly their microporosity, as an early step in assessing their feasibility as scaffolds for bone tissue engineering. Therefore, this study aimed to characterize the microporosity of decellularized gambier leaves using Scanning Electron Microscopy (SEM) as an initial assessment of their potential application as plant-based scaffolds for bone tissue engineering.

MATERIALS AND METHODS

This was an in vitro laboratory design with a post-test-only control group approach. Gambier leaves (*Uncaria gambir* Roxb.) were collected from plantations in Babat Toman Village, Musi Banyuasin Regency, South Sumatra, Indonesia. The selected leaves met the following criteria: light green, approximately five months old, measuring 8-13 cm in length, and structurally intact. Leaves that were wilted or showed signs of pest damage were excluded from the study. Freshly harvested leaves were rinsed with distilled water and stored at −20 °C until further processing. Before decellularization, the leaves were cut with a biopsy punch while submerged in distilled water to maintain tissue stability (Fig. 1).

Figure 1. (A). Native gambier leaf (B). The gambier leaf samples are being cut using a biopsy punch

Decellularization was performed using a detergent-based protocol. Leaf samples were immersed in 10% sodium dodecyl sulfate (SDS) for five days at room temperature (20–25 °C) on a low-speed orbital shaker. Samples were then rinsed thoroughly with distilled water to remove residual SDS[11]. Subsequently, the leaves were immersed in a mixed solution of 1% Tween-20 (v/v) and 10% sodium hypochlorite (NaClO, v/v). This solution was replaced every 24 hours until the leaves became fully transparent. After complete decellularization, samples were washed with deionized water.[11]

A total of five decellularized gambier leaf samples (n=5), prepared as circular disc-shaped specimens, were used in this study. No experimental grouping or treatment comparison was used because all samples underwent the same preparation protocol for physical characterization.

The decellularized samples were fixed in 4% formaldehyde prepared in Phosphate-Buffered Saline (PBS) and incubated overnight. Samples were then rinsed with PBS and dehydrated through a graded ethanol series (30%, 50%, 80%, and 95%) for 15 minutes at each concentration. Following dehydration, the samples were treated with Hexamethyldisilazane (HMDS) and air-dried. Dried samples were sputter-coated with a 50 nm gold layer and examined using *Scanning Electron Microscopy* (SEM) at magnifications of 600×, 1200×, and 2400×.

RESULT

The decellularization and sample preparation procedures were carried out at the Integrated Biology Laboratory at Raden Fatah State Islamic University, Palembang, and SEM examination was then performed at the Engineering Laboratory at Sriwijaya University. The outcomes of the decellularization process are presented in Figure 2.

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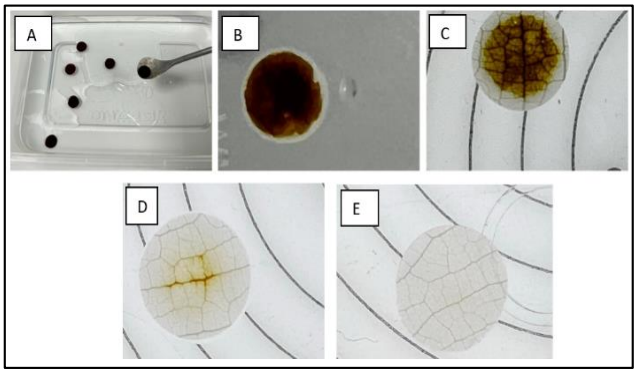

Figure 2. Gambir leaves post-decellularization. (A). After 5 days of immersion in SDS (B-D). Color changes occurred during immersion in the bleach solution (E). After 5 days in the bleach solution, the leaf samples were completely colorless.

Figure 2 shows the gambier leaves following SDS-based decellularization. After decellularization, visual observation revealed that the natural green pigmentation had completely disappeared, and the leaf appeared translucent. The internal tissue architecture remained intact, with the vascular channels clearly visible and exhibiting no tearing or structural damage.

SEM analysis was conducted at three different magnification levels on transverse sections of decellularized gambier leaves. The observations in Figures 3 and 4 revealed surface wrinkling within the internal structure of the leaves after decellularization. Uneven sample edges were observed in all specimens, likely caused by mechanical cutting during sample preparation.

