



RESEARCH ARTICLE

EPOXY COMPOSITES REINFORCED WITH FISH-DERIVED HYDROXYAPATITE FOR LOAD-BEARING BIOMEDICAL APPLICATIONS

Ismail Zainol^{1*}, Alhussein Arkan Majhool², Che Nor Aiza Jaafar³, Mustafa Mudhafar²

¹*Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Proton City, 35900 Tanjung Malim, Perak, Malaysia.*

²*Department of Medical Physics, College of Applied Medical Sciences, University of Kerbala, Karbala, Iraq.*

³*Department of Mechanical and Manufacturing Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43000 Serdang Selangor, Malaysia.*

Abstract. The combination of epoxy resins and hydroxyapatite (HA) has emerged as a promising material system in various medical applications, particularly in the fields of tissue engineering, drug delivery, and orthopaedic implants. This synergy exploits the favourable properties of both materials, including the mechanical strength of epoxy and the bioactivity of hydroxyapatite. In this study epoxy resin was mixed with micron size of natural hydroxyapatite powder from fish scales (FsHA). The mechanical properties, chemical and biological properties of the composite was investigated by means of flexural strength, impact strength, chemical properties and biocompatibility study and morphological observation. Addition of micron size FsHA in the epoxy matrix significantly improved the impact and flexural strength of epoxy/FsHA composite. At 10% FsHA loading, flexural strength was improved about 77% and the impact strength increased about 65% as compared to neat epoxy. Fourier transform infrared spectroscopy and X-ray diffraction (XRD) has confirmed the present of the FsHA in the matrix. From FTIR analysis no chemical interaction between FsHA and epoxy matrix was detected. XRD results indicated that the FsHA is in crystalline phase. The morphology analysis of the fractured surface was studied using a scanning electron microscope (SEM) revealed that the homogeneous distribution of the FsHA particles in the matrix especially at 10% FsHA content. The overall results indicated that the epoxy/FsHA composite has potential for load bearing application in medical application.

Keywords: Epoxy, hydroxyapatite, epoxy/hydroxyapatite composite, fish scales.

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*Corresponding author: ismail.zainol@fsmt.upsi.edu.my

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1. INTRODUCTION

Epoxy resin has been widely used to prepare biomedical components such as material for dental applications, medical imaging, bone plate applications, tissue engineering scaffold and bone implant [1]. The combination of epoxy resins and hydroxyapatite (HA) has emerged as a promising material system in various medical applications, particularly in the fields of dental and orthopedic applications. This synergy exploits the favorable properties of both materials, including the mechanical strength of epoxy and the bioactivity of hydroxyapatite.

Synthetic hydroxyapatite (HA) is widely used in medical applications due to its structure closely resembles the mineral component of bone, making it an excellent candidate for bone tissue engineering. Marcela et al. (2022) demonstrated that the presence of HA in 3D-printed PLA/HA scaffolds induced osteogenic differentiation of human mesenchymal stem cells (MSC) thus making them suitable as bone replacements for in vivo applications [2]. Epoxy/HA composite not only provide structural support but also promotes cell attachment and growth. The bioactive properties of hydroxyapatite facilitate mineralization, while the epoxy matrix provides necessary mechanical strength. Epoxy/HA composites are also being explored for use in orthopedic implants. The mechanical properties of epoxy make it suitable for load-bearing applications, while HA contributes to the biocompatibility and osteoconductive of the implant. A recent 2024 study by Alam et al. [3] showed that adding hydroxyapatite (HA) and a small amount of silica to epoxy resin significantly improved its strength and stiffness. The composite was over twice as strong and more than three times stiffer than plain epoxy. These improvements suggest it could be useful for medical applications like bone repair or joint replacements.

