

Fabrication and Testing of Nano Bio-silica, Hemp, and Bamboo Fibre-Reinforced Chitosan Bio-composite Material

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Abstract

This article investigates the mechanical, thermal, and biocompatible properties of chitosan-reinforced bamboo, hemp, and nano-bio-silica-reinforced composites (CP, CF, C1, C2, C3) using hand layup. Nano bio-silica at 1%, 2%, and 3% was used to fabricate C1, C2, and C3. C2 exhibited superior mechanical strengths, with a tensile strength of 142.44 MPa, flexural strength of 186.58 MPa, and compressive strength of 64.20 MPa. It demonstrated low thermal conductivity (0.2460 W/m-K) and minimal mass loss in TGA. MTT assays confirmed non-cytotoxicity for CP, CF, C1, C2, and C3, highlighting C2's potential as a biocompatible material against *S. aureus* gram-positive bacteria.

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Keywords: Biodegradable material, Natural fibre composite, Mechanical testing, Hand-layup method, Thermal analysis.

1. Introduction

Nowadays many natural fibre-reinforced materials researches are done for meeting eco-friendly and sustainable materials. Although human body implants are one of the emerging fields in biomedical engineering, Bio implants need new materials with excellent biocompatibility and strength. Bone plates in orthopaedic implants' primary objective are to correct the position of fractured bone and resist the tensile stress at the fractured surface.[1]. Although titanium[2], stainless steel high strength material, corrosion resistant materials are used for bone fracture plates the young modulus of this material is very high thus the stiffness is mismatched to the human bone thus more load is taken by these plates and stress shielding in bone occur.[3]. Thus designing the orthopaedic bone plate and minimizing the effect of stress shielding would be done when the composite material is fabricated with similar stiffness to bone and replace the use of titanium and stainless steel based bioimplants .[4]. Natural Composite material are defined as matrix reinforced natural fibres like Banana [5], bamboo , hemp fibres, rice husk[6] etc. Mechanical[7] , thermal[8][9] ,wear [10], biocompatible testing are necessary for the analysis of composite material[11]. Thus, finding new fibres and nano-bio filers is the new trend in research [12]. Bioimplants come in direct contact with the human body's tissue, fluid, and bone. Thus, bio-implants should be made up of biomaterial, where biomaterial is defined as material that is not considered in medicine or any type of synthetic

substance that may be employed in the human body to replace any part, organ or tissue of the body to improve the quality of life[13]. Biomaterials that are utilized in biomedical applications are commonly in four major forms polymers[14], metals, ceramics, and composites. Polymeric biomaterials are commonly classified as polysaccharides, proteins and biodegradable polymers[15]. Natural Polysaccharides are the most abundant naturally occurring macromolecular polymers obtained from algae, fungi, plants, bacteria, and microorganisms[16]. Natural Polysaccharides from different origins play an important role in many physiological and biological activities like antioxidant, anticancer, antihyperglycemic, and immunological regulatory properties[17]. Chitin is a present as one of the crucial structural components in living organism. It is second most abundant biopolymer after cellulose. It is a long-chain polymer and it is composed of $\beta(1\rightarrow4)$ -linked 2-acetamido-2-deoxy- β -D-glucose(N-acetylglucosamine) as shown in Figure 1, its presence is found in many unicellular like diatoms, fungi etc. and multicellular like sponges, corals, worms, and arthropods etc.[18]. Chitin has three isoform according to the arrangement of the backbone's side chain: α -chitin, β -chitin and γ -chitin[19]. Chitosan is a sugar like polysaccharide which is obtained from hard surface of skeleton of shellfish, crab, lobster, and shrimp. It can also be obtained from deacetylation and its also copolymer of N-acetylglucosamine and glucosamine. Chitosan is also a linear polymer of $\alpha(1\rightarrow4)$ -linked 2-amino-2-deoxy- β -D-glucopyranose (Figure 2)[20].

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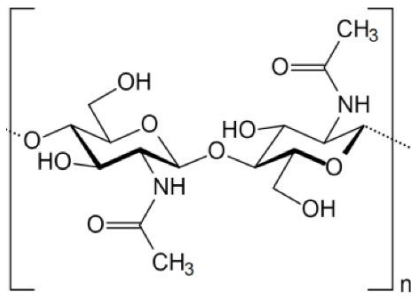


Figure 1. Structure of Chitin

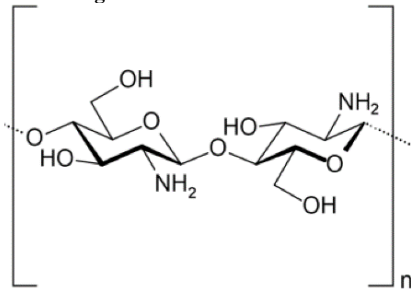


Figure 2. Structure of Chitosan

Chitosan's mechanical properties are found to be poor, so it needs some improvement for using in biomedical application, bio adhesives, waste materials etc[21]. Chitosan-spirulina extract tensile strength are tested with maximum value of 29 MPa among antimicrobial, graphene oxide and glycerol extracts [20], [22]. Thermal degradation is also prone in chitosan. Chitosan cannot resist the temperature beyond 220°C [23]. Chitosan has high solubility, it allows to be produced in various forms like films, hydrogel, pastes etc. so chitosan can be formed in various forms and structure in many biomedical industries[24].

