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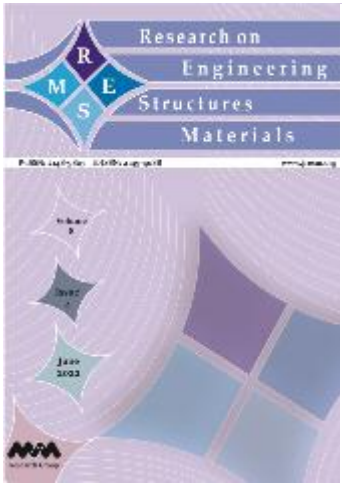

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Research Article

Influence of MWCNTS on mechanical and in vitro biocompatibility properties of PMMA bone cement for orthopedic application

T V Vineeth Kumar[1,a], N Shanmugapriya[2,b], Arun S[3,c], Madeva Nagaral[4,d]

[1,2]*Department of Mechanical Engineering, Siddaganga Institute of Technology (Autonomous Institution Affiliated to Visvesvaraya Technological University, Belagavi), Tumakuru 572 103, Karnataka, India*
[3]*Advanced Composites Division, CSIR - National Aerospace Laboratories, Bengaluru 560 017, Karnataka, India*
[4]*Aircraft Research and Design Centre, Hindustan Aeronautics Limited, Bengaluru 56003, Karnataka, India*

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Abstract

Polymethyl methacrylate (PMMA) serves as sealing material in securing the implant and distributing the load between the implant and bone. Fast polymerization and speedy patient recovery after surgery are the main benefits of using PMMA bone cement. Considering PMMA for orthopedic applications, the mechanical properties and biocompatibility studies are important. In this study, Simplex P bone cement is reinforced with carboxyl functionalized multiwall carbon nanotubes (MWCNTs-COOH) to evaluate compressive strength, Shore D hardness, and in vitro biocompatibility properties. MWCNTs are added to the PMMA powder in different amounts using a geometric dilution technique. The PMMA/MWCNT nanocomposite is prepared with MWCNTs varying from 0.1 wt. % to 0.7 wt. %. The compressive strength and Shore D hardness values increased to a maximum of 69.21% and 4.84%, respectively for 0.3 wt. % loading. The in vitro cytotoxicity studies on MG-63 cells show a percentage cell viability of 81.37 % for 0.3 wt. % and 83.25 % for 0.7 wt. % MWCNTs loading. Hemolysis studies on human B+ve blood exhibited a low hemolytic potential of 15.12% for 0.3 wt. % and 16.38% for 0.7 wt. % MWCNTs loading on human RBCs. It is concluded that the prepared PMMA/MWCNT nanocomposites are found to have enhanced mechanical properties compared to Simplex P bone cement.

1. Introduction

Bone cement, also known as polymethyl methacrylate, is a widely used biomaterial in orthopedic surgeries [1]. It is a thermosetting material that is used to fix artificial joint implants to the bone, as well as to fill voids or defects in bones caused by fractures or disease. The bone cement is injected into the bone in a viscous state, and then hardens and sets within a few minutes, creating a stable and durable bond between the implant and the bone. PMMA bone cement is often used in joint replacement surgery, where the cement mantle acts as an interface in transferring loads between the implant and bone [2]. PMMA bone cement offers several advantages in the preparation of the cement mantle and its application. One of the main advantages is its ability to provide immediate fixation and stability to the implant or bone [3]. This allows for early weight-bearing and faster rehabilitation for the patient. However, there are a few disadvantages, such as impaired mechanical properties due to pore entrapment, and monomers that can cause toxicity or adverse effects. The poor abrasion resistance can make bone cement susceptible to wear and lose its original shape or surface finish, leading to implant loosening and failure [4].

*Corresponding author: madev.nagaral@gmail.com
[a] orcid.org/0000-0002-0814-8531; [b] orcid.org/0000-0002-4028-3813; [c] orcid.org/0000-0001-5266-7542; [d] orcid.org/0000-0002-8248-7603
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The development of novel bone cement has undergone significant improvements over the years in terms of its composition and properties. The researchers have added different fillers like graphene, hydroxyapatite, glass-ceramic, silver nanoparticles and chitosan to augment the mechanical properties and biocompatibility of PMMA bone cement [5][6][7][8][9]. Nanomaterials have gained special importance and extensively investigated as fillers due to their unique properties [10]. Nanoparticles are typically less than 1000 nm in size and have a high reactivity and substantial surface area-to-mass ratio, distinguishing themselves from bulk materials of the same composition [11]. Among the nanomaterials, carbon nanotubes possess superior mechanical and thermal properties [12]. Carbon nanotubes are cylindrical structures formed by folding single or several sheets of graphene and are characterized by a zigzag, chiral, or armchair configurations based on the direction of hexagons [13]. Multi-walled carbon nanotubes (MWCNTs) were incorporated into PMMA bone cement at a low weight percentage and resulted in notable improvements in the mechanical properties as well as cytotoxicity [14][15]. The MWCNT's performance mainly depends on the homogeneous dispersion in the matrix material. The MWCNTs dispersion within the bone cement microstructure was mainly decided by the method used to integrate into the bone cement [16]. Three different methods have primarily been described for the preparation of composite bone cement. In the first, an ultrasonic disintegrator was used to incorporate MWCNTs into liquid monomers by dispersion. In the second method, high-temperature mechanical shear mixing or Rheomix was used to blend MWCNTs with commercial bone cement powder. In the third method, MWCNTs were incorporated into the methylmethacrylate (MMA) component using a magnetic stirrer. Ormsby et al. (2010) [17] evaluated the mechanical properties of 0.1 wt. % loaded both functionalized and unfunctionalized MWCNTs. The compressive strength of PMMA bone cement was reduced by 13% when 0.1 wt. % functionally loaded MWCNTs were added using the magnetic-stirring method. However, the same was increased by 4% and 13% in the dry blending and sonication synthesis process, respectively.