The combination of HA and polymers has also been investigated for drug delivery systems, particularly for localized drug delivery in bone diseases. The porous structure of HA can be utilized to encapsulate drugs, while the polymer matrix can control the release profile. Recent review by Farnaz et al. (2024) showed that combination of HA with polymers is a perfect combination for drug delivery system. They reported the latest synthesis methods of HA nanocomposites and newly developed nanocarriers [4]. In dentistry, HA composites have been utilized in the development of restorative materials and dental implants. The bioactive nature of hydroxyapatite enhances the integration of dental implants with bone, while the mechanical properties of epoxy ensure durability and resistance to wear [5].

Most of the hydroxyapatite used in commercial polymer/HA composite using synthetic HA. Due to the high production cost of synthetic HA, biogenic HA has become an alternative. Natural occurrence of HA in fish scales has become the promising materials to replace synthetic HA since the study proved their similarity structure to synthetic HA and biocompatible [6]. It was reported that hydroxyapatite fish scales (FsHA) are biologically safe, economical and biocompatible [7, 8] and have been used as fillers in HDPE [9]. Thus, in this research the suitability of natural hydroxyapatite from fish scales as fillers in the epoxy matrix was explored.

2. MATERIALS AND METHODS

2.1 Materials

Epoxy resins, Dow 331 (Dow Chemical Company, USA) and curing agent, Joint Mine 905-3S (Epochemie International Pte Ltd, Singapore) was purchased from a local supplier. Biogenic FsHA was prepared through thermal degradation technique. The fish scales ash was ball milled overnight and the particle size was determined around 5 to 10 μm .

2.2 Epoxy/FsHAp Composite Preparation

The FsHA powder was dried in the oven overnight before mixing with epoxy resin using mechanical stirred for 2 hours and followed by Sonicator Q700. The curing agent was then added and further stirred for 30 min and cast in the waxed aluminium mould. The composition of epoxy/FsHA composite is shown in Table 1. The casted resin composite was cured at 80 °C for 2 hrs and post cured for 2 hrs at 110 °C on the oven.

Table 1: Sample coding and their epoxy/FsHA content in the composites

Sample	Epoxy (wt.%)	FsHA (wt.%)
E0HA0	100	0
E5HA	95	5
E10HA	90	10
E15HA	85	15

2.3 Mechanical Testing

Flexural properties were measured by a universal testing machine (Instron, Model 3366). The distance between supports was 40 mm and the load was applied to the centre of the specimen. The tests were carried out at room temperature at a crosshead speed of 1 mm/min as per ASTM D790. The samples were prepared in dimensions of 8 mm x 12.7 mm x 101 mm. The impact properties were observed using a Charpy impact test. The composite samples were cut in accordance with ASTM A370. The standard specimen size for Charpy impact testing is 10 mm × 10 mm × 55 mm. A v-notch was cut into the specimens using a sample notcher with an angle of 45°. An average of at least five tests per sample was performed to report flexural strength and flexural modulus.

2.4 FTIR Spectroscopy Analysis

FTIR analysis was conducted on a Thermo Nicolet 6700 FTIR spectrometer using the KBr disc technique. The surface of the composite sample was scratched into powders and mixed with KBr powder and pressed into disc using hydraulic press. The FTIR analysis was performed in the wavenumber ranged from 4000 to 400 cm⁻¹.

2.5 Differential Scanning Calorimetry (DSC)

Thermal properties of the epoxy were monitored by DSC (Mettler Toledo). The sample was hermetically sealed in an aluminium pan and was scanned from room temperature to 300 °C in nitrogen gas at a heating rate of 10 °C/min.

2.6 Field Emission Scanning Electron Microscopy (FESEM) and EDX

Morphology of the sample was taken from the fractured surface of the sample. In this study Hitachi SU8020 FESEM was used. Prior to imaging, the samples mounted on aluminum stubs and coated with platinum for better conductivity using Quorum Q150RS. FESEM was also equipped with an EDX (Horiba EMAX) detector that can be used to determine the elemental compositions of the samples. The samples were radiated by electron beam, and EDX analysis needed 60 s for each point in the samples.

