Enhanced mechanical properties of hBN–ZrO₂ composites and their biological activities on Drosophila melanogaster: synthesis and characterization†

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In this study, six compositions in the system [x(h-BN)–(100 – x)ZrO₂] (10 ≤ x ≤ 90) were synthesized by a bottom up approach, i.e., the solid-state reaction technique. XRD results showed the formation of a novel and main phase of zirconium oxynitrate ZrO(NO₃)₂ and SEM exhibited mixed morphology of layered and stacked h-BN nanosheets with ZrO₂ grains. The composite sample 10 wt% h-BN + 90 wt% ZrO₂ (10B90Z) showed outstanding mechanical properties for different parameters, i.e., density (3.12 g cm⁻³), Young’s modulus (10.10 GPa), toughness (2.56 MJ m⁻³), and maximum mechanical strength (227.33 MPa). The current study further checked the in vivo toxicity of composite 10B90Z and composite 90B10Z using Drosophila melanogaster. The composite 10B90Z showed less cytotoxicity in this model, while the composite 90B10Z showed higher toxicity in terms of organ development as well as internal damage of the gut mostly at the lower concentrations of 1, 10, and 25 μg mL⁻¹. Altogether, the current study proposes the composite 10B90Z as an ideal compound for applications in biomedical research. This composite 10B90Z displays remarkable mechanical and biological performances, due to which we recommend this composition for various biomedical applications.

1. Introduction

Increasing interest in the development of advanced composites for various biomedical applications has impelled a large amount of research to synthesize easily scalable, low-density, high-strength, wear-resistant and non-toxic biomaterials. The elemental dullness with low density pooled with high strength makes h-BN one of the capable materials for these applications. Boron (B) and nitrogen (N) atoms are bonded with strong covalent bonding, whereas weak van der Waals forces bind the layers, which allow the layers to glide next to each other similarly. This special sparkler arrangement and unique bonding between as well as distinctive performances such as proportional elemental and thermal dullness, mechanical properties, hardness and strength, good corrosion resistance and stability at high temperatures, brilliant electrical lagging properties and good manufacturability make h-BN promising for various applications.1–12 Recently, three dimensional (3-D) interrelated nanostructures of h-BN and graphene architectures were synthesized using numerous novel methods.13–18 Hence, it is predictable that the growing 3-D h-BN architectures will enhance their significance in a wide range of applications.18–20 Owing to the heterostructures and similarity with carbon, h-BN represents elevated thermal resistance and high mechanical strength.21–25 The surprising performance of h-BN provides a novel material for the production of numerous technological applications. h-BN-based bioceramics with good chemical stability and high oxidation resistance have been used in diverse research areas at a large scale such as metallic and chemical industries and thermal security systems.26–32 ZrO₂ is still being employed as a biomaterial for various implants; however, owing to high brittleness, it undergoes terrible failure when a load is applied beyond a certain limit.33,34 Hence, scientists are much attracted towards the development of such ZrO₂-based novel composites, which reveals relatively high mechanical properties and low density with notable...
biological performances for bone implant applications. However, plentiful investigations have been carried out in the research field of biocomposites for numerous biomedical applications such as in jawbone, joint, hip, and dental implants but the stability and biocompatibility of these implants are not adequate due to their high density, highly fragile nature, high coefficient of thermal expansion, and low resistance to corrosion with respect to acid and humid environments.35-42

Hence, scientists having enormous interest for the fabrication of new biocomposites such as zirconia doped alumina, ceria/yttria stabilized zirconia (CeY-TZP), and 84ZrO2-8Al2O3-8SrAl2O19, which show notable mechanical strength, stiffness, venerable biocompatibility, and substantial requirements for several dental/bone implant applications.43-50 Recently, three dimensional nanostructured biocomposites of hBN–B2O3 were fabricated using plasma sintering technique for bone restoration applications and 3-D interconnected architecture of h-BN reinforced ZrO2 composites for structural evolution and enhanced mechanical properties of bone implant applications.18,51 They found remarkable osteogenic properties with enough compressive strength. Besides the mechanical fidelity, they also report pretty good chemical stability and biocompatibility. Initially, in 1969, ZrO2 was used for medical devices in biomedicine as an innovative implant for head, knee, and jaw/teeth in dentistry owing to superior mechanical characteristics.52-54 Due to the high utility, its efficacy and malicious effects on humans is a matter of concern. Animal studies have revealed the toxicity of zirconia nanoparticles in zebra fish and Drosophila.55,56 Drosophila melanogaster is used as a model to test the toxicity of various nanoparticles57-58 because of its short natural life, simple translucent larval structure, small size, ease of handling, and ease of visualization under a microscope. The evolutionary conserved diseased genes can be used to check the toxic effect in the current study.