The literature review indicates that MWCNTs were used to enhance the properties of bone cement. However, the incorporation of MWCNTs into PMMA bone cement may present some challenges, including:

- Dispersion - Achieving a uniform and stable dispersion of MWCNTs in PMMA bone cement is challenging due to the tendency of MWCNTs to agglomerate. Poor dispersion can lead to heterogeneous mechanical properties and may negatively affect the overall performance of the cement.
- Cytotoxicity - The use of MWCNTs in biomedical applications raises concerns about their potential cytotoxicity. While some studies have shown that MWCNTs can induce cell death and inflammation, others have suggested that MWCNTs may not be toxic at low concentrations. Further research is needed to fully understand the toxicity of MWCNTs in PMMA bone cement.

Mechanical properties - The addition of MWCNTs to PMMA bone cement can alter its mechanical properties, such as strength and stiffness. Achieving the desired balance between the mechanical properties of the cement and the MWCNT content can be challenging.

In this study, the geometric dilution method was used to distribute MWCNTs in the powder medium. The geometric dilution method is expected to achieve uniform distribution of MWCNTs into MMA powder without causing any damage to the walls of nanotubes. However, the lower weight percentage of MWCNTs was selected to minimize the possibility of agglomeration [18]. The mechanical properties and in vitro biocompatibility of the nanocomposite bone cement were evaluated and compared with the commercial Simplex P bone cement. X-ray diffraction analysis (XRD), Scanning electron microscopy (SEM), and Differential scanning calorimetry (DSC) are used for material characterization.

2. Materials and Methods

The two-part acrylic-based Surgical Simplex® P radiopaque bone cement consisting of liquid and powder components is purchased from Stryker Howmedica Osteonics in the Republic of Ireland. The monomer used in this solution is methyl methacrylate, and N, N-Dimethyl-p-Toluidine is employed as an activator, while a small amount of hydroquinone is included in the solution to prevent polymerization during storage. The powder component of the solution contains polymethyl methacrylate polymer units, benzoyl peroxide - polymerization initiator, and either barium sulfate or zirconium dioxide, which are radiopaque substances that facilitate X-ray imaging. In the composite composition, carboxyl functionalized MWCNTs of 10-15 nm outer diameter and 2-10 μm length are used as nanofillers, which are purchased from Platonic Nanotech Pvt. Ltd. in Kachwa Chowk, Mahagama, Jharkhand.

The weight proportions of the composite bone cement are shown in Table 1. The powder and liquid medium are mixed in a 2:1 proportion according to the manufacturer's guidelines. A geometrical dilution method with the measured size is employed by combining fine powders of an equal proportion of MWCNTs with MMA powder. This MMA/MWCNTs powder is then added to N, N-Dimethyl-p-Toluidine (liquid monomer solution) to initiate self-polymerization. The sticky, working, and hardening phases are involved in the mixing of the powder and liquid components. The resulting dough stage mixture is then transferred into polypropylene molds of diameter ⌀6 mm and height 12 mm to form cylindrical specimens. After 24 hours of curing at room temperature, the cylindrical specimens are sectioned and polished using SiC paper with grits ranging from 220-2400.

Table 1. Composite identification and composition

Sl. No.	Sample identification	Material composition	MMA g	Liquid mL	MWCNT wt. %
1	C	PMMA (control)	10	5	-
2	C1	PMMA+0.1% MWCNT	9.99	5	0.1
3	C2	PMMA+0.3% MWCNT	9.97	5	0.3
4	C3	PMMA+0.5% MWCNT	9.95	5	0.5
5	C4	PMMA+0.7% MWCNT	9.93	5	0.7

2.1. Mechanical Tests

The hardness of the composites is measured using a Yuzuki Shore D Durometer with a 5% tolerance (ASTM D2240-05). The samples are loaded against an indenter with a diameter of 1.15 to 1.40 mm and a height of 6.5 mm. Measurements are taken at room temperature using five samples of each composition, with dimensions of 10x40x3 mm.

Cylindrical nanocomposite samples of⌀⌀6x12 mm are prepared and allowed to harden in PBS at pH 7.4. After drying for 24 hours, the samples are tested for compressive strength using the Tinius Olsen Universal Testing Machine (Model 50ST) at a crosshead speed of 5 mm/min following ASTM F-451-08 standard. The compressive strength is estimated using Equation 1.

$$\text{Compressive strength} = \frac{4F}{\pi d^2} \quad (1)$$

where, 'F' is the fracture load in Newton, and 'd' is the cylindrical specimen diameter in mm.