2.7 Cytotoxicity Study

The epoxy/FsHA was immersed in complete media with a weight/volume ratio of 200 mg/mL for 24 h at 37 °C without agitation. The positive control was represented by the extraction vehicle (complete media) with no material. The pure extract was then filtered through a 0.2 µm syringe and diluted with complete media to establish weight/volume ratios of 100, 50 and 25 mg/mL. The pure extract and the diluted extracts were added to healthy monolayer L929 cells, which were already seeded with 1×10^5 cells/mL in 24-multiwell plates for 24 h and incubated in a CO₂ incubator at 37°C/5% CO₂ for 24 h. After 24 h incubation, cell viability was tested using Alamar Blue assay (Invitrogen, USA). The culture was stained with Alamar Blue solution (1:10) and then incubated for 4 h at 37 °C in a CO₂ incubator. After incubation, the absorbance of the stained culture was detected at 570 nm using a Universal Microplate Reader (Bio-Tek Instruments, USA). Cell viability above 80% was considered to indicate non-toxicity as biocompatible materials.

3. RESULTS AND DISCUSSION

3.1 FTIR Analysis

FTIR analysis was conducted to study the presence of functional groups of materials used and to confirm the formation of composite. FTIR is also a powerful tool to identify any possible chemical interactions between epoxy resin and hydroxyapatite. Figure 1(a and b) shows the overlaid FTIR spectra of cured epoxy and epoxy/FsHA composite respectively. The FTIR spectrum of pure epoxy resin (Figure 1a) showed several characteristic absorption peaks typical to post cured epoxy. The results show the broad peak at around 3200–3600 cm⁻¹ representing the O-H stretching from the hydroxyl group of the epoxy ring or probably from residual moisture. The absorption peaks observed from 2800–3000 cm⁻¹ were corresponded to C–H stretching (CH₂ and CH₃) of epoxy alkyl groups backbone [9].

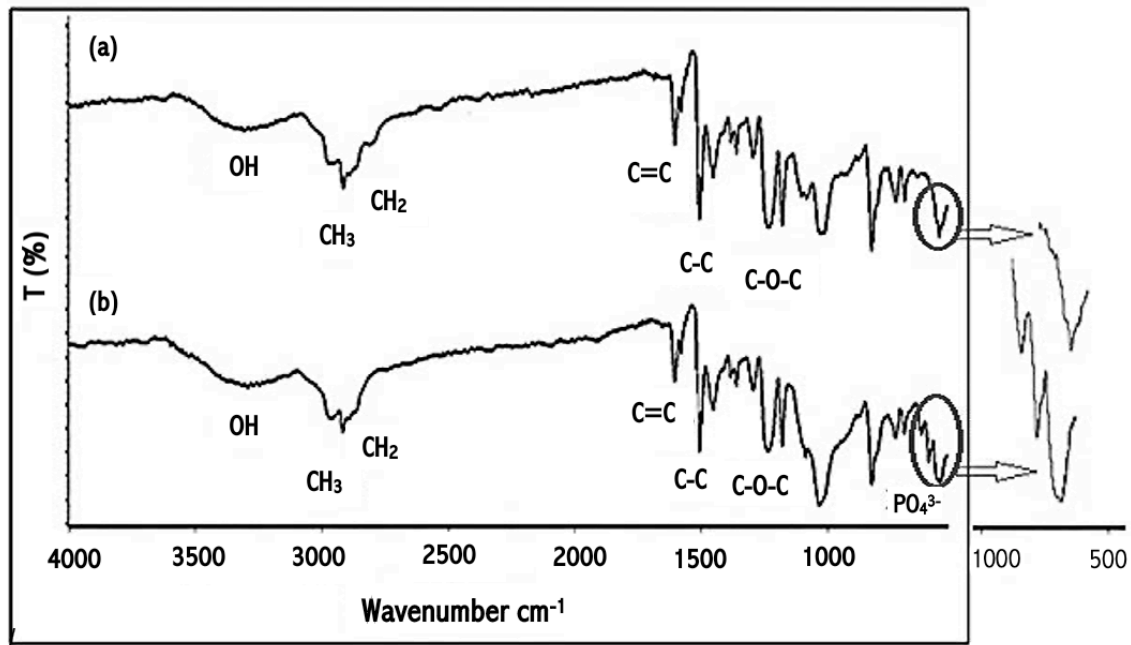


Figure 1: FTIR spectra of (a) post cured E0HA and (b) epoxy composite (E10HA)

