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# Fabrication and Biological Evaluation of Bio-Epoxy Hybrid Composites Reinforced with Flax, Glass, Carbon, and Polyethylene Fibers for Bone Plate Applications

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**ABSTRACT**

The long-standing use of metallic implants for bone fracture fixation, such as stainless steel and titanium, often results in adverse biological responses, including inflammation and stress shielding, due to their mismatched mechanical behavior relative to bone. To address this, the study reports the fabrication and evaluation of novel hybrid bio-based polymer composite plates developed via a hand layup technique. The matrix comprises a bio-epoxy resin integrated with 2 wt.% pumpkin powder and reinforced using natural (flax) and synthetic (ultra-high molecular weight polyethylene [UHMWPE], carbon, and glass) woven fibers. Seventeen distinct laminate configurations were synthesized and structurally optimized. The material properties were characterized through contact angle analysis (wettability), antibacterial activity (against Staphylococcus aureus and Pseudomonas), and in vitro cell viability using the MTT assay. All hybrid composites exhibited hydrophilic surfaces (contact angle < 90°), with flax fiber inclusion promoting smoother textures and improved wettability compared to UHMWPE. Antibacterial testing revealed superior performance against S. aureus, with UHMWPE-based composites showing the most effective inhibition. Biocompatibility assessment indicated high cell viability, with flax-glass hybrid laminates achieving up to 96.73% viability at 72 hours. These results highlight strong correlations between composite structure and biological performance. The optimized hybrid laminate—comprising bio-epoxy, 2% pumpkin powder, four flax layers, and two glass fiber layers—demonstrated properties closely aligned with cortical bone. This study contributes to the advancement of solid bio-composite materials with tailored mechanical and biological characteristics for orthopedic applications.

## 1. INTRODUCTION

Bone fractures are a common medical condition that often requires surgical intervention using internal fixation devices such as bone plates. Traditionally, these plates have been manufactured from metals like stainless steel and titanium due to their mechanical strength and durability. However, while effective in stabilizing fractures, metal implants can pose several challenges. They often cause stress shielding—where the implant bears too much load, leading to bone resorption—and may also trigger inflammation or allergic reactions in some patients [1, 2]. As a result, researchers have been exploring alternative materials that not only match the mechanical demands of bone repair but also support better biological integration with the body. One promising direction involves the use of bio composites, which combine natural fibers with bio-based polymers to produce materials that are lighter, more compatible with human tissue, and customizable in terms of strength and biodegradability [3-5].

In recent years, bio-composite materials have gained attention for orthopedic use, particularly due to their tunable mechanical properties and improved biological responses [4, 6, 7]. By reinforcing biodegradable or bio-based resins with fibers such as flax, jute, or synthetic polyethylene, researchers have created structures that better mimic the mechanical behavior of natural bone while also encouraging tissue integration [8, 9]. The inclusion of natural additives—such as plant-derived powders—has further enhanced the surface activity and biological interaction of these materials [10, 11]. These hybrid systems offer a promising balance between strength, flexibility, and bio-functionality, which makes them suitable candidates for replacing traditional metal plates in long bone fracture fixation. Building on this approach, the current study focuses on developing a series of laminated bio-epoxy composites using a combination of natural and synthetic fibers, alongside functional fillers, to evaluate their potential for biomedical applications [12, 13]. These adverse effects are primarily attributed to the corrosion of metallic implants, which leads to the release of metal ions into surrounding tissues and causes local inflammation, allergic responses, and stress shielding due to the significant mismatch in elastic modulus between the implant and natural bone.

Hybrid composites have emerged as a promising class of materials capable of combining the desirable properties of different constituents to achieve superior performance [6, 14]. When applied to orthopedic bone plates, these materials can offer enhanced mechanical behavior while addressing the limitations of traditional single-fiber systems. Studies on polyester-based composites reinforced with varying ratios of kapok and glass fabric have shown that increasing the glass fiber content significantly improves overall strength and stiffness [4, 15]. Similarly, epoxy composites reinforced with combinations of glass fiber, sisal fiber, and natural additives like chitosan have demonstrated high tensile, flexural, and compressive strengths, which indicates their potential suitability for load-bearing medical applications. For instance, pumpkin powder has been incorporated to enhance antioxidant and antibacterial activities, while turmeric and neem leaf extract have shown effectiveness in promoting osteogenic differentiation and enhancing biocompatibility in bio-epoxy composites [16, 17].