Bamboo fibres are observed with high density of 1.5g/cm³ and maximum tensile strength of 575 MPa [18]. Bamboo fibres are also evidenced with high young modulus of 27-40 GPa among many natural fibres [19]. Bio silica is also a material present in unicellular organisms, plants, and animals which are crucial for their structural integrity, defense mechanisms, and physiological processes [20]. The biocompatibility, biodegradability, and cell proliferation capability of silica-derived biomaterials make them useful in bone tissue engineering [21]. The high mechanical strength of bio-silica makes it appropriate for use in bio-implants[25], [26] Silica derived material is hydrophilic in nature because of presence of silanol group (Si-OH) [23]. Bio-silica's good mechanical strength makes it suitable to use in bio implants[27]. Bioactive glass derived from silica is shows bone formation properties[28]. Tensile strength of

chitosan blended with One-fifth of bioactive glass is found as 67MPa[29]. Young modulus of silica and chitosan is found to be approximately 6 GPa and 7 MPa respectively[30], [31].Hemp fibres are one of the highest strength natural fibres with young modulus of maximum 15GPa , tensile strength of 637 MPa[32]. FEA simulation are used to solve complex problems [33]–[36].A number of researchers used the simulations for bio implants using CAD software's [37], [38]. Finite element method is the numerical technique to solve the complex problems using mathematical equations[39]–[43].Stress intensity factors and cracks can be evaluated using FEM simulations [44].

In this study, nano bio-silica, bamboo fibre, and hemp fibre are reinforced in chitosan matrix to determine its mechanical, thermal, and biocompatible properties, as well as to develop a new type of orthopaedic long bone material to improve osseointegration and reduce bone stress shielding.

2. Material and Methods

2.1. Materials

In this experiment, a bamboo and hemp woven mat is used as primary reinforcement and bio-silica nano-particles are used as the epoxy-chitosan matrix's secondary strengthening. The base material chitosan (marine derived) is used in powder form of size 2-3µm and density 0.38g/ml and 1526g/mol molecular weight supplied by Chenchems Chennai, India. Resin (Epoxy LY556) and hardener (HY951) are a density of 0.96 g/cc and 1.18g/cc respectively supplied by Chenchems Chennai, India. Bio-silica (wheat husk derived) size of 20nm, density 2.7gg/cc and white colour is supplied from Herinba Instruments and Chemicals, Chennai, India. Bamboo woven mat and hemp mat is of 220GSM with a density of 1.3 g/cc and 1.4gg/cc respectively which is supplied from Gogreen Products, Chennai.

2.2. Composite Fabrication

Blending of a chitosan with solvent (Acetic acid) in ratio 1:100[45]. The mixture is stirred in a magnetic stirrer for 10 minutes at 430 rpm at room temperature. Then the mixture is blended with resin solution in 1:5 and solution is kept on ultrasonic cleaner for 20 min at room temperature and finally nano biosilica particles are added with 1%, 2% and 3% by volume in the resin solution(Table 1). The resin-chitosan blend was mixed with 1wt.% of hardener and mixed for 10 min using a sonicator. This process is shown and described in Figure 3.

Table1. Composite designation according to chitosan, resin, fibre, the bio-silica nanoparticle composition

Bio-composite Designation	Chitosan-resin blend (vol%)	Fibre (vol%)	Bio-silica nanoparticles (vol%)
CP(Composite Plain)	100	-	-
CF(Composite with Hemp, bamboo and hemp(hbh) Fibre)	50	50	-
C1(Composite with hbh Fibre+1% silica oxide)	49	50	1
C2(Composite with hbh Fibre+2% silica oxide)	48	50	2
C3(Composite with hbh Fibre+3% silica oxide)	47	50	3

Using the hand layup method composite material is fabricated, in which a mould of 30x30x3.5cm was prepared using silicon rubber and waxed fully for the easy removal of the composite. Once the complete solution is made, the solution was poured into the mould and the fibre of three lamina is laid one by one (Figure 4). Finally using a compression moulding machine, it is pressed at 5 bar pressure under room temperature for 1hr. After the compression, the material is kept to cure for 12 h and a post-cured for 2 hours at 120°C is done for final fabrication. The bamboo, Hemp mat in hand layup process and Composite material are shown in Figure 4. Using the hand layup process Five categories of samples are fabricated and designated as shown in Table .1. Post-cured composites according to designation (Table.1) are cut into samples as per the ASTM standard using Abrasive Jet Machining (AJM). The AJM cutting process was performed with an operating pressure of 220psi with a stand-off distance of 3mm and nozzle diameter is 1.1mm and a flow rate of abrasives is 0.42 g/s. [46]. However the samples cutting process effects the fabricated composites due to its cutting parameters , feed etc.