Microporous features were identified in representative well-preserved samples. At 1200× magnification (Figure 4B), distinct microporous features were observed in localized regions of the decellularized leaf section. Two micropores were measured directly from the SEM image using the analysis software, with diameters of 0.689 µm and 0.5512 µm. These measurements represent localized pore dimensions observed at specific regions of interest and do not reflect mean pore size values across all samples. Due to the exploratory and descriptive nature of this study, a quantitative pore size distribution analysis was not performed.

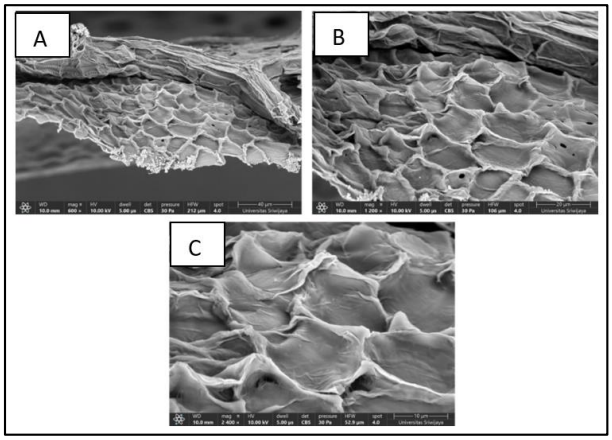

Figure 3. The SEM images of sample 1 with 600x magnification (A), 1200x (B), and 2400x (C).

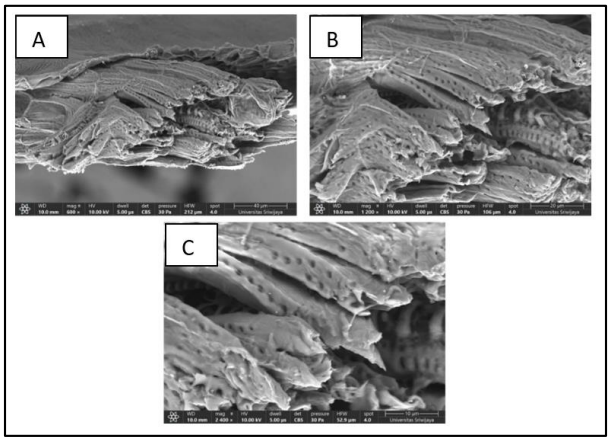

Figure 4. The SEM images of sample 3 with 600x magnification (A), 1200x (B), and 2400x (C). Localized microporous features were visible within the leaf matrix at higher magnifications.

DISCUSSION

This study examined the microporosity of gambier leaves after decellularization. This process successfully changed the leaf color from green to translucent, as shown in Figure 5. An SDS-based ionic detergent method was used to remove cellular membranes and nuclei by solubilizing lipid components and denaturing proteins.[9] This technique is widely recognized as the gold standard for decellularization in both plant and mammalian tissues.[6,7] Because SDS is cytotoxic, an extensive washing procedure

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using 10% NaClO and Tween 20 was performed to remove detergent residues and remaining cellular debris.[11]

Following this process, decellularized gambier leaves preserved vascular-like structures consistent with xylem architecture, as shown in Figure 3E. The vascular architecture remained intact and clearly defined. These channels play an essential role in facilitating nutrient transport, gas exchange, and the removal of metabolic by-products during subsequent cellular regeneration. Preservation of such structural complexity indicates that a properly optimized decellularization protocol can maintain the intrinsic morphological characteristics of plant tissues.[10]

To assess microstructural changes after decellularization, SEM analysis was performed on samples prepared using the *Hexamethyldisilazane* (HMDS) drying method. This approach followed the protocol described by Melis Toker et al. (2020).[20] HMDS served as an alternative to the *Critical Point Drying* (CPD) technique, functioning by gradually replacing ethanol and removing residual moisture through evaporation. This method has demonstrated drying performance comparable to CPD for preparing plant-derived samples for SEM analysis.[21]