Other hybrid systems, such as those combining Kevlar and flax fibers with epoxy, have been shown to achieve mechanical properties exceeding those of human cortical bone to provide both structural support and reduced risk of stress shielding. More recent developments involving the integration of carbon and glass fibers with natural flax in epoxy matrices have produced composites with superior flexibility and resilience under tensile, compressive, and bending loads, highlighting their potential as alternatives to conventional metal bone plates [18, 19]. Generally, these bio-composites exhibit tensile strengths in the range of 50 to 300 MPa and elastic moduli between 3 and 30 GPa, which are comparable to those of cortical bone, with typical tensile strength of 100–150 MPa and an elastic modulus of 7–30 GPa [18, 19].

Therefore, the primary objective of this study was to fabricate and evaluate a range of bio-epoxy hybrid composite laminates reinforced with combinations of flax, UHMWPE, carbon, and glass fibers, and enhanced with 2 wt.% pumpkin powder, for potential use as bioactive bone plate materials. The mechanical and biocompatibility properties of the developed composites were systematically investigated. It was hypothesized that the integration of natural fibers and functional fillers would significantly improve the composites' surface wettability and antibacterial activity. All this with achieving mechanical performance comparable to cortical bone to offer a promising alternative to conventional metallic implants.

## 2. MATERIALS AND METHODS

This study focused on developing a hybrid polymer matrix composite from natural and synthetic fabrics. We evaluated its suitability for specific applications. Using a hand layup technique, the hybrid bone plate combines a biopolymer, a bio epoxy thermosetting material, with 2% pumpkin powder and various woven fabrics, including UHMWPE, flax, carbon fiber, and glass fiber. We examined seventeen types of bio composites, detailed in Table 1.

**Table 1.** Type of laminated composite material

| No. of Laminations | Total No. of Layers | Layers' Symbol | Lamination Layup Procedures |
|---|---|---|---|
| laminate (0) | - | - | Bio epoxy +2%P |
| laminate (1) | | 2%P+1 Flax (1F) | - |
| laminate (2) | Bio epoxy + pumpkin powder + | 2%P+2 Flax (2F) | - |
| laminate (3) | flax fibres | 2%P+3 Flax (3F) | - |
| laminate (4) | | 2%P+4 Flax (4F) | - |
| laminate (5) | Bio epoxy + pumpkin powder+ | 2%P+4 flax (4F) +1 Carbon (1C) | 2F+ 1C+ 2F |
| laminate (6) | flax fibers+ Carbon fiber | 2%P+4 flax (4F) +2 Carbon (2C) | 2F+ 2C+ 2F |
| laminate (7) | Bio epoxy + pumpkin powder + | 2%P+4 flax (4F) +1 Glass (1G) | 2F+ 1G+ 2F |
| laminate (8) | flax fibers+ Glass fiber | 2%P+4 flax (4F) +2 Glass (2G) | 2F+ 2G+ 2F |
| laminate (9) | | 2%P+1 UHMWPE (1U) | - |
| laminate (10) | Bio epoxy + pumpkin powder+ | 2%P+2 UHMWPE (2U) | - |
| laminate (11) | UHMWPE fibers | 2%P+3 UHMWPE (3U) | - |
| laminate (12) | | 2%P+4 UHMWPE (4U) | - |
| laminate (13) | Bio epoxy + pumpkin powder+ | 2%P+4 UHMWPE (4U) + 1 Carbon (1C) | 2U+1C+2U |
| laminate (14) | UHMWPE fibers+ Carbon fiber | 2%P+4 UHMWPE (4U) + Carbon (2C) | 2U+2C+2U |
| laminate (15) | Bio epoxy + pumpkin powder+ | 2%P+4 UHMWPE (4U) + 1 Glass (1G) | 2U+1G+2U |
| laminate (16) | UHMWPE fibers+ Glass fiber | 2%P+4 UHMWPE (4U) + 2 Glass (2G) | 2U+2G+2U |