2.2. Characterization Techniques

The phase and crystalline nature of the nanocomposite samples are investigated using X-ray diffraction (XRD). Nanocomposite bone cement samples of ⌀8x2 mm are prepared as described previously and hardened in PBS at pH 7.4. After 24 hours, the samples are subjected to XRD testing using an X-ray diffractometer (PAN Analytical). The measurements are taken at room temperature using Cu-Ka source radiation with 1.541 Aº wavelengths, 40 kV voltage, and 30 mA current. The XRD data are acquired at a scanning frequency of 0.50 min-1 with a step size of 0.001º over a Bragg angle range of 10-80º.

The morphology of the specimens of composite bone cement reinforced with MWCNTs is examined by SEM (JEOL 6500, JSM, Japan) at working voltages of 5.0 kV and 4 Torr vacuum pressure. Energy dispersive spectroscopy is obtained to investigate the elemental composition of the composite bone cement. Samples of size ⌀8x5 mm are prepared as previously noted and allowed to harden in PBS with a pH of 7.4. The samples are then dried for 24 hours, polished using SiC paper (220-2400 grits), sputter coated and analyzed.

The thermal endurance and glass transition temperature (Tg) of the bone cement are studied using DSC in a nitrogen gas atmosphere, with a heating rate of 100°C/minute from room temperature up to 700°C. The samples are allowed to harden in a pH 7.4 PBS solution and air-dried for 24 hours, then crushed and analyzed.

2.3. In Vitro Biocompatibility Tests

2.3.1 Cytotoxicity Evaluation

Cytotoxicity is the degree to which a substance can cause damage to a cell. In this work, cell growth and cytotoxicity are measured using a colorimetric test. The samples are tested for cytotoxicity on mitochondrial lactate dehydrogenase produced by MG-63-Human Osteosarcoma cell lines (NCCS, Pune). It turns MTT into insoluble formazan crystals that, when dissolved in the right solvent, show a purple color, the intensity of which is related to the number of live cells and is measured spectrophotometrically at 570 nm. Since the composite bone cement is meant to encourage osteointegration, MG-63 cells are chosen as a model of osteoblast cells. This cell line is frequently used to conduct preliminary in vitro research on the cytocompatibility of biomaterials for bone substitutes.

Maintenance of cell lines: The MG-63 cell line is purchased from NCCS, Pune, India. The cells are kept in Dulbecco's modified eagle medium, a high glucose medium supplemented with 10% fetal bovine serum and the 1% antibiotic-antimitotic solution at 37°C in a CO2 incubator. The cells are subcultured every two days.

Cell viability: The MG-63 cell suspension (1000 µl) is seeded in a twelve well-plate at a density of 50,000 cells per well and allowed to grow for about 24 hours. The composite bone cement samples are sterilized beneath UV light for 30 min and washed with PBS for 2 mins to ensure sterility. After sterilization, the samples are carefully kept in each well of twelve well plates. Cells without any sample are considered untreated and cells treated with doxorubicin at 1 uM/ml concentration are considered positive controls for the study. The plates are kept in an environment of 5% CO2 and incubated for 24 hours at 37°C. After incubation, the plates and used medium are removed. Finally, MTT reagent is added, and plates are incubated for 3 hours. Upon removing MTT, 100 µl of solubilization medium is added. The absorbance is read on a spectrophotometer at 570 nm wavelength. The % cell viability is calculated from Equation 2,

$$\% \text{ cell viability} = \left(\frac{\text{Mean abs of treated cells}}{\text{Mean abs of untreated cells}}\right) \times 100 \quad (2)$$

2.3.2 Hemolysis

A hemolysis assay is performed as per ASTM F756 to check whether the composite bone cement contains compounds that can induce the lysis of red blood cells (RBCs). To assess the lysis of hemoglobin spectrophotometrically, RBCs are separated from the blood sample and treated with the test chemicals. A positive control is one in which the detergent lyses the cells, whereas a negative control shows completely undamaged cells. A healthy individual donated about 5 mL of blood, which is then centrifuged at 1000 rpm for 10 minutes at 4°C to separate the RBCs from the new blood and keep the blood healthy (B+ve). After removing the supernatant (plasma), 1 ml of PBS is used to wash the collected erythrocytes. Diluted RBCs (500µL) are added to each well of 12 well-plate and sterilized bone cement samples are placed. The untreated, standard control of 1% sodium dodecyl sulfate (SDS) and blank controls are used for comparison. The RBCs are incubated for 24 hours at 37°C, and the reaction is centrifuged for 5 minutes at 300 rpm. Each reaction's supernatant is put on a 96-well ELISA plate, and the absorbance is checked at 590 nm. The proportion of hemolysis is calculated using Equation 3.

$$\text{Haemolysis} = \left(\text{Mean abs}\frac{\text{Sample}}{\text{Positive Control}}\right) \times 100 \tag{3}$$

2.3.3 Statistical analysis

Two sample t-test statistical analysis is used to determine whether the results for compressive strength and hardness tests. The mean standard deviation is used to represent the findings. The level of statistical significance is set at $p<0.05$.