3. Characterization of Bio-Composites material

3.1. Mechanical Properties

3.1.1. Tensile, Compressive, and flexural testing

Universal Testing Machine (UTM), FIE(Fie Electronic Universal testing Machine), India was used to analyse the mechanical strength, including the flexural, tensile, and compressive properties of CP, CF, C1, C2, and C3. According to ASTM D 3039 standard, the tensile

characteristics are assessed on samples measuring 229x25x3.3 mm at a transverse speed of 1.5mm/min. According to the ASTM D695 standard, the compression test is carried out at a transverse speed of 1.5 mm/min on samples of 38.1x12.7x3.3 mm. According to ASTM D790, the flexural test is carried out on samples measuring 127x12.7x3.3 mm at a transverse speed of 1.5 mm/min.

3.2. Surface Morphology of Tensile failure

The morphology behaviour of fractured tensile specimens is examined using SEM images using Joel JEM(Japan Electron Optics Laboratory Company) machine; a working image of approximately 25mm and voltage of 5KV.

3.3. Thermal Properties

3.3.1. Thermal conductivity and Thermogravimetric Analysis

A thermal conductivity test is performed on Metro Precision Testing Equipment on the principle of the Lee disc method is performed on the disc specimen of 40 x 3.3 mm. The specimen's test temperature is recorded on a heat input of 1°C/min. The thermal behaviour of chitosan-based matrix composite material using thermogravimetric analyser (TG/DTA SIINT 6300 Japan) under Air atmosphere. A sample weight Samples were scanned from 0°C to 800°C at a heating rate of 20°C and the pan material Alumina (Al_2O_3). The thermo-gravimetric study is carried out to look at how much mass a polymer composite material loses as the temperature rises.

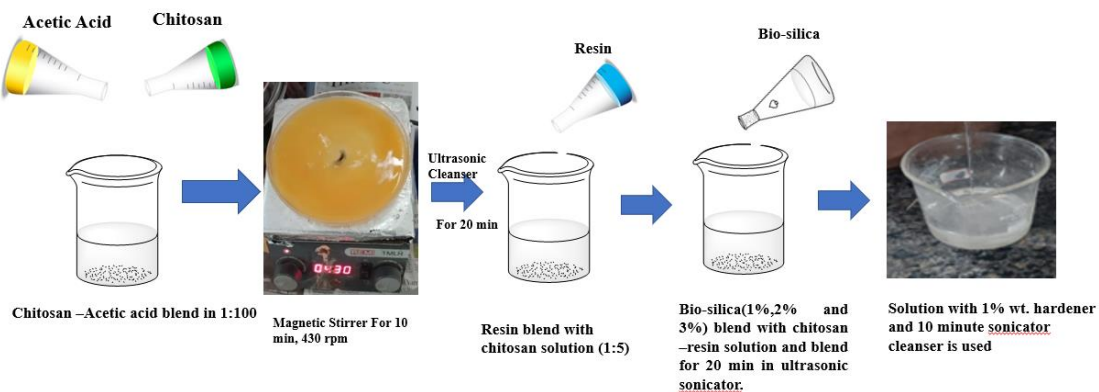


Figure 3. Fabrication of Chitosan-Bio-silica Matrix material using solution making method.

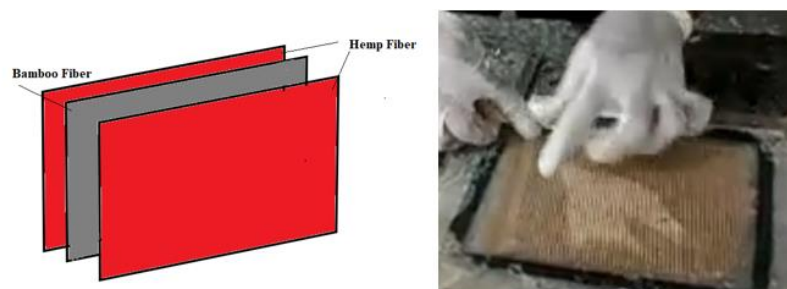


Figure 4. Fabrication of Chitosan-Bio-silica Composite material (A) Bamboo and hemp woven mat Fibre(hbh) (B) Hand layup method