The C–O–C stretching (epoxide group) is characteristic to absorption band between 1250 - 1150 cm⁻¹, which is indicative of the oxirane ring in the epoxy [10]. The aromatic C=C stretching is shown near 1600 cm⁻¹ associated with the aromatic ring structure in the epoxy. A peak around 900 cm⁻¹, confirming the presence of the epoxy group. Figure 1(b) shows the FTIR spectrum of epoxy/FsHA composite. From the spectrum it was observed that most of the typical absorption peaks corresponding

to epoxy were observed except the peaks corresponding to hydroxyapatite was clearly seen at around 570–600 cm^{-1} , which are specific to the phosphate groups (P-O) in hydroxyapatite [11]. The present of this peaks in the composite demonstrating that hydroxyapatite is well incorporated into the epoxy matrix.

However, no significant chemical interactions between the two components are typically observed since no peak changes occurred in the absorption band for epoxy matrix in composite. This is typically due to the non-reactive surfaces of HA and epoxy resin, which do not naturally form strong covalent bonds without surface modification or coupling agents. Similar observation was also reported by several other researchers [12].

3.2 Mechanical Properties of Epoxy/FsHAp composite

Figure 2 shows the flexural strength of epoxy/FsHA composites at different FsHA loading. The results show that the flexural strength increased with increasing FsHA loading amount. Addition of 5 wt% FsHA to the epoxy resin improved the flexural strength from 60 MPa to 90 MPa. The highest flexural strength, 106 MPa was achieved when the FsHA filler loading was 10 wt%. The improvement of flexural strength is attributed to the filler's dispersion and physical characteristics rather than chemical bonding as shown in FTIR results. High contact area between the micron size of FsHA fillers and the polymer matrix efficiently transfer the load from the epoxy matrix to the rigid HA particles [13]. Fu et al., 2008 demonstrated that filler particles may also act as barriers to stop the cracks thus preventing catastrophic failure. This mechanism enhances the overall toughness and flexural strength of the composite [14]. They also reported that addition of particulate fillers to polymers significantly improved the composite's stiffness, which directly contributed to enhancement of flexural strength.