3. Results and Discussion

3.1. Mechanical Tests

The Shore D hardness of the composite bone cement is depicted in Table 2. It is observed that the addition of MWCNTs enhanced the composites' Shore D hardness. The reason is that MWCNTs-COOH reinforcement helps in better interaction with the PMMA matrix and acts as a barrier for crack propagation [19]. Although MWCNTs increase the hardness of the PMMA, the trend is not proportional to the MWCNTs added. Hardness increased significantly ($p=0.023$) by 4.84% for 0.3 wt. % loading, but the increase is marginal for 0.5 and 0.7 wt. % of MWCNT concentrations. Greater hardness is seen in C2 samples of which had fewer voids and a more evenly dispersed matrix. The undissolved beads spotted in SEM images (Figure 7) may have caused the creation of voids and poor adherence of the MWCNTs to the PMMA in samples C3 and C4. Therefore, there is no substantial increase in the C3 and C4 sample's hardness.

Table 2. Percentage variation in Shore D hardness

Bone cement samples	Shore D hardness %↑
C1	3.03↑
C2	4.84↑
C3	3.63↑
C4	2.42↑

The compressive strength of the bone cement samples is depicted in Figure 1, it can be observed that with increasing MWCNTs up to 0.3 wt. % (p=0.03), compressive strength rose significantly. This is attributed to the nanotubes' high density of interfaces, and a tendency to resist crack propagation. The homogeneous distribution of MWCNTs in the PMMA matrix also helped in effective load transmission between the PMMA and MWCNT [20]. However, with a further increase of MWCNTs in C3 and C4 samples (0.5 and 0.7 wt %), due to the existence of undissolved PMMA beads and voids inside the PMMA matrix, the compressive strength reduced[21][22].

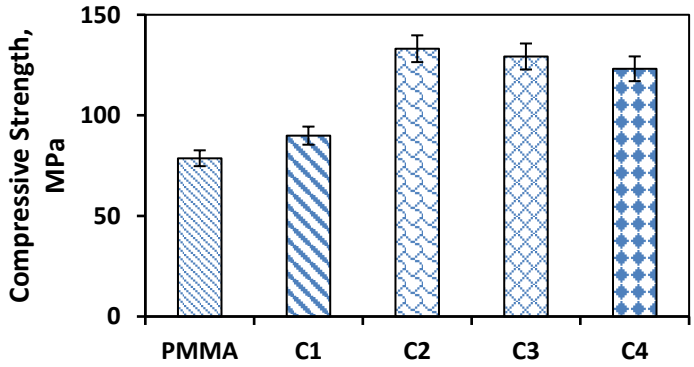

Fig. 1 Compressive strength of composite bone cement composition

The compressive strength of the C1, C2, C3, and C4 samples increased by 14.28%, 69.21%, 64.29%, and 56.27%, respectively, compared to PMMA bone cement. These findings are consistent with previous studies by Mu et al. (2018) [23] and Nien et al. (2010) [24] , which also reported increased compressive strength due to the addition of MWCNTs.

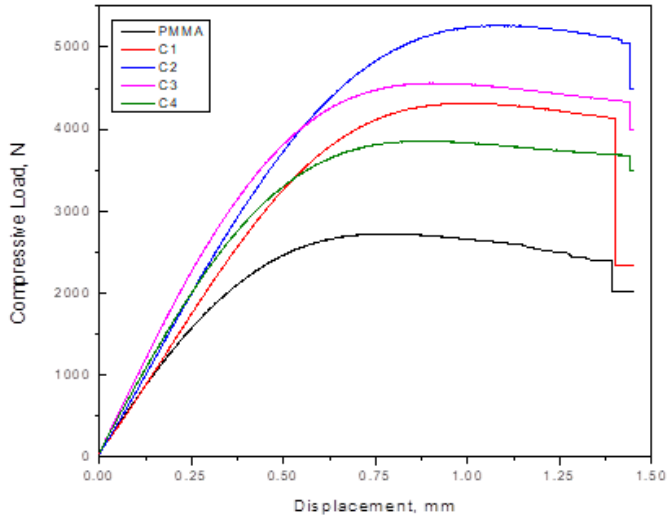

Fig. 2 Behavior of composite bone cement under compressive loading

The load versus compression plots obtained for test samples are shown in Figure 2. All the samples exhibited similar loading trends for a compressive load up to 25kN and are found to be elastic below 20kN. The resistance to the applied load is notably high up to 0.3 wt. % and beyond which it decreased gradually.

Figure 3 displays the compressive modulus of the bone cement composites. The highest modulus of elasticity, reaching 3854 MPa, is achieved at 0.3 wt.% MWCNT loading. The optimal value at 0.3 wt.% is attributed to the efficient stress transfer between the MWCNTs and PMMA, facilitated by a strong interfacial bonding [25]. However, the modulus decreases beyond this point, which is mainly attributed to agglomerations of MWCNTs.