3.4. Biocompatibility Test

3.4.1. MTT assay

The MTT assay is done to check the cell viability of CP, CF, C1, C2 and C3. In this cell lines are taken from National Centre for cell science pune, India. The cell lines are cultured Dulbeccos Modified Eagles and incubated with humidity in Galaxy® 170 Eppendorf, Germany. The cells then seeded in well plates and acclimatize for 24hour with 37°C and 5% CO₂ environment.[47]. The absorbance at 570 nm was measured with micro plate reader. Two wells per plate without cells served as blank[48]. All the experiments were done on CP, CF and C3 samples in triplicate format where the cell viability was expressed using the Equation 1.

$$\text{viability} = \frac{\text{Percentage of cell}}{\text{Average absorbance of treated}} \times 100 \quad (1)$$

4. Results and Discussion

4.1. Mechanical Properties

Tensile, compressive, and flexural properties of composite material are observed and the influence of bamboo and hemp fibre and inclusion of nano particles of bio-silica on the chitosan based composite materials. The mechanical properties of CP, CF, C1, C2 and C3 composite materials for the five samples are shown in Table 2. The tensile stress-strain behaviour, Compressive Load-

displacement behaviour and flexural Load-deformation of all five-sample composite material are shown in Figure 5, 6 and 7 respectively and their statistical analysis of one-way ANOVA with three specimens of each sample are represented in the form of $N (=3) \pm SD$ with their P value in Figure 8, 9 and 10 respectively.

The tensile behaviour of CP, CF, C1, C2 and C3 is shown in Figure 5. From Figure 5 and Figure 8, CF is evidenced with more strength than CP due to the presence of three layer of bamboo and hemp mat. The tensile strength is increased to 128.80 ± 2.9 MPa and 142.44 ± 2.0 MPa in C1 and C2 from CF. However C3 is evidence 115.69 ± 4.3 MPa due to agglomeration of nano bio-silica material particles[49]. The compressive load vs displacement shows CP with premature failure and C1, C2 and C3 with secondary failure are shown in Figure 6. From Figure 9 it is evidenced that the compressive strength and compressive modulus of hemp-bamboo-hemp fiber reinforced chitosan is increases by 40% and 30 % respectively compared to plain chitosan matrix. The average ultimate flexural strength and flexural modulus of composite material were observed as 156.86 ± 9.412 MPa and 5.42 ± 0.2856 MPa (Figure 7 and Figure 10) respectively. The flexural properties of chitosan based composite materials are tabulated in Table 2. Figure 7 shows that the reinforcement of three-layer of hemp-bamboo-hemp fibre in chitosan-resin gives high flexural strength of 157.90 MPa in CF composite material when compared to CP (107.45MPa). C2 composite material is observed with secondary failure but the C1 and C3 composites are evidenced with only premature primary failure.

Table 2. Mechanical Properties of Bio-Composite material.

Composite samples	Tensile stress (MPa)	Tensile Modulus (GPa)	Flexural Stress (MPa)	Flexural Modulus (MPa)	Compressive Stress (MPa)	Compressive Modulus (GPa)
CP	67.14±2.32	2.08±0.47	107.45±10.40	2.41±0.355	30.95±4.788	2.09±0.47
CF	113.49±2.30	4.75±0.53	151.90±13.50	6.17±0.329	54.81±3.248	4.82±0.54
C1	128.80±2.96	4.29±0.29	176.01±4.33	6.13±0.113	57.31±4.22	4.31±0.31
C2	142.44±2.14	4.60±0.541	186.58±19.9	6.51±0.478	64.20±1.7133	4.71±0.55
C3	115.69±4.31	4.57±0.494	162.24±8.23	5.89±0.196	62.59±2.212	4.2±0.48