This study utilizes liquid bio epoxy resins from Dow Chemical Company in China, with an equivalent weight of 182 to 192 g/eq and a 1.16 g/cm³ density. The bio-epoxy resin used was D.E.R.™ 331 (Dow Chemical, China), an unmodified liquid epoxy resin based on bisphenol-A and epichlorohydrin, with curing performed at room temperature (25℃) for 48 hours using a polyamide hardener at a resin-to-hardener weight ratio of 100:43.

We incorporate prepared pumpkin powder with an average particle size of 1.5 micrometers. We use Changzhou Doris Textile Co., Ltd. flax fibers, parallel-aligned carbon fibers, and glass fibers from Otto Bock Corporate for reinforcement. The flax fibers had a tensile strength of approximately 800 MPa, modulus of 60 GPa, and elongation at break of 1.5%; the UHMWPE fibers exhibited a tensile strength of 2,900 MPa, modulus of 117 GPa, and elongation of 3.5%; the carbon fibers possessed a tensile strength of 3,500 MPa, modulus of 230 GPa, and elongation of 1.4%; while the glass fibers displayed a tensile strength of 2,450 MPa, modulus of 85 GPa, and elongation of 2.5%.

A glass mold measuring 25 cm × 25 cm × 0.4 cm is employed, with its inner surface covered in nylon thermal paper to prevent resin from sticking. We accurately measure resin, hardener, and pumpkin powder for testing using a digital scale, while woven fibers are cut to size with digital Vernier calipers. For fabrication, the hand layup technique is employed. The resin is mixed with 2% pumpkin powder, followed by hardener at a ratio of 100:43. After pouring a thin layer into the mold, we alternate layers of fibers and matrix material, repeating until the desired thickness is achieved. The laminates are then cured for 48 hours and cut to precise dimensions with a water jet machine for experimental testing. Figure 1 illustrates the preparation steps for the hybrid laminated bio-composite.