In this study, we intend the preparation of composites of h-BN and ZrO2 through a simple solid-state reaction to obtain better malleability and improvement in the mechanical and tribological properties without varying the other properties, using a typical sintering method for biomedical applications.

3. Sample characterizations

In order to examine the crystallographic structures of the composites, viz., 10B90Z, 20B80Z, 40B60Z, 60B40Z, 80B20Z, and 90B10Z, the XRD patterns were obtained within the range of 15–60° 2θ values at the scanning rate of 3° min⁻¹ by employing Rigaku Miniflex-II XRD consisting of Cu-Kz radiation having the wavelength, λ = 0.15418 nm, monitored at 40 kV and 40 mA. The density values of the sintered composites were evaluated by using a mass/volume formula.

The respective amounts of all the powder samples were mixed with potassium bromide in weight fraction of 1 to 99 using mortar and pestle. Further, various uniformed and cylindrical pellets having diameter of 12 mm were shaped and dried at 120 °C for 60 min in an oven. Finally, IR spectroscopic measurements were performed to understand the bonding mechanisms and interaction between the constituent molecules of the fabricated composites within the wavenumber range from 4000–400 cm⁻¹ using an IR Affinity-1S spectrometer (Shimadzu). Raman spectroscopic studies were also carried out using a Renishaw In-Via Reflex Micro-Raman spectrometer having a 514.5 nm Argon laser. To analyze the surface composition and chemical states of the composite sample, X-ray photoelectron spectroscopy was performed using PHI-5000C ESCA Versaprobe-II, FEI, Inc., with Al KR X-ray as the excitation source. A BET analyzer (BELSORP-MINI-II, Japan Inc.) was employed to investigate the surface area and average pore diameter under nitrogen gas adsorption–desorption.

To obtain the surface morphological information from the sintered, polished, and Ag-Pd coated composite pellets, SEM was performed using a scanning electron microscope (JEOL JSM-6400). The coating on the pellet samples was done by an Auto Fine Coater sputtering machine (JEOL, JEC-3000FC). Eventually, the coated samples were then fixed upon the copper stubs by conducting carbon tape and the SEM images were recorded at appropriate magnifications.

To analyze the particle shape and size of the composite, transmission electron microscopy was also conducted by a field emission gun electron microscope (JEOL 2100). A fraction of milligram of the powder sample 10B90Z was subjected to bath sonication up to 25 min in CH3CHOHCH3. Further, few tiny
drops were cast on the top surface of the holey carbon grid and then dried in a vacuum chamber for 24 h. Thus, the TEM images were recorded for the vacuum dried sample.

For mechanical characterisation, the Universal Testing Machine (Instron 3639) was employed in the compression mode for the sintered pellets (diameter and height $1.2 \times 1.2 \text{ cm}^2$). The load bearing capability of all the samples was investigated by stress–strain curves, using which the Young’s modulus and fracture toughness were determined. The fracture toughness values were estimated using the area under the stress–strain curves.

The wear and sliding friction tests were performed using a pin-on-disk apparatus (Magnum Engineers, Bangalore, India). A rotating disk of EN31 steel hardened to 60 HRC was also utilized as a counter face for testing the composite pins, which were fixed during the measurements. The load was applied to the pin through the lever, resulting in a continuous contact between the pin and the counter face. Moreover, the wear tests were performed for a total duration of 800 seconds and then the weight loss was determined using an analytical balance having 0.0001 g accuracy. Three tests were performed for each composite under the same environment. The machine associated with a control panel reveals the frictional force and therefore, the friction force was noted after every 30 s and the same method was used to calculate the coefficient of friction.

3.1. Fly stock maintenance

Control Oregon-R flies were used to check the toxic effects of the composite samples. The stock was provided by C-CAMP, Fly Facility Bengaluru India. The flies follow a generalised short period of lifecycle of 10–15 days, which allows us to study all the positive and negative effects of the nanoparticles. They were fed with regular fly food, which consisted of corn meal, yeast powder, sucrose, and agar-agar type I. The fabricated nanocomposites were fed to the flies by mixing them in their food with their respective concentrations. Five different concentrations were chosen to check the toxicity of the nanocomposites 10B90Z and 90B10Z, i.e., 1 µg mL$^{-1}$, 10 µg mL$^{-1}$, 25 µg mL$^{-1}$, 50 µg mL$^{-1}$, and 100 µg mL$^{-1}$, along with the control. All the vials having these flies were reared with 12 h light–dark conditions at 25 °C.