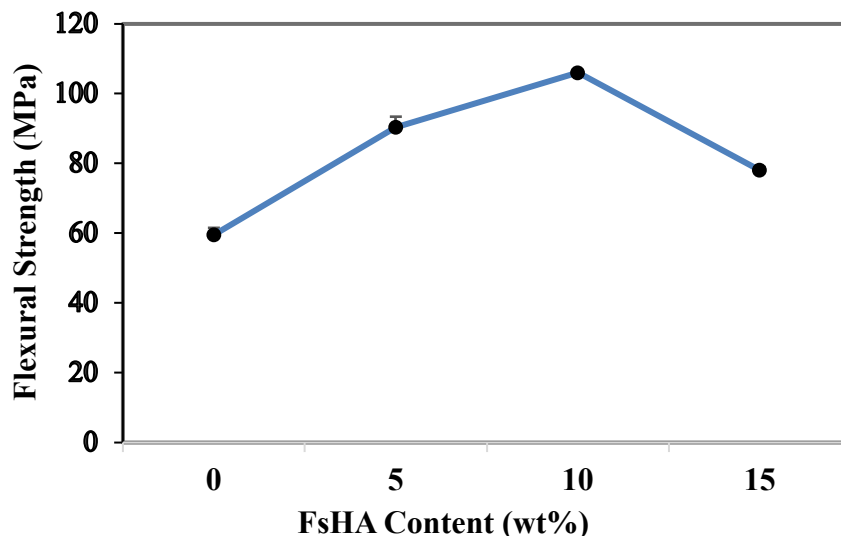


Figure 2: Flexural strength of epoxy/FsHA composites at different filler content

The amount and dispersion of particulate fillers also influence the improvement in flexural strength. For optimal reinforcement, the filler particles need to be well-dispersed throughout the matrix, preventing agglomeration and ensuring a uniform stress distribution. In this study, the optimum filler loading is found to be 10 wt% FsHA (E10HA) to achieve maximum reinforcement without compromising the matrix's integrity. However, when the fillers is above 10 wt%, it is lead to agglomeration, while too little filler (below 10 wt%) may not provide significant reinforcement. These results obtained in this study is an agreement with the study by Oladele et al. (2018) where they reported that the mechanical properties of epoxy/HA composite were improved as the content of HA is increased. They also reported that the addition of 5 to 10 wt% HA significantly improved flexural strength of the composite [15].

The effect of filler loading on impact strength of epoxy/FsHA composites is shown in Figure 3. Results showed that the impact strength increased with increasing FsHA loading amount. The result shows a similar trend to flexural strength results as shown in Figure 2. Addition of rigid filler such as FsHA in epoxy can act as a reinforcing agent in the matrix. The composite ability to absorb and dissipate energy during impact will be increased. The increase in impact strength indicates that the toughness of epoxy composite was improved with the addition of FsHA fillers. The highest impact strength was 6.8 kJ/m^2 is shown for E10HA composites with filler content of 10 wt%.

The presence of FsHA particles increases the composite's toughness by introducing mechanisms like crack deflection, crack pinning, and energy dissipation. The rigid FsHA particles can absorb part of the energy during impact, reducing the energy transferred to the matrix and thus preventing the failure. The energy absorbed by the particles is redistributed through the composite, which can increase the overall toughness [12]. FsHA particles can act as obstacles in the matrix, causing cracks to deflect, slow down, or stop. This process, known as crack deflection or crack pinning, prevents rapid crack propagation, improving the toughness and impact resistance of the composite [14].

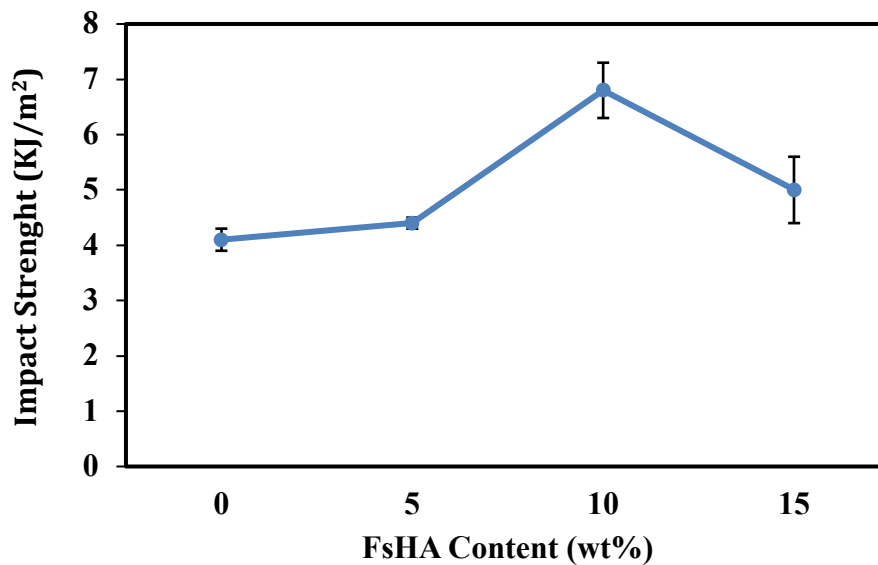


Figure 3: Effect of filler content on impact strength of epoxy /FsHA composite

However, the impact strength is reduced when filler content is above 10 wt%. The reduction of impact strength is expected due to agglomeration of the FsHA in epoxy matrix. Similar observation was also reported by Joseph et al, 2024 when they studied the effect of fish scales and calcium carbon loading on epoxy resin. They reported that at high filler concentration, the mechanical properties reduce due to filler agglomeration, void formation and reduce composite homogeneity and ductility [16].