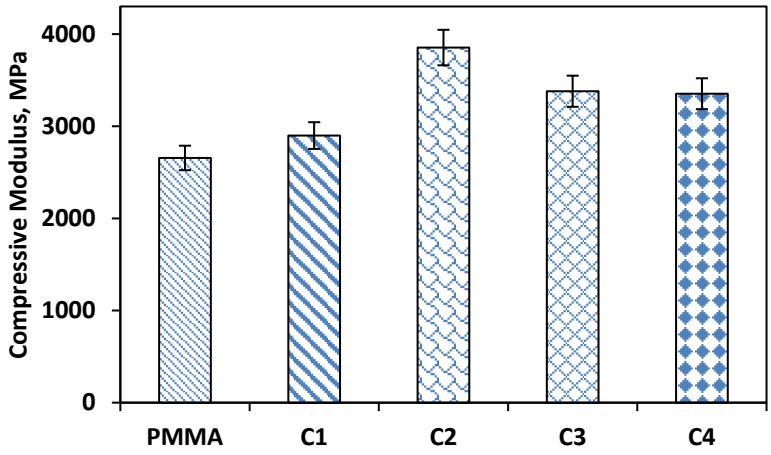

Fig. 3 Influence of MWCNTs on the compressive modulus of composite bone cement

3.2. Reasons for the Enhanced Mechanical Property

The plausible justifications for the increase in mechanical properties of composite bone cement up to 0.3 wt. % MWCNTs loadings are discussed in this section. The mechanical properties of PMMA/MWCNTs composites are improved due to MWCNT's intrinsic features. The modulus of elasticity of an individual CNT was reported to be up to 1TPa [26]. In this study, the modulus of elasticity and compressive strength of PMMA is experimentally determined as 2.66 GPa and 78.67 MPa, respectively. Hence, it is evident that the modulus of elasticity and strength of nanotubes is superior compared with PMMA bone cement. So, the addition of MWCNTs to PMMA is expected to improve the mechanical properties. The improvement in compressive strength for lower loadings of MWCNTs is due to MWCNTs' proclivity to withstand compression load and high-density interfaces (bonding between matrix and MWCNTs) of nanomaterial [27].

The effect of MWCNTs on the degradation temperature of PMMA is shown in Figure 4. The weight loss pattern is observed to be identical for the synthesized samples. However, the degradation temperature of PMMA is increased with the MWCNTs reinforcement, which acts as an impediment to PMMA degradation. The rise in degradation temperature observed from the derivative of the thermogravimetric curve (DTG) confirms the interfacial bonding of PMMA with MWCNTs [28].

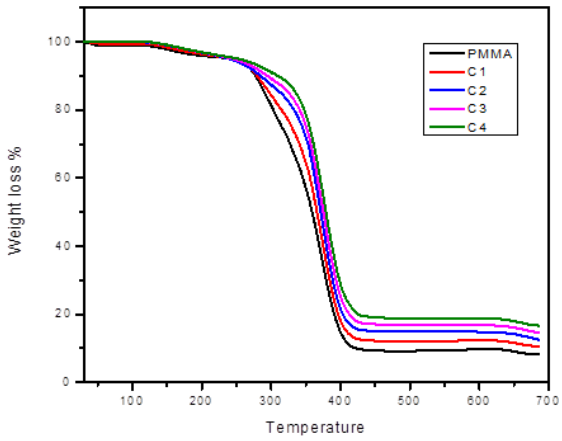

Fig. 4 TGA curves of PMMA/MWCNT composites

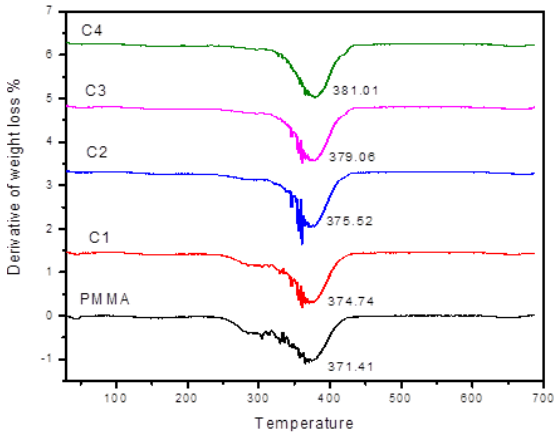

Fig. 5 Derivative of TGA curves of composite bone cement

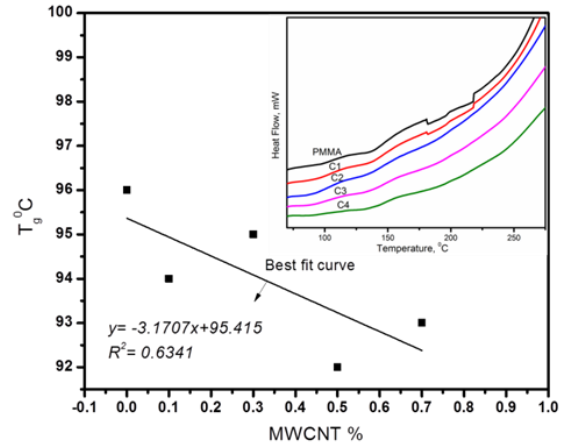

Fig. 6 Glass transition temperatures of composite bone cement

The DTG of PMMA/MWCNTs composites are illustrated in Figure 5. The DTG plot revealed that the addition of MWCNTs delayed the thermal degradation of PMMA and increased its thermal stability. The degradation temperature of PMMA is obtained as 371.41ºC, which is increased by 0.89%, 1.1%, 2.1%, and 2.58% for C1, C2, C3, and C4 samples, respectively.

The behavior of Tg of PMMA/MWCNT composites with varying MWCNT concentrations is represented in Figure 6. The variations in Tg are observed to decrease prominently compared to PMMA with the increase in MWCNT concentration. However, among the PMMA/MWCNT composites, both increasing and decreasing trends are observed, which was also reported by Tomova et. al (2017) [29]. The Tg value is found to be maximum for PMMA and among the composites, it is maximum for 0.3 wt. % and minimum for 0.5 wt. % MWCNTs loading. The linear fitting of Tg revealed a high slope, indicating the considerable effect of MWCNTs on Tg due to the strong interfacial bonding among PMMA and MWCNTs [23]. The correlation between PMMA and MWCNTs is demonstrated by the elevated thermal stability and Tg of the composite, resulting in improved compressive strength and hardness. However, the decrease in Tg may be attributed to the plasticizing effect of MWCNTs on PMMA [24].