3.2. Developmental cycle analysis

The life cycle of Drosophila was tested from the egg to the adult stage of development at an interval of 6 h and was compared with the control to check any delay in the developmental stages. All the developmental changes of the fly were carefully observed. The number of pupa was observed in each vial for three days after pupa formation at an interval of 24 h and they were compared with the control.

3.3. Larval crawling assay

Wild type larvae or the control larvae choose to crawl in a straight line towards the periphery of the plate; however, the treated larvae or the larvae with some defects generally take a longer path by stopping and changing their direction multiple times. This assay was done by following Mishra and Barik’s study. Briefly, third instar larvae were collected from the food vials and washed in $1 \times$ PBS (phosphate buffer saline) to get rid of the extra amount of particulate food stuck on their body. Then, they were allowed to move over a 2% solidified agar gel prepared in a 90 mm petri dish. The trailing path of the larvae was marked. To check the distance travelled by the larvae, we put a graph paper below the petri dish and recorded the speed of the larvae for 1 min. The speed was determined by counting the number of squares travelled by the larvae within 1 min. A graph was plotted using the distance travelled by the larvae.

3.4. Trypan blue staining

To verify the dead cells inside the gut tissue of Drosophila, trypan blue staining was done after feeding with the composite samples. Any kind of injury in the tissue allows trypan blue to enter through its membrane and get attached to the damaged cells, thus giving blue colour to that area. In this way, the live cells can be distinguished from the dead cells. Trypan blue staining was done by following the study by Mishra et al. Briefly, six third instar larvae were taken from each concentration along with the control to perform this assay. One wash with PBS was done prior to staining. 0.5 mg mL$^{-1}$ of trypan blue (HIMEDIA) was used to stain the larvae for 30 minutes in dark. After incubating with the stain, the larva were rewash with PBS and then observed under a stereo microscope.

3.5. Nitro blue tetrazolium (NBT) reduction assay for reduced oxygen species (ROS) production

ROS production is the result of a normal metabolic pathway that maintains homeostasis in one organism. NBT assay is performed to analyze the amount of free radical yield inside the larvae after treatment with the nanocomposite. The NBT test was performed by following the method reported by Mishra et al. Briefly, the haemolymph was extracted in ice-cold temperatures in order to avoid melanisation. For the extraction of the haemolymph, 15 larvae of all concentrations were pinched by means of a sterilized needle in a micro centrifuge tube of 0.5 mL with a small cut at its base. The tube was kept in a tube of 1.5 mL and centrifuged at 4 °C in 6000 rpm for 5 min to collect the larval haemolymph. Then, to the 1.5 mL tube, 10 µL of 1 × PBS was added. 10 µL of NBT was added to the tube and the sample was kept in dark for 1 h at room temperature. Then, 20 µL of acetic acid glacial (100%) was mixed with the sample and incubated for 5 min. Finally, 150 µL of acetic acid glacial (50%) was mixed with it. After mixing properly, the sample was taken in a 96 well plate to check the OD at 595 nm.

3.6. Adult phenotype assay

The fly grew on nanocomposite mixed food and laid eggs in the vial. The embryo completed the developmental stages and the first generation fly (F1) was enclosed. 50 adult flies were taken...
from each of the different concentrations along with the control. They were anesthetized and observed carefully under any body part with special reference to thorax, eyes, and wings. The arrangement of ommatidia in each eye was observed. The wings were placed over slides and scanned under a digital stereo microscope to check defects in the venation patterns. For the thorax, the number of bristle pairs was counted from the images and searched for abnormal pattern of bristle. Any phenotypic concentration of the flies were recorded and plotted in the graph.