3.3 Morphological Analysis Of Epoxy And Epoxy/FsHA Composite

The fracture surface morphology of fully cured neat epoxy (E0HA) and epoxy composites reinforced with hydroxyapatite fillers (E10HA and E25HA) was examined via scanning electron microscopy (SEM), as presented in Figure 4. The SEM image of the neat epoxy (Figure 4a) revealed a smooth and featureless surface, indicative of brittle fracture behavior. This is attributed to the highly crosslinked network structure of the epoxy matrix, which restricts molecular mobility and inhibits plastic deformation, resulting in rapid crack propagation. Correspondingly, the neat epoxy exhibited the lowest impact strength, as shown in Figure 3. These findings are consistent with those reported by Arulmozhivarman et al. [17], who observed similar brittle fracture characteristics in unmodified epoxy systems.

In contrast, the E10HA composite (Figure 4b) displayed a rougher and more irregular fracture surface, reflecting improved energy dissipation mechanisms such as crack deflection and matrix–filler interfacial interactions. The microstructure also demonstrated homogeneous dispersion of the FsHA particles with minimal agglomeration, which correlates with the enhanced flexural and impact strengths observed in Figures 2 and 3. However, at higher filler loading (25 wt%) in the E25HA composite (Figure 4c), the SEM image revealed increased filler agglomeration and non-uniform dispersion. These morphological features are likely to introduce stress concentration sites, negatively impacting the composite's mechanical performance.

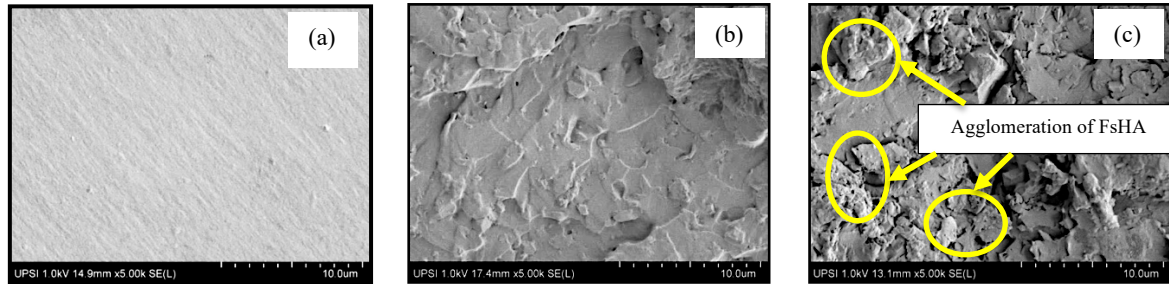


Figure 4: Scanning electron micrographs for (a) E0HA (b) E10HA composite and (c) E25HA composite

At high filler loading, particle agglomeration occurs due to increased van der Waals forces and reduced dispersion efficiency. These agglomerates lead to a decrease in the effective surface area available for interaction with the polymer matrix. Consequently, the interfacial bonding between the matrix and fillers weakens, reducing the ability to transfer stress efficiently. This phenomenon negatively affects the mechanical properties, including tensile and impact strength, of the composite [18].