The markedly smaller pore sizes observed in the present study compared to those reported by Ali Salehi et al. (2020) may be attributed to several factors. First, the pore architecture in plant-derived scaffolds is highly dependent on species-specific leaf anatomy.[22] Gambier leaves possess a relatively dense mesophyll structure with tightly packed parenchymal cells, which may inherently limit the formation of large interconnected pores following decellularization.[9] Second, micropore measurements in this study were obtained from localized regions of interest within decellularized leaf sections rather than from clearly identifiable vascular channels. As the SEM analysis was focused on surface-level morphology, the observed pore dimensions likely represent microstructural features of the leaf matrix rather than larger transport channels. This localized observation approach may have contributed to the detection of submicron-scale pores. While these submicron pores are insufficient for cell penetration or vascular ingrowth, they may still enhance surface area, protein adsorption, and initial cell attachment, which are critical during the early stages of tissue regeneration.

Furthermore, structural shrinkage or partial collapse of the extracellular matrix may have occurred during decellularization and dehydration processes, particularly during ethanol dehydration and HMDS drying, resulting in reduced pore dimensions, as illustrated in Figures 3 and 4. This deformation is likely attributable to the drying protocol: using 100% HMDS at two drops per sample may have induced excessive shrinkage of the cell walls. Similar phenomena were reported by Moritz Schu et al. (2021), who found that drying with 10% HMDS can cause cracking in biological specimens.[21] These observations suggest that drying conditions influence the structural appearance of the samples.

Beyond preparation effects, the inherent morphology of gambier leaves also contributed to the SEM visualizations. Based on previous anatomical descriptions, gambier leaves are reported to possess a rigid anatomical framework composed of several distinct layers: the upper epidermis, a double palisade mesophyll layer, vascular bundles, a spongy mesophyll with abundant air spaces and oil droplets, and the lower epidermis.[9] Their rigidity is largely due to the thick sclerenchyma tissue, which consists of dead cells with heavily lignified secondary walls that provide mechanical strength and protection. The thickness and density of this layer may limit SEM visualization primarily to surface and near-surface features, thereby restricting observation of deeper microporous structures.[23]

Additional challenges emerged during sample processing, particularly due to the lack of established HMDS concentration and dosage

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guidelines for gambier leaf tissues. Despite careful handling, noticeable shrinkage occurred. Suboptimal sample manipulation further contributed to the wrinkling observed in the SEM micrographs. Moreover, the delicate structure of the leaves, combined with extensive internal air cavities, likely increased the samples' susceptibility to collapse during drying.[24]

Finally, this study acknowledges technical limitations related to specimen preparation for SEM. The irregular cut surfaces seen in several images may have resulted from variability during manual trimming of leaf specimens before imaging. Since the cutting process was performed mechanically, inconsistencies in blade pressure, angle, or sample stabilization could introduce artifacts unrelated to the true morphology of the decellularized specimens. Moreover, operator-related factors during sample handling and mounting may have contributed to minor microstructural distortions, particularly when performed by individuals with limited experience in leaf–tissue preparation for SEM. These artifacts must be considered when interpreting morphological features, as they may affect the surface appearance without reflecting true structural changes caused by decellularization.

Taken together, the findings in the present study suggest that decellularized gambier leaves may require additional structural modifications, such as mechanical perforation, chemical treatment, or combination with secondary biomaterials, to achieve pore sizes optimal for bone tissue engineering applications. Future research should focus on optimizing drying protocols, particularly the HMDS concentration and volume, to minimize sample shrinkage and structural distortion. Refining the decellularization process, including varying detergent concentrations or adding additional washing steps, may also improve microstructural preservation. Further evaluation of the mechanical properties, pore interconnectivity, and biocompatibility is necessary to assess the feasibility of gambier leaves as a potential scaffold for bone tissue engineering.

CONCLUSION

This study successfully characterized the surface morphology of decellularized gambier leaves and identified microporosity with diameters of 0.689 µm and 0.5512 µm in one of the analyzed samples. Another samples displayed pronounced surface wrinkling, which limited detailed interpretation of their microstructural features. To the best of our knowledge, this is the first study to document microporosity measurements in decellularized gambier leaves, providing essential baseline data for future structural optimization of this plant-derived material.

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