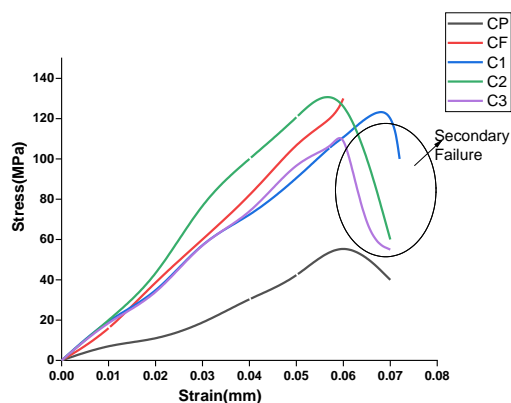


Figure 5. Tensile Stress-strain curve of bio-composite material

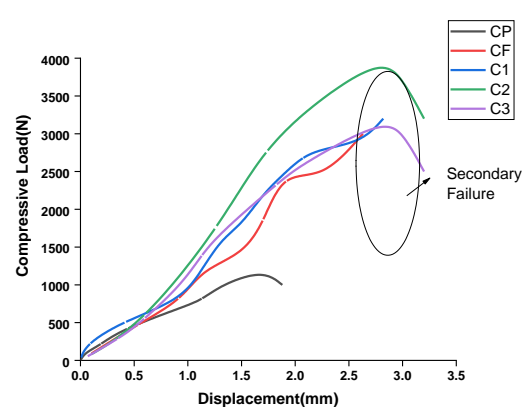


Figure 6. Compressive Load-Displacement curve of bio-composite material

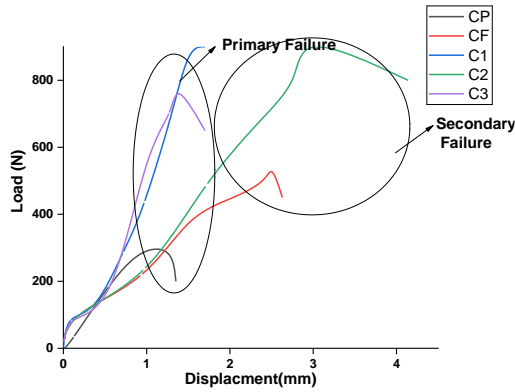


Figure 7. Flexural Load-Displacement curve of bio-composite material

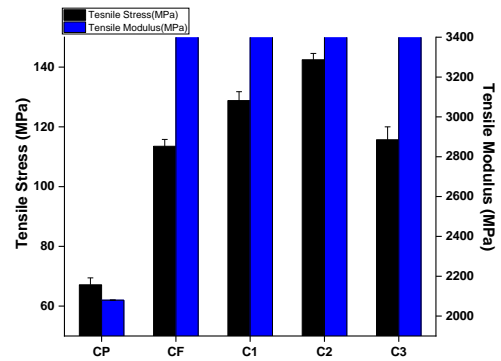


Figure 8. Tensile Modulus and Flexural strength of CP, CF, C1, C2 and C3 biomaterial are represented. Data are expressed in mean \pm SD and $p < 0.0001$.

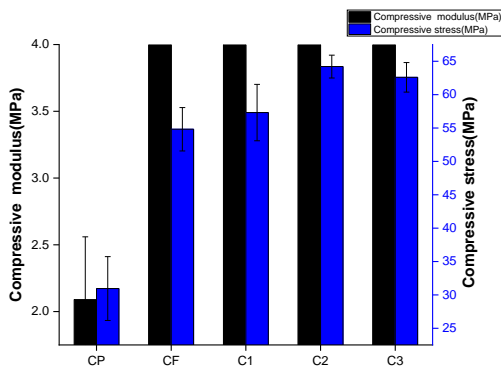


Figure 9. Compressive Modulus and Flexural strength of CP, CF, C1, C2 and C3 biomaterial are represented. Data are expressed in mean \pm SD and $p < 0.0001$.

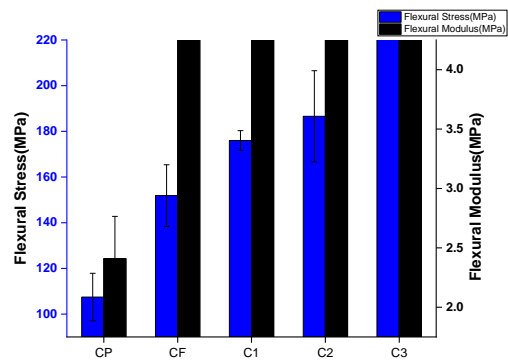


Figure 10. Flexural Modulus and Flexural strength of CP, CF, C1, C2 and C3 biomaterial are represented. Data are expressed in mean \pm SD and $p < 0.0001$.

The statistical analysis of One-Way ANOVA is done tensile and tensile modulus, compressive strength and compressive modulus, flexural strength and flexural modulus and found that P value is less than 0.0001. Which neglects the Null hypothesis and proves that all the material strength and modulus are different from each other and a significant impact is done. The Figure 8, 9 and 10 it is observed that C2 has highest strength and rigidity among all the five samples and adding 3% volume of nano bio-silica (C3) particles decreased the strength and modulus in tensile [50], compressive and flexural testing [51]. This proves the agglomeration of nano bio-silica particle in the bio-composite material.

4.2. Surface Morphology of Tensile failure

To comprehend the morphological behaviour of the composite material, SEM micrographs of all fractured tensile specimens are examined (Figure 11).