3.3. Reasons for Diminution of Mechanical Properties

This section outlines the most likely reasons for the reduction in mechanical properties of composite bone cement upon the addition of more than 0.3% MWCNTs, as evidenced by SEM images. The SEM images (300 nm scale) of PMMA/MWCNT composites are depicted in Figure 7.

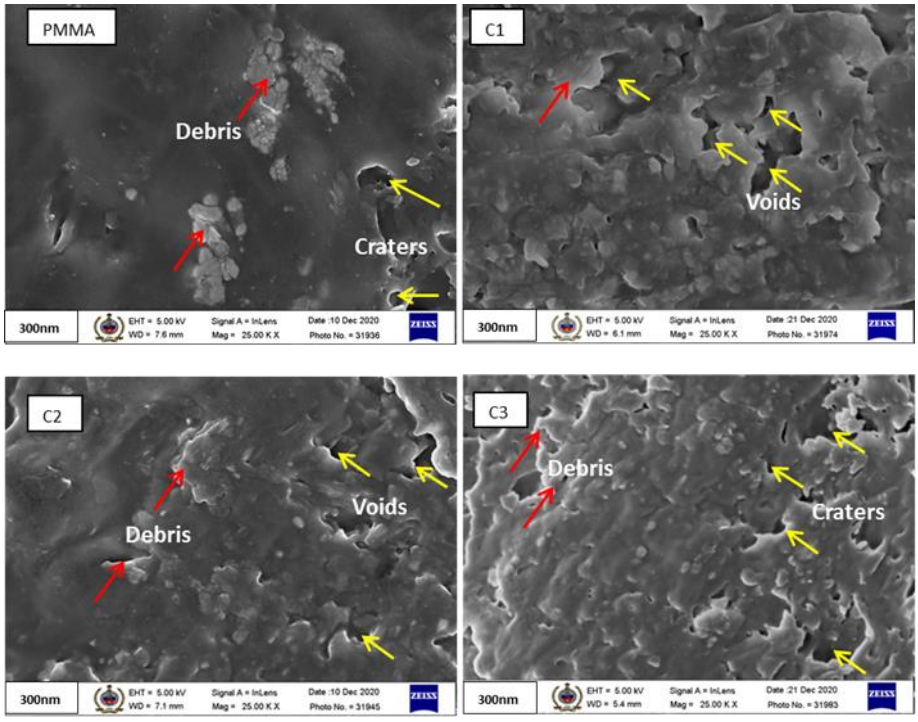

Fig. 7 SEM images of composite bone cement

The nodular-like particles and voids are observed on C1 and C2 samples. The nodular-like particles present in the images are likely to be Benzoyl peroxide and Zirconium dioxide,

which serve as initiator and radio-opacifier, respectively, in the MMA powder. When the MWCNTs loading exceeds 0.3 wt. %, there is an increase in voids, craters, and agglomerations observed in the SEM images (Figure 7-C3). Muthu et al. (2018) [30] attributed the agglomerations to the inherent van-der Waals forces among the individual Nanotubes. Incorporating more wt. % of MWCNTs into the PMMA matrix increased the composite's density, resulting in a higher viscosity. However, due to the higher viscosity of the PMMA matrix, it was unable to wet the surface area of the MWCNTs completely during composite preparation by hand mixing. Thus, the micro-voids and agglomeration of MWCNTs in the cement matrix are responsible for the drop in compressive strength beyond 0.3 wt. % loading [31][18].

The XRD pattern of PMMA/MWCNTs composites with 2θ ranges of 10–80º is depicted in Figure 8. PMMA is an amorphous polymer with two large peaks found at 2θ values of 30.67º and 31.96º. The strong peak around 42 and the next prominent peaks around 30 and 26 are observed in all prepared samples. The presence of sharp and narrow peaks implies that the nanotubes served as nucleating agents, initiating the formation of new crystallites [32].

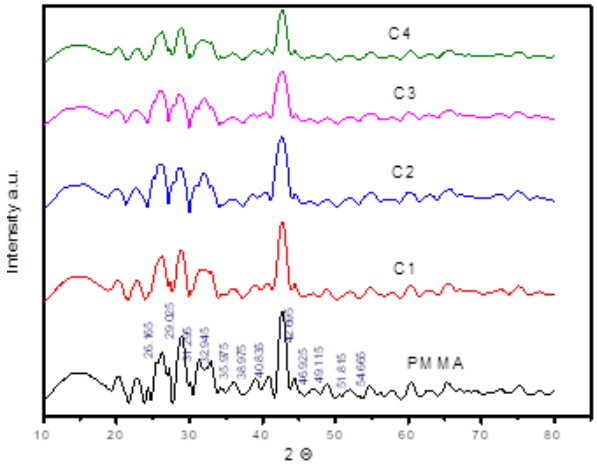

Fig. 8 XRD of PMMA composite bone cement

The composite's crystallinity is a factor that influences the mechanical properties of polymer composites. The presence of strong Bragg peaks in the XRD pattern is a direct indication of material's crystalanity. The area of crystalline peaks divided by the total area of all peaks yields the percentage crystallinity. The crystallinity measurement by XRD is due to the total coherent scattering as a constant independent of the arrangement of the atoms in the sample [33]. This study found that the crystallinity of PMMA is 46.83%. However, the crystallinity of composite bone cement reinforced with 0.1, 0.3, 0.5, and 0.7 wt. % MWCNTs is calculated to be 49.52%, 53.87%, 52.25%, and 50.50%, respectively. The interfacial bonding between MWCNTs and PMMA has resulted in maximum crsytallanity of 52.25% at 0.3 wt. % loading.