Particle dispersion of FsHA fillers based on calcium (Ca) content in the polymer matrix was determined by EDX mapping analysis as shown in Figure 5. The EDX mapping results shows no Ca element was found in epoxy resin (Figure 5(a)) whereas well distribution of Ca element in E10HA sample (Figure 5(b)) which indicated well distributions of fillers in epoxy matrix with 10% loading. On the other hand, pure fillers distribution and agglomeration is clearly seen in EDX mapping of composite with 25 wt% filler loading as shown in Figure 5(c). These results in agreement with the mechanical properties of composite obtained and confirm filler distribution will affect the mechanical properties of epoxy composite [19].

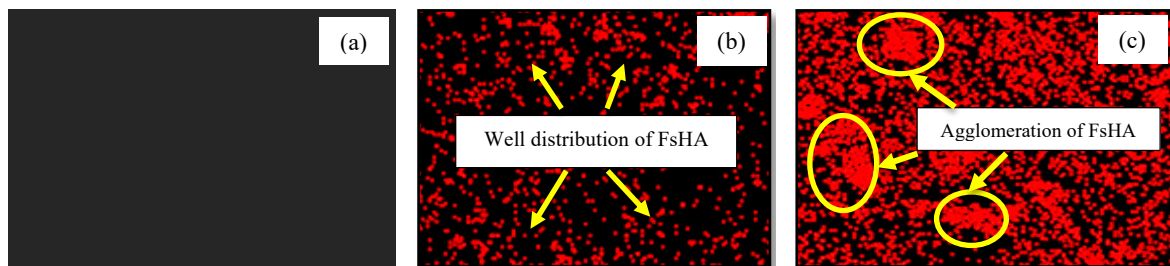


Figure 5: EDX mapping distribution of calcium (Ca) on surface of epoxy matrix for (a) E0HA (b) E10HA composite and (c) E25HA composite

3.4 DSC Analysis of Composite

Figure 6 shows the DSC curve of the post-cured epoxy and epoxy composite. The DSC curves did not show any exothermic peak which indicating fully cured epoxy and composite. The results indicates that heat treatment used for curing the sample is sufficient to fully cured the epoxy resins. From the results, the glass transition temperatures (T_g) for both samples are clearly seen. The T_g for

neat epoxy is observed at 56.7 °C whereas for epoxy composite is observed at 59.4 °C. The T_g of a composite is slightly higher compared to pure epoxy because the fillers within the composite restrict the molecular mobility of the epoxy matrix. This restricted mobility makes it more difficult for the polymer chains to move freely when heated, thus requiring higher temperatures for the material to reach its glass transition [20].