To better comprehend the images, magnification (20X to 500X) is applied. Figure 11 depicts the uniform distribution of chitosan particles in CP composite material. There is no evidence of non-homogeneity in the specimen, and a clean fracture is observed. Bamboo fibres are indicative of CF-fractured specimens. This decreases the tensile strength of specimen C3[51].

CP samples shows no reinforcement thus resins can soften under mechanical loading due to the conversion of mechanical energy into heat. This thermal softening effect can lead to a reduction in stress at the onset of plastic deformation, resulting in a low yield point. While voids may not be observable macroscopically, there could still be defects at the microscale, such as micro-voids or regions of incomplete polymerization, which could influence the material's mechanical behaviour and contribute to the low yield point.

The need of attaining a balance between filler content and matrix integrity for optimal material performance is shown by the physical relevance of voids in C2 and C3. C2 exhibits the highest level of strength despite the existence of voids, suggesting the presence of effective reinforcement mechanisms. Conversely, c3 displays the lowest strength because of the detrimental impact of voids on material properties. To attenuate the negative impacts of void formation and improve the mechanical characteristics of materials with high bio-silica concentrations, it is possible to address this issue by implementing enhanced processing techniques, such as improved dispersion methods or optimising filler-matrix interactions.

4.3. Thermal Characteristics

Thermal conductivity evaluation of Chitosan-based materials Samples of composite materials are examined based on their classification (Table 1). Consideration is given to three specimens for each CF, CP, C1, C2, and C3. The mean ± SD values of all the samples are shown in Figure 12. Figure 13 depicts the thermo-gravimetric values of chitosan-based matrix. Initial (220 °C to 480 °C) and intermediate (450 °C to 800 °C) decomposition are observed for all composites (CP, CF, C1, C2, and C3) and are listed in Table 3.

Table 3. The area of change in mass percentage loss in Thermogravimetric Analysis

Designation	Mass loss in Initial decomposition Percentage (%)	Mass loss in middle decomposition Percentage (%)
CP	2	54
CF	1.9	51
C1	1.8	32
C2	1.56	34
C3	1.56	54

According to Figure 12, bamboo, and hemp mat increases thermal conductivity by 6% from CP to CF. There is evidence that the thermal conductivity of CF increases by 4.2%, 8.13 %, and 13% for every 1%, 2%, and 3% composite volume increase of nano-bio-silica particles[52].

According to Table 3 and Figure 13 (CP, CF, C1, C2, and C3), the chitosan material undergoes two stages of thermal degradation. All bio composite materials decompose between 40 and 60 percent at temperatures between 220 and 480 degrees Celsius. This is evidenced due to the release of volatile compounds such as water, and acetic acid in chitosan, [53], bamboo[54] and silica[55].

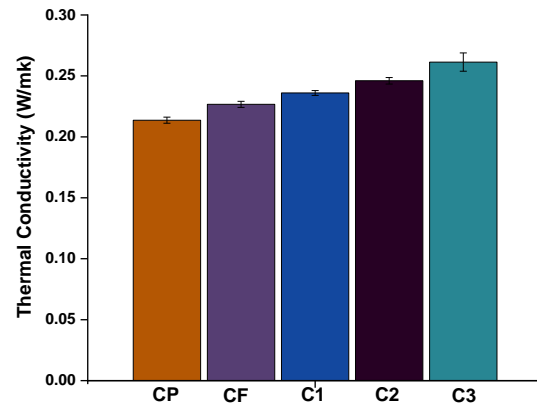


Figure 12. Thermal Conductivity of Composite material of CP, CF, C1, C2 and C3. Data are expressed in mean ±SD.

In the second stage of CP and CF composites, the stability of CP is 40% and that of CF is 35%, so the addition of bamboo and hemp fibre enhances the stability between 450 and 800 degrees Celsius. In the second stage of decomposition, C1, C2, and C3 lose 34%, 30%, and 40% of their weight, respectively. This is observed between 400 and 700 degrees Celsius on average. Thus, it is demonstrated that the addition of nano-silica particles accelerates the rate of decomposition at 700 °C; however, the material is more stable than CP and CF materials.

4.4. Biocompatibility Test

4.4.1. MTT Assay

The cell images of CP, CF, C1, C2 and C3 for 6.25µg/ml is shown in Figure 14. Figure 15 displays the percentage viability of various test sample percentages for CP, CF, and C3. For CP, CF, and C3, the concentrations of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml are indicated with the cell viability %.