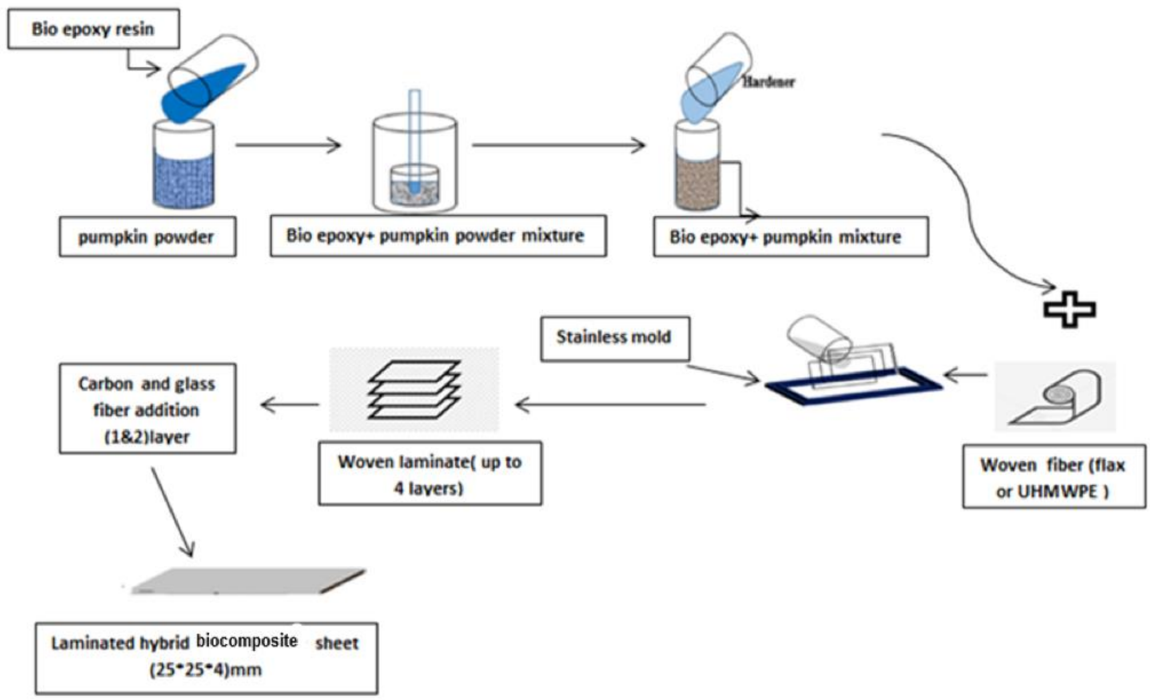



**Figure 1.** Cavity geometry

## 3. EXPERIMENTAL SETUP

### 3.1 Contact angle (wettability) test

Many biological, chemical, and physical processes depend on a surface's wetness. We use contact angle measurement to measure this, which indicates how well a liquid interacts with a solid. The contact angle reveals the wetting property of the surface, showing if it is hydrophobic, meaning it repels water. We measured the contact angle using an optical contact angle and interface tension meter, following the ASTM standard D5946-04. We placed the specimen on a glass slide and secured the tissues for viewing during contact angle measurements. Then, we added an 8 ml droplet of distilled water to the bio-composite surface, measured the contact angle, and recorded the droplet's shape with a camera.

### 3.2 Antibacterial activities test

We used the agar well diffusion method to test the antibacterial efficacy of different samples against Staphylococcus aureus (S. aureus) and Pseudomonas. First, bacterial samples were grown in the nutrient broth at 37℃ for 18 to 24 hours. Then, 0.1 ml of each suspension was spread on nutrient agar and incubated for 24 hours. To prepare a standardized bacterial suspension (about $1.5 \times 10^8$ CFU/mL), we added a single colony to 5 mL of normal saline. This suspension was spread on Mueller-Hinton agar and allowed to settle for 10 minutes. We created wells in the agar, added 50 µl of purified and crude EPS to each, and used distilled water as a control. We measured the inhibition zones after incubating the plates at 37℃ for 18 hours. A larger zone indicated stronger antibacterial activity.

### 3.3 MMT assay (cell availability) test

We conducted an MTT assay using a 96-well plate to assess the toxicity of specimens on cells. The MG-96 is a human osteoblast-like cell line derived from osteosarcoma (ATCC® CRL-1427™), cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), 1% penicillin-streptomycin (Gibco), and maintained at 37°C in a humidified incubator with 5% $CO_2$ [20].

Cell lines were seeded at $1 \times 10^4$ cells per well after 24 hours. Once a full layer of MG-96 cells was established, we treated them with the specimens and measured viability 72 hours post-treatment. To evaluate this, we removed the medium, added 100 µL of a 2 mg/mL MTT solution, and incubated for 2.5 hours at 37℃. We then dissolved the resulting crystals with 130 µL of Dimethyl Sulphoxide (DMSO) and incubated for 15 minutes at 37℃ while shaking. We measured absorbency at 492 nm using a microplate reader and conducted the test thrice. The cell growth inhibition rate (cytotoxicity percentage) was calculated with the following equation [20, 21]:

$$Inhibition\ rate \ = A - B/A * 100 \qquad (1)$$

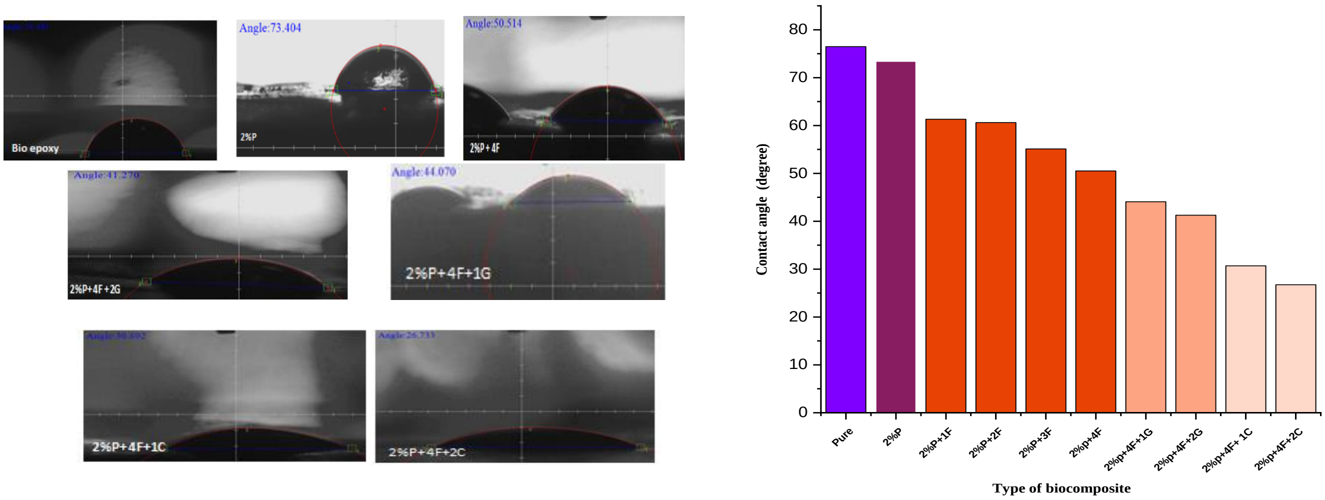
where, A is the optical density of the control, and B is the optical density of the samples [22]. Data are provided as average SD (n = 3).

## 4. RESULTS AND DISCUSSION

### 4.1 Contact angle (wettability) results

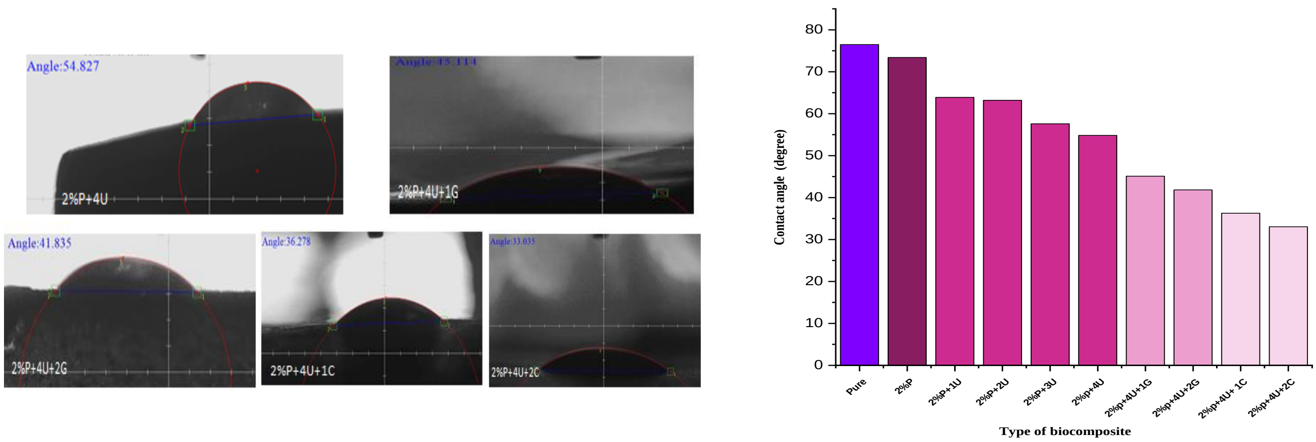
We assess the wettability of composites by measuring the contact angle, which indicates how liquids spread on the surface. Rough surfaces typically have higher contact angles, while smooth surfaces have lower ones. A contact angle under 90° indicates hydrophilicity (water-attracting), while over 90° indicates hydrophobicity (water-repelling). All our composites exhibit contact angles below 90°, making them hydrophilic.