4. Results and discussion

4.1. XRD analysis

XRD patterns of the prepared nanocomposites of hBN–ZrO$_2$ were recorded and are depicted in Fig. 1(a–f). The diffraction peaks were sharp for all the compositions and found to be slightly shifted towards higher angle side with increasing concentration of h-BN. A high intensity characteristic peak was observed at 2$\theta$ value $\sim$28.32$^\circ$ that confirmed the formation of the major phase of zirconium oxynitrate [ZrO(NO$_3$)$_2$] (JCPDS file no. 049-1062). The secondary phase formation of zirconium boride (B$_{12}$Zr) (JCPDS file no. 044-1081), hexagonal boron nitride (h-BN) (JCPDS file no. 035-1365), and zirconium nitrite (Zr$_2$O(N$_2$)) (JCPDS file no. 050-1170) were also confirmed and marked in their respective XRD patterns.

4.2. Infrared spectroscopic analysis

In order to study the mechanisms of bond formation, FTIR measurements of the pure h-BN, pure ZrO$_2$, and their nanocomposites were performed and the results are shown in Fig. S1(a, b)$^\dagger$ and Fig. 2(a–e) respectively. Fig. 2(a–e) depicts the IR spectra recorded for (a) 10B90Z, (b) 20B80Z, (c) 60B40Z, (d) 80B20Z, and (e) 90B10Z. The different shoulders that appeared in the IR spectra of various h-BN–ZrO$_2$ nanocomposites are represented in Table 2 and their assignments are given in Table 5. The peaks are shown in a rising sequence of 1 to 15. The IR spectra exposed different distinctive absorption peaks that confirm the existence of different stretching and bending vibrational modes of h-BN and ZrO$_2$. The asymmetric stretch of N–B–N appears in the wavenumber range from 1383–1490 cm$^{-1}$; however, the same vibration for B–N–B appeared in the wavenumber range from 767–775 cm$^{-1}$.$^{60,62}$ The absorption bands (1) and (2) arose due to the presence of ZrO$_2$ in the

![Fig. 1 XRD patterns of the prepared hBN–ZrO$_2$ biocomposite of (a) 10B90Z, (b) 20B80Z, (c) 40B80Z, (d) 60B40Z, (e) 80B20Z, and (f) 90B10Z sintered at 1000 °C for 3 h.](image)

![Fig. 2 IR spectra of the prepared hBN–ZrO$_2$ composite samples (a) 10B90Z, (b) 20B80Z, (c) 60B40Z, (d) 80B20Z, and (e) 90B10Z sintered at 1000 °C for 3 h.](image)
Table 1 Sample code, h-BN compositions, density, mechanical strength, Young’s modulus, toughness, surface area, pore size and pore volume for all the composite samples.

| Sample code | BN compositions (%) | Density (g cm⁻³) | Mechanical strength (MPa) | Young’s modulus (MPa) | Toughness (MJ m⁻³) | Surface area (m² g⁻¹) | Pore size (nm) | Pore volume (cm³ g⁻¹) |
|-------------|---------------------|------------------|---------------------------|----------------------|------------------|------------------------|---------------|------------------------|
| 10B90Z      | 10                  | 3.12             | 227                       | 10.10                | 2.56             | 21.011                 | 1.21          | 3.71                   |
| 20B80Z      | 20                  | 3.04             | 129                       | 9.93                 | 0.96             | —                      | —             | —                      |
| 40B60Z      | 40                  | 2.82             | 95                        | 6.60                 | 0.57             | —                      | —             | —                      |
| 60B40Z      | 60                  | 2.79             | 76                        | 5.44                 | 0.53             | 31.073                 | 1.64          | 7.63                   |
| 80B20Z      | 80                  | 2.70             | 38                        | 3.70                 | 0.45             | —                      | —             | —                      |
| 90B10Z      | 90                  | 2.67             | 34                        | 3.57                 | 0.16             | 65.666                 | 1.66          | 12.24                  |

Table 2 Composite samples code, peak positions in IR spectra of all the composite samples.

| Samples code | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|--------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|
| 10B90Z       | — | — | — | — | 767| 876| 923| 1060|—— | 1383| 1634|—— |—— |—— |3451|
| 20B80Z       | — | — | — | — | 769| 882| 923| 1060|1194| 1386| 1456|1627|—— |2258|2358|2515|3221|
| 60B40Z       | — | — | — | — | 769| 884| 922| 1071|1193| 1338| 1517|—— |2030|2257|2358|2515|3242|
| 80B20Z       | 507|586|646|814|884|923|1072|1193|1366|1487|—— |2032|2257|2358|2515|3199|
| 90B10Z       | — | — | — | — | 775|883|922|1066|1193|1361|1490|—— |2257|2358|2515|3223|