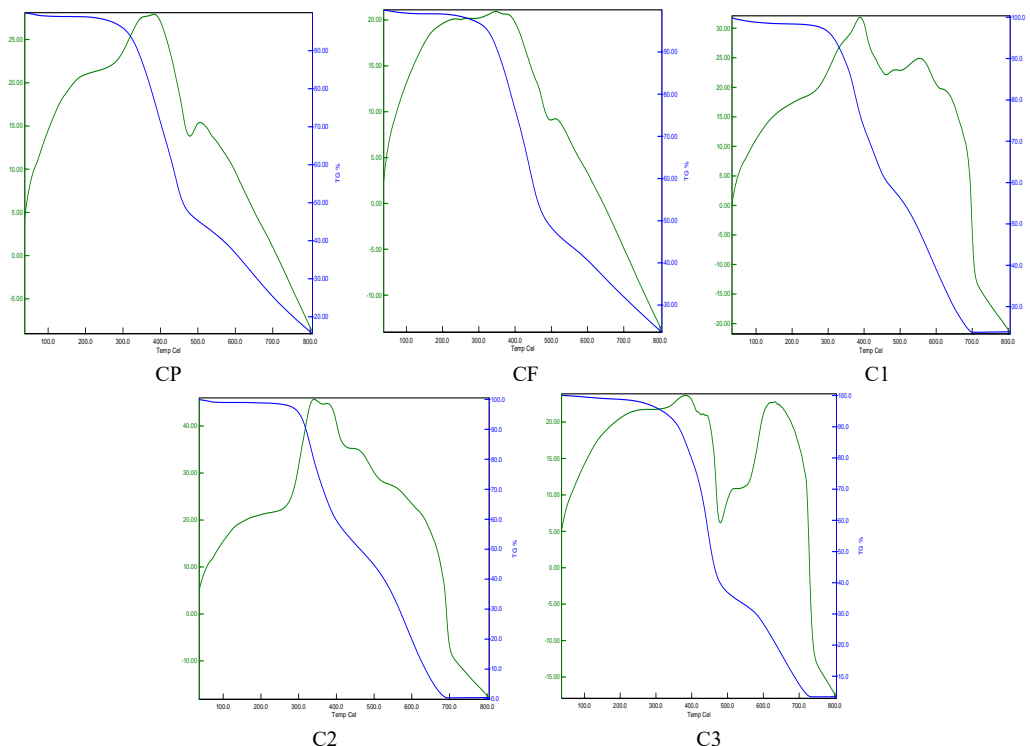


Figure 13. TGA Curves of CP, CF, C1, C2 and C3 composite material.

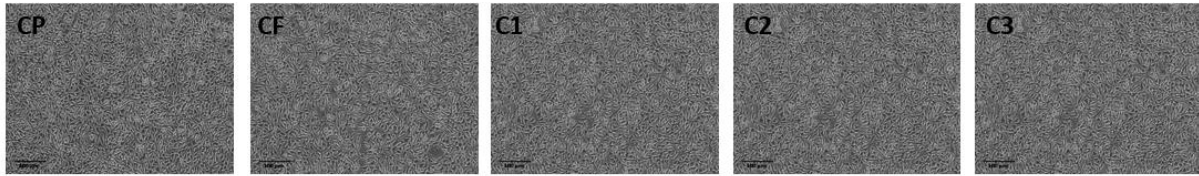


Figure 14. The cell images at 100µm microscopic scale for CP, CF, C1, C2 and C3.