Figures 2 and 3 illustrate the impact of different reinforcements on the wettability of the bio composites [23]. The pure bio epoxy matrix had the highest contact angle of 76.481°. Adding 2% pumpkin powder reduced it to 73.4°, as the powder particles smoothed and compacted the surface, decreasing hydrophobicity [24]. Incorporating flax fiber into composite materials reduces the contact angle and enhances wettability due to the cellulose in flax and the smooth surface finish of hybrid composites [23]. Adding UHMWPE fibers functions similarly to pumpkin powder in hybrid composites. Composites with pumpkin powder and flax fibers had a medium contact angle, with flax fibers resulting in a smoother surface than UHMWPE fibers. When carbon fiber was included, the contact angle decreased to 26.7 for the flax hybrid composite and 33.07 for the UHMWPE hybrid composite, lower than that observed with glass fibers.




(a) Scan (b) Contact angle

**Figure 2.** Contact angle for pure bio epoxy, bio composite, and flax hybrid bio composites as a function of the number and types of reinforcement

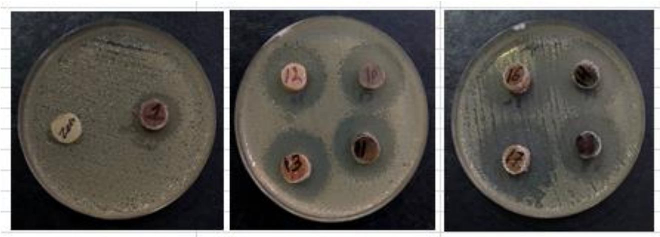



(a) Scan (b) Contact angle

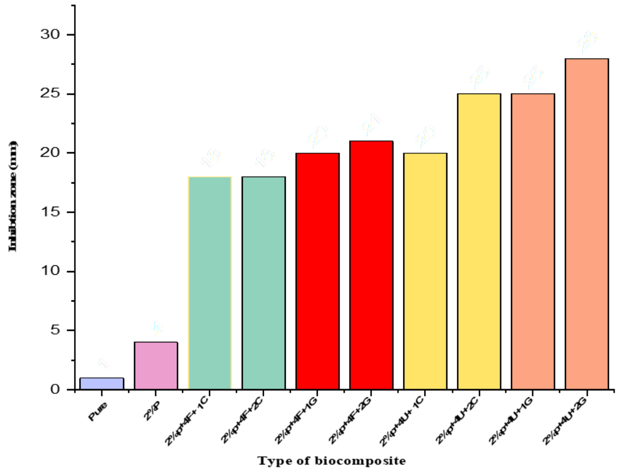
**Figure 3.** Contact angle for pure bio epoxy, bio-composite, and UHMWPE hybrid bio-composites as a function of the number and types of reinforcement

### 4.2 Antibacterial activity results

The study evaluates how effectively bio-composite samples combat Gram-negative and Gram-positive bacteria. Figures 4 and 5 illustrate where the samples inhibited the growth of Pseudomonas and S. aureus. Figure 4 presents the antibacterial activity of bio-composite samples against Pseudomonas (Gram-negative bacteria). The pure bio-epoxy sample showed the lowest inhibition zone at 1 mm, followed by the pumpkin-reinforced epoxy with 4 mm. Both the flax-based hybrid composites (1F and 2F) exhibited inhibition zones of 18 mm, while the 3F and 4F composites demonstrated enhanced antibacterial effects with 20 mm and 21 mm, respectively. The addition of glass fibers further improved the performance, with the 2%P + 4F + 1G and 2%P + 4F + 2G laminates showing inhibition zones of 22 mm and 23 mm. The highest inhibition, at 28 mm, was recorded for the composite with 2% pumpkin powder, four flax layers, and two glass fiber layers (2%P + 4F + 2G), indicating a strong antibacterial synergy. Adding up, Figure 5 shows the inhibition zones of the same bio-composites against Staphylococcus aureus (Gram-positive bacteria), where overall antibacterial activity was notably higher. The pure epoxy displayed an inhibition zone of 2 mm, and the pumpkin-enhanced matrix increased it to 6 mm. The flax-based laminates again started at 20 mm for both 1F and 2F, and increased to 21 mm and 22 mm for 3F and 4F, respectively. With the integration of one and two layers of glass fiber, the zones expanded to 24 mm and 26 mm, while the composite with the highest reinforcement (2%P + 4F + 2G) achieved the maximum inhibition zone of 28 mm. These results demonstrate that Gram-positive S. aureus was more susceptible to the bio-composites, especially those with multilayer flax and glass fiber reinforcement.