3.3. In Vitro Biocompatibility Tests

3.3.1 Cytotoxicity Evaluation

The composite bone cement samples, C2 and C4 are subjected to cytotoxicity effect on MG-63 cells. Figure 9 illustrates the results of the cytotoxicity study, which is conducted using the MTT assay. The study found that the test samples are non-cytotoxic on MG-63 cells,

with a percentage cell viability of 81.37% and 83.25% after a treatment period of 72 hours. Doxorubicin is used as a standard control for the study, which showed effective cytotoxicity on MG-63 cells with 11.32% cell viability.

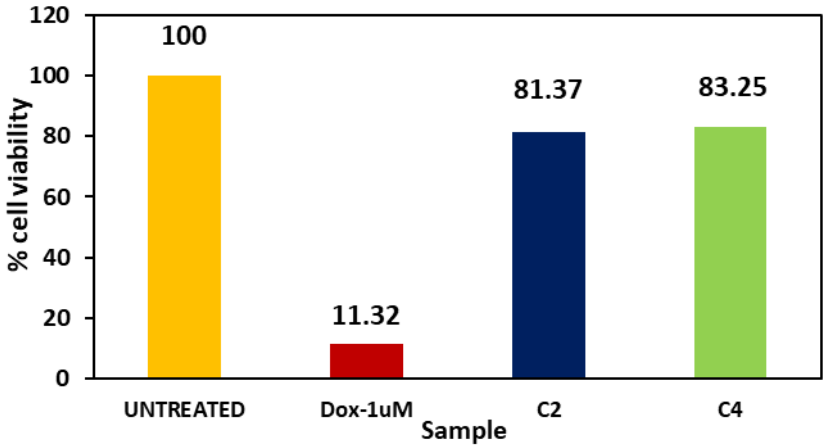

Fig. 9 Percentage of cell viability against MG-63 cells

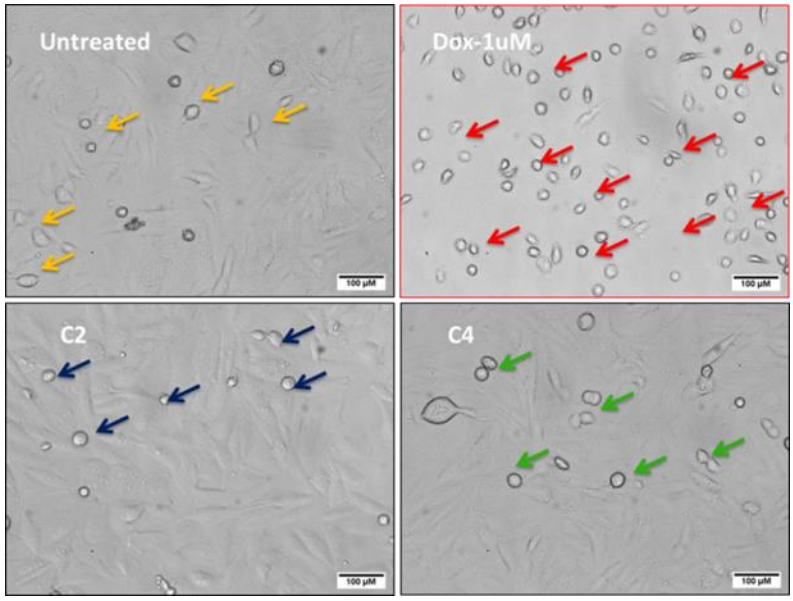

Fig. 10 Optical images of samples after cytotoxicity evaluation

The microscopic observations of cell-treated images of test samples after 72 hours of incubation are shown in Figure 10. The MTT assay results showed that the samples are non-cytotoxic on human bone cancer cells and further studies could be carried out to control the molecular mechanism.

3.3.2 Hemolysis

The composite bone cement samples, C2 and C4 are subjected to hemolysis test on a healthy human blood (B+ve). After a treatment period of 24 hours, the samples did not demonstrate significant hemolytic potential on human RBCs in comparison to 1% SDS, which exhibited effective hemolysis on human RBCs. Figure 11 displays the comparative percentage hemolysis potency of the samples C2 (15.12 %) and C4 (16.38%), along with the control, after an incubation period of 24 hours. The results indicate that the samples, C2 and C4, are non-toxic and safe for human health, without any adverse effects on normal human health.