wavenumber range from 0–07 cm⁻¹ and 586–590 cm⁻¹, respectively; however, bands (3) and (4) occurred in the wavenumber range from 643.28–646.18 cm⁻¹ and 767–814.95 cm⁻¹, respectively, band (5) in the wavenumber range from 876–883 cm⁻¹, band (6) in the wavenumber range from 922–923 cm⁻¹, and band (7) in the wavenumber range from 1060–1072 cm⁻¹ (h-BN vibrational modes) occurred due to the presence of h-BN. The peak (8) situated at 1193 cm⁻¹ might represent the B-O-H bending vibrations. Further, a high intensity band (9) that appeared in the wavenumber range from 1338–1386 cm⁻¹ and 1456–1517 cm⁻¹ is associated with the B-N stretching vibrations and B-O, respectively. In Fig. S1(b), a strong thick envelop in the wavenumber range from 1386–1456 cm⁻¹ was observed that could be attributed to the very close involvement of both h-BN as well as ZrO₂ vibrations. The absorption band (10) appeared due to ZrO₂. The absorption bands (11), (12), (13), and (14) occur at different wavenumber ranges from 1627–1634 cm⁻¹, 2030–2032 cm⁻¹, 2257–2258 cm⁻¹, 2358 cm⁻¹, and 2515 cm⁻¹, which appeared owing to h-BN correspondingly. Fascinatingly, as the concentration of h-BN was increased, the absorption bands (10) vanished due to h-BN, as shown in Fig. 2(c–e). Moreover, this might be due to the suppression of vibrations of h-BN due to the accumulation of excess amount of h-BN. The excess quantity of h-BN (90 wt%) beyond a certain limit into ZrO₂ may restrict these particular usual vibrations. However, some peaks appeared in the wavenumber range from 586–590 cm⁻¹, 767–814 cm⁻¹, 1338–1383 cm⁻¹, and 2358 cm⁻¹, and confirmed the presence of ZrO₂. Eventually, an absorption band (15) was observed in the wavenumber range from 3199–3451 cm⁻¹ in all the IR spectra of the nanocomposite samples, which was accredited to O–H stretching.

Table 3 Density, percentage of porosity and mechanical properties of different bone implant materials.

| Bone implant materials | Density (g cm⁻³) | Porosity [%] | Compressive strength (MPa) | Young’s modulus (MPa) | References |
|------------------------|-----------------|-------------|-----------------------------|-----------------------|------------|
| Titanium (porous)      | 4.5             | 48          | 54 ± 5                      | 7.7 ± 2               | 61 and 73  |
| HAp                    | 2.1             | 41          | 34 ± 2                      | —                     | 74         |
| 0.5 La₂O₃-HAp (solid state route) | 2.85 | 9.66 | 108.89 ± 1 | 90.81 ± 0.5 | 75         |
| 50BN–50B₂O₃ (thin film) | 1.3             | ~36.4       | 37 ± 5                      | —                     | 16         |
| Tricalcium phosphate   | 3.14            | 50          | 11 ± 1.3                    | —                     | 76         |
| 3D graphene (SPS)      | 4.12            | 42          | 40 ± 3                      | 4.1 ± 0.5             | 77         |
| hBN–ZrO₂ (solid state route) | 2.34 | ~10 | 174 ± 1 | 9.84 ± 0.5 | 18         |
| hBN–ZrO₂ (solid state route) | 3.12 | —     | 227 ± 1 | 10 ± 0.1 (GPa) | Current work |
| Pure ZrO₂ (sintered at 1000 °C for 3 h) | 5.68 | —     | 181 ± 1 | 9.053 ± 0.1 | —          |
4.3. Raman spectroscopic analysis