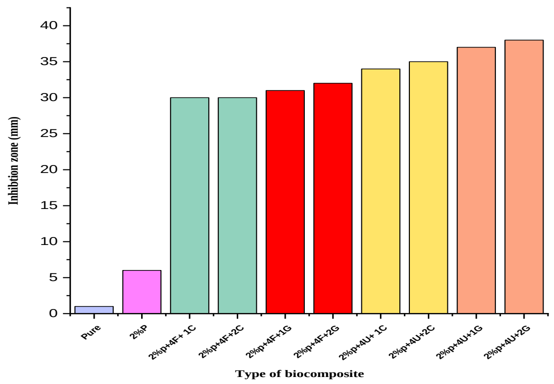
(a) Experimental pictures




(b) Inhibition zone

**Figure 4.** Inhibition zone of the pure bio epoxy matrix, bio epoxy reinforced by pumpkin powder (2% P), and hybrid bio-composites against Pseudomonas bacteria

(a) Experimental pictures




(b) Inhibition zone

**Figure 5.** Inhibition zone of the pure bio epoxy and bio epoxy reinforced by pumpkin powder (2% P) and hybrid bio-composites against S. aureus bacteria

The composite material exhibits strong antibacterial effects, likely due to the natural agents in pumpkin powder and flax fibers. This bio-composite is a promising option for products designed to combat bacteria. The inhibition zones indicate the effectiveness of these natural materials. The antibacterial agents break down bacterial cell walls, inhibiting growth. When combined with a bio-epoxy matrix, the fibers retain and potentially enhance their antibacterial properties, as the matrix protects them and extends their effectiveness. The synergy of natural fibers and the bio-epoxy matrix creates surfaces discouraging bacterial growth. The composite's surface texture also prevents bacteria from adhering and proliferating [25]. Bacteria adhere less to rough surfaces, allowing us to design flax-epoxy and UHMWPE–epoxy composites with beneficial textures. Controlled porosity in the composite can release natural antibacterial agents from the fibers over time, providing long-lasting protection. Additionally, incorporating antibacterial agents like pumpkin microparticles or organic compounds during manufacturing can enhance the material's germ-fighting abilities [26]. Hybrid bio-composites are more effective against S. aureus than Pseudomonas, with the UHMWPE-reinforced composite performing best against both.

### 4.3 MMT assay (cell availability) results

We studied the biocompatibility of samples with the MC3T3-E1 cell line using the MTT assay. The results indicated good cell viability, as shown in Figures 6 and 7. The pure bio-epoxy sample had viabilities of 85.24%, 86.07%, and 93.93% at 24, 48, and 72 hours, respectively. Adding pumpkin powder to the bio-epoxy matrix significantly increased cell viability over time due to pumpkin's known medicinal benefits, including lower cholesterol and antioxidant properties [27]. The results indicated a significant increase in cell viability with fiber-reinforced bio-composites (flax, UHMWPE, carbon, and glass) at 24, 48, and 72 hours, for both flax and UHMWPE samples, laminated bio-composites with glass fiber showed higher cell viability than those with carbon fiber [28].