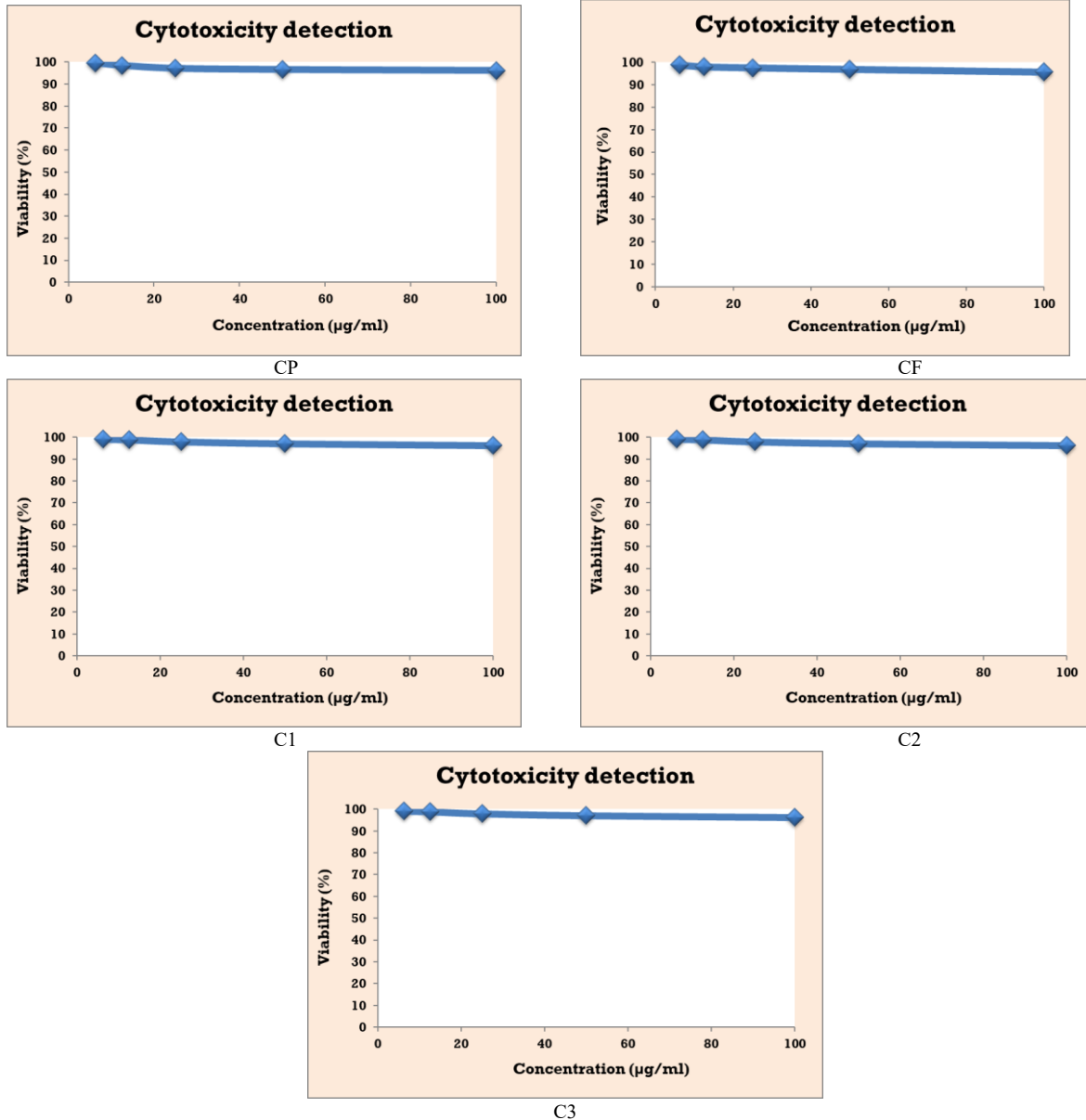


Figure 15. Percentage of cell viability vs concentration of CP, CF and C3 exposed to cell for CP, CF, C1, C2 and C3.

Figure 14 depicts the percentage of viable cells versus the concentration of sample CP, CF, C1, C2 and C3 administered to Cell. As the concentration increases from 6.25 g/ml to 100 g/ml, the percentage of viable cells decreases, but it is always greater than 95% for CP, CF, C1, C2 and C3. This demonstrates that none of the samples are cytotoxic. Figure 15 demonstrates that C1, C2 and C3 are most of the most biocompatible materials, whereas CP is the least biocompatible material at 100 g/ml concentration.

Therefore, bio silica is effective at enhancing biocompatibility. This indicates that the majority of sample cells are still viable and in excellent health after exposure to these substances at this concentration[56].

5. Conclusion

Numerous studies are conducted to find new materials for bio-implants employing zirconia, ceramics, titanium alloys, stainless steel, and other materials. These materials are stiffer than human cancellous and cortical bone and have high youthful moduli, thus a novel material with stiffness like human bone tissue is needed to reduce the stress shielding effect. In this study, a bio-composite material is employed to lessen the stress shielding effect and it was discovered that:

- The mechanical characteristics of the bio-composite materials C2, C1, C2, and C3, C2 has the highest tensile, compressive, and flexural strength and modulus.
- The maximum thermal conductivity of C3 is 0.2613 ± 0.0075 W/mK, and thermal degradation of C2 composite material is proved to be most stable in thermal analysis.
- SEM images depict the agglomeration of nano bio-silica particles, and bamboo and hemp fibre and chitosan with nano-silica particles.
- The addition of reinforcing nano bio-silica particles increases cell viability and reduces biofilm formation in C3 bio-composite material.
- The addition of 3% nano bio-silica particles from volume decreases the mechanical strength, thermal conductivity, and loss of mass in TGA which restricts the addition of nano-silica particles.
- The materials fatigue life, wear rate, impact and biofilm analysis etc. can be done for better understanding behaviour of material and its suitability for orthopaedic bone fracture plates/bio implants.

Disclosure statement

The co-authors don't have any relevant financial or non-financial competing interests.

Declaration

No conflict of interest.

Data Availability Statement

Data will be available on request from author.

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Declaration of interests

- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.