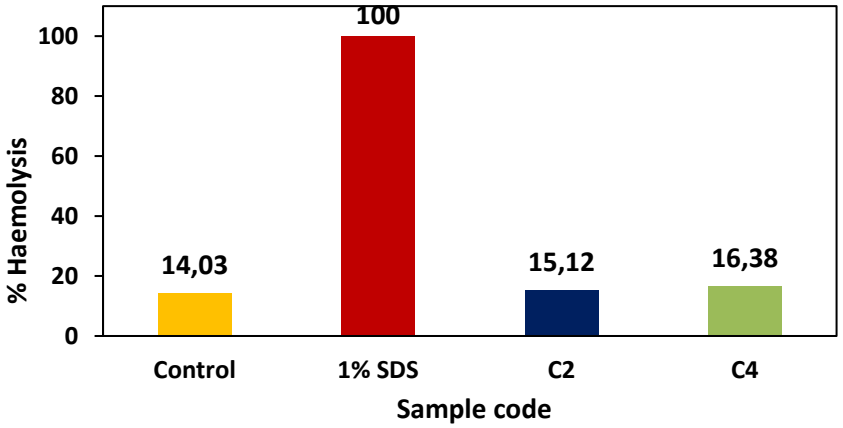

Fig. 11 Percentage Haemolysis of composite bone cement samples

3.4 Discussion

The two major purposes of bone cement are to quickly secure implants after surgery and to distribute implant stresses to the surrounding bone bed. When incorporating fillers to meet bone cement specifications, it is crucial to ensure that the fundamental properties of the cement are not significantly altered. In this work, the post-fabrication drying time of 24±2 hours is followed for testing the static mechanical properties. It's interesting to note that, compared to commercial Simplex P cement, the compressive strength and Shore D hardness of the cement improved with the inclusion of MWCNTs.

The mechanical properties of bone cement play a critical role in serving as a linkage between the prosthesis and bone, as it is responsible for transmitting the load to the surrounding bone tissue. Compressive strength and hardness tests have revealed that this characteristic holds for all composite cement that is loaded with multi-walled carbon nanotubes (MWCNTs). The present study demonstrates a notable 69.21% increase in compressive strength upon the addition of 0.3 wt. % MWCNTs-COOH to the bone cement. In comparison, earlier research by Nien (2011) [18] observed a 23% increase in compressive strength for MWCNT-modified bone cement, while Ormsby et al. (2014) [34] reported a mere 2% increase in compressive strength for PMMA/MWCNT bone cement. Xu et al. (2013) [35] dispersed MWCNTs using ultrasonic disintegration and observed a 24.5% increase in compressive strength. Therefore, the increase in compressive strength exhibited by the composite bone cement developed in this study is significantly superior to the previous findings [18] [34] [35]. The incorporation of 0.3 wt. % MWCNTs-COOH into the bone cement results in a modulus of elasticity of 3.85 GPa. In contrast, cancellous bone has Young's modulus of 0.33 GPa, while metals commonly utilized for implants possess

Young's modulus values ranging from 110 GPa to 230 GPa [36]. Notably, the composite bone cement developed in this study exhibits a 44.7% increase in modulus of elasticity compared to conventional bone cement. This enhanced stiffness characteristic may aid in reducing the stress shielding effect (due to difference in stiffness) at the interface between the cement and bone [37]. The addition of 0.3 wt. % MWCNTs-COOH, the Shore D hardness of the bone cement increased by 4.84%. This improved hardness can enhance the wear resistance of the cement and enable the cement mantle to regain its original shape and surface finish without failure and causing implant loosening.

In this study, the MWCNTs are integrated into the bone cement matrix using a geometric dilution technique, which resulted in a relatively well-dispersed cement mixture at 0.3 wt. % optimum loading. However, there are certain acknowledged limitations in the present study. Firstly, only one type of cement (Simplex P) is employed, and the results may vary depending on the chemical composition and viscosity of other types of cement. Simplex P is chosen as it is one of the most commonly used bone cement formulations for total hip and knee replacements [38]. Secondly, although fatigue is a crucial factor in in vivo cement failure, the fatigue parameters of the MWCNT-loaded cement are not evaluated [39]. This is because testing for fatigue is beyond the scope of the current project. Lastly, the biocompatibility assessment only measured cell viability, while other relevant markers such as cell proliferation and lactate dehydrogenase are not examined. Future studies should explore dynamic mechanical properties and biocompatibility in greater detail.

4. Conclusions

This study builds upon previous research to enhance the mechanical properties and biocompatibility of Simplex P bone cement. Specifically, the geometric dilution method is utilized to incorporate MWCNT-COOH into the cement, avoiding the possible agglomerations without damaging MWCNTs. The influence of 0.1, 0.3, 0.5, and 0.7 wt. % MWCNTs on the MMA powder of Simplex P bone cement on mechanical properties and in vitro biocompatibility of the cured cement are examined. The results of this study indicate that the C2 samples exhibited the highest increase in compressive strength (69.21%) and compressive modulus (44.75%). The observed positive interaction between PMMA and MWCNTs at a concentration of 0.3 wt.% is likely due to the uniform distribution of MWCNTs in the PMMA matrix, which is also supported by the increased thermal stability of the resulting bone cement. The results of cytotoxicity (82.31% average cell viability) and hemolysis (15.75% average) tests confirm that the composite bone cement formulations prepared in this study are non-toxic and safe for use in therapeutic applications related to human bone treatment. The overall findings of this research propose that it is difficult to predict the performance of synthesized bone cement by in vitro characterization only. It is worthwhile to conduct in vivo biocompatibility studies of PMMA loaded with -COOH functionalized MWCNTs to assess the viability and potential clinical usage impairments.

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