Hybrid bio-composites with flax fabric exhibited better cell availability than those with UHMWPE fabric over 24, 48, and 72 hours. Laminated composites (2%P +4F+2GF) achieved a high cell availability of 96.733% at 72 hours, with all samples being non-toxic to human fibroblast cells. These findings indicate that bio-composites support strong cell viability, making them suitable for bone plate fixation in living organisms.

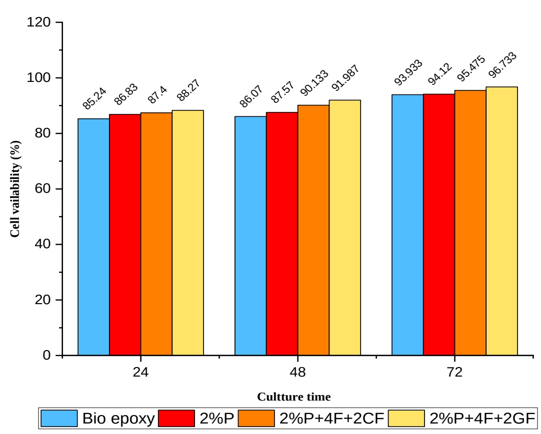



**Figure 6.** Cell availability of bio composite bone plates fixation as a function of flax fabric

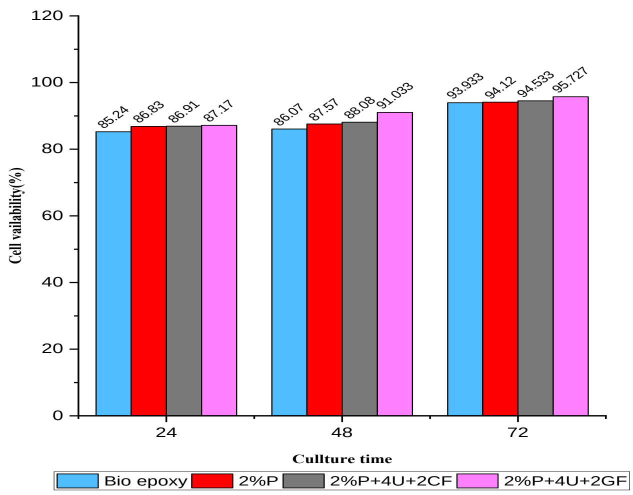



**Figure 7.** Cell availability of bio composite bone plates fixation as a function of UHMWPE fabric

## 5. CONCLUSIONS

This study investigated the fabrication and biological performance of laminated hybrid bio-composites developed from a bio-epoxy matrix enhanced with 2% pumpkin powder and reinforced with natural (flax) and synthetic (UHMWPE, carbon, and glass) fibers. Contact angle analysis revealed that all composites exhibited hydrophilic behavior with values below 90°, confirming enhanced surface wettability—a desirable trait for biological applications. The pure bio-epoxy sample showed a contact angle of 76.48°, which decreased to 73.4° with pumpkin powder addition. Flax-based hybrid laminates demonstrated even lower angles, with the flax-carbon and flax-glass hybrids reaching as low as 26.7°, indicating superior wettability. These results highlight the role of fiber type and lamination in modifying the surface characteristics essential for cell adhesion.

Antibacterial tests showed significantly improved inhibition zones in hybrid bio-composites compared to pure epoxy, particularly against Staphylococcus aureus. The highest inhibition zone against Pseudomonas was 28 mm, and against S. aureus was also 28 mm, both achieved by the flax-glass hybrid laminate (2%P + 4F + 2G). In vitro MTT assays confirmed excellent biocompatibility, with cell viability improving over time. The flax-glass composite reached 96.73% viability at 72 hours, significantly higher than the pure epoxy's 93.93%. Flax-based composites consistently outperformed UHMWPE-based ones in supporting cell growth. Overall, the hybrid laminate combining pumpkin powder, flax, and glass fibers demonstrated the best biological profile, suggesting its potential as a bioactive alternative to metal bone plates in orthopedic applications.

However, further in vivo investigations are necessary to confirm the long-term biocompatibility, mechanical stability, and biological integration of these hybrid bio-composites within complex physiological environments.

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