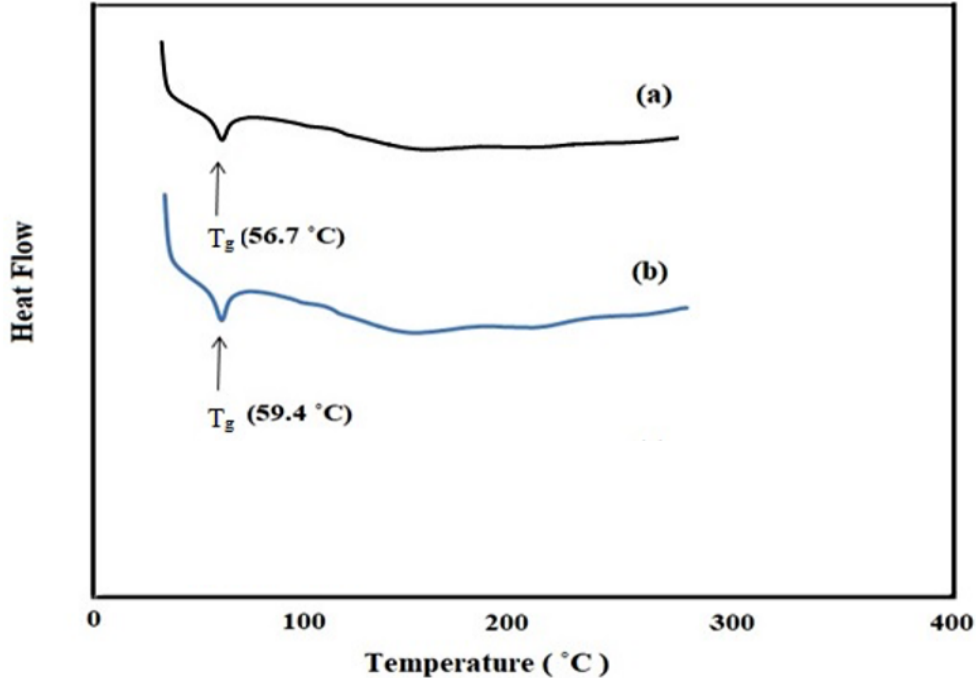


Figure 6: The DSC thermogram of (a) E0HA (b) E10HA composites.

3.5 Cytotoxicity Analysis

The cytotoxicity of the E10HA composite was evaluated using the Alamar Blue assay in accordance with ISO 10993-5:2009, which outlines standardized procedures for assessing the in vitro cytotoxicity of medical device materials. L929 mouse fibroblast cells were used for the assay, and the results, shown in Figure 7, demonstrated cell viability exceeding 100% at all tested concentrations (25, 50, 100, and 200 mg/ml). This indicates that the composite material is non-cytotoxic and supports cell proliferation. These findings suggest the epoxy/FsHA composite exhibits promising biocompatibility for potential biomedical applications, particularly in implantable devices. Nonetheless, further investigations are warranted to evaluate its biological performance in human tissue environments.

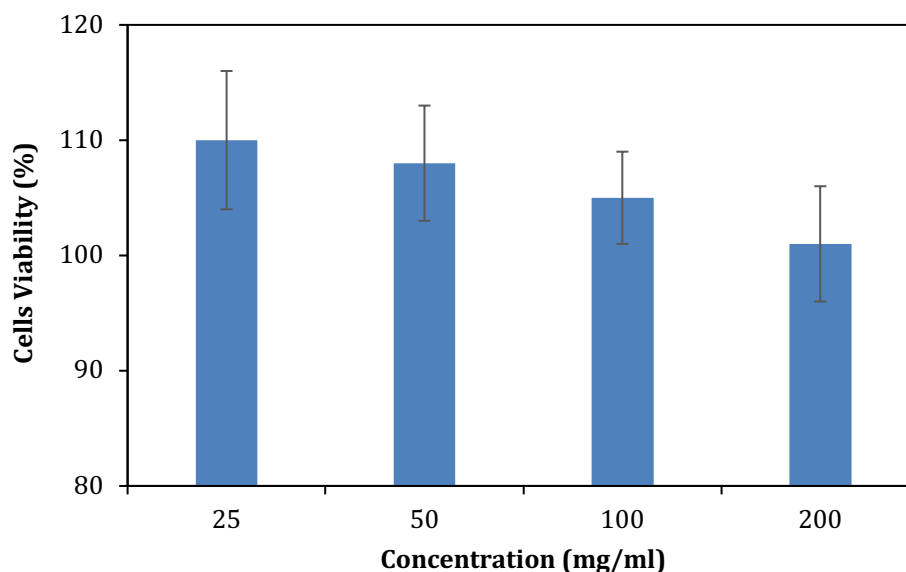


Figure 7: Percentage of cell viability (L929) at various concentration of E10HA composite.

4.0 CONCLUSION

Epoxy composites were successfully fabricated using fish scale-derived hydroxyapatite (FsHA) as particulate fillers. The composite containing 10 wt% FsHA exhibited optimal mechanical performance, achieving a flexural strength of 106 MPa and an impact strength of 6.8 kJ/m². FTIR analysis confirmed the absence of chemical interaction between the fillers and the epoxy matrix, while FESEM micrographs demonstrated uniform filler dispersion at the optimal loading. Furthermore, cytotoxicity assessment confirmed the composite's non-toxic nature. Collectively, these findings suggest that the epoxy/FsHA composite possesses favourable mechanical and biological properties, making it a promising candidate for biomedical applications.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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