Fig. S2(a and b)† represents the Raman spectra for (a) pure h-BN, (b) pure ZrO$_2$ whereas, Fig. S3(a–e)† depicts the Raman spectra for h-BN–ZrO$_2$ nanocomposites denoted as (a) 10B90Z, (b) 20B80Z, (c) 60B40Z, (d) 80B20Z, and (e) 90B10Z, respectively. The bands are represented by (1), (2), (3), (4), ... (16) in the ascending order of the wavenumber of their appearance, similar to the IR results. The obtained Raman spectra of the composite samples yield a consistent peak at 1367 cm$^{-1}$, matching with the Raman peak of high quality crystalline h-BN, as shown in Fig. S3(a).†† Pure ZrO$_2$ exhibited its characteristic Raman active bands at various wavenumbers such as 45, 178, 189, 260, 330, 377, 476, and 642 cm$^{-1}$.66 As h-BN was introduced into ZrO$_2$, few new bands at 882 cm$^{-1}$ and 1367 cm$^{-1}$ (already mentioned) appeared due to the characteristic bands of h-BN, some existing bands of ZrO$_2$ at 223, 307, 505, and 557 cm$^{-1}$ disappeared dramatically and the remaining bands of ZrO$_2$ were shifted by the 0 to +5 cm$^{-1}$ wavenumber and finally appeared at 179, 191, and 382 cm$^{-1}$ in the spectra of all the composites.78–73 Therefore, the typical bands of hBN–ZrO$_2$ nanocomposites were centred at 103, 333, 349, 476, 615, and 638 cm$^{-1}$. A strong reduction in the intensities of the characteristic bands of h-BN and ZrO$_2$ was observed on increasing the amount of h-BN from 20 wt% in ZrO$_2$. Thus, the results obtained from the Raman study are consistent with the IR results (Table 4).

4.4. X-ray photoelectron spectroscopic (XPS) analysis

The composition of the surface and chemical states of Zr, O, B, and N elements in the sample 10B90Z was further tested by using XPS technique. The XPS spectra are shown in Fig. 3(a–d), representing the existence of Zr, O, B, and N elements on the surface layer of the powder sample 10B90Z without any impurities. Moreover, Fig. 3 shows the observed binding energies corresponding to particular atomic states that constitute different elements that were recognized as 182.4 and 180.15 eV for Zr 3d, 528.4 eV for O 1s, 396.1 and 395.9 eV for N 1s, and 182.45 eV for B 1s.67,49 Fig. 3(a) shows the high-resolution spectrum of Zr 3d, in which two peaks were observed at 180.15 eV (Zr 3d$_{5/2}$) and 182.4 eV (Zr 3d$_{3/2}$) with enough

| Wave number range (cm$^{-1}$), IR | Wave number range (cm$^{-1}$), Raman | IR assignments | Raman assignments |
|----------------------------------|----------------------------------|----------------|-----------------|
| 0–507 & 586–590                   | 145, 178, 189, 260, 330, 377, 476, 642 | Occurred due to ZrO$_2$ | Characteristic Raman active bands of pure ZrO$_2$ |
| 643.28–646.18, 767–814.95, 876–883, 922–923, 1060–1072 | 882, 1367 | h-BN vibrational modes$^{59}$ | Characteristic bands of h-BN |
| 1193                             | —                               | Bending vibrational mode of B–O–H | — |
| 1338–1386                        | 1367                            | Stretching vibrations of B–N$^{60}$ | — |
| 1456–1517                        | —                               | Stretching vibrations of B–O | — |
| 1386–1456                        | —                               | It produced that might be due to the nearly the same involvement of the intensities of vibrations of both h-BN and ZrO$_2$ | — |
| 1634–1627                        | —                               | Appeared due to ZrO$_2$ | — |
| 1627–1634, 2030–2032, 2257–2258, 2358 & 2515 | —                               | Appeared owing to h-BN | — |
| 586–590, 767–814, 1338–1383 & 2358 | —                               | Confirmed the presence of ZrO$_2$ (ref. 61) | — |
| 3199–3451                        | —                               | Stretching vibration of O–H group of atmospheric moisture | — |
separation of 2.25 eV. However, Fig. 3(b and c) depicts the spectra of O 1s and B 1s, in which a single peak in each spectrum was observed at 528.4 eV and 182.45 eV that implies the formation of B–O and B–N bonding. The N 1s peaks of the particular XPS spectrum revealed two different peaks of nitrogen at 396.1 eV and 395.9 eV, which could be the binding energy of B–O and B–N (Fig. 3(d)). Therefore, the B 1s binding energy in B–(O, N) atmosphere is superior than that in B–O atmosphere, where the N atom replaces the O atom.

4.5. BET analysis

In this study, the surface area and average pore size of few selected nanocomposites 10B90Z, 60B40Z, and 90B10Z were measured with the help of nitrogen (N2) adsorption/desorption isotherms using BET analyzer (Fig. 4(a–d)). These nanocomposites exhibit plentiful microporous channels (2 nm < width). The adsorbed content of N2 with respect to relative pressure, P/P0, was found to be maximum in the range of 0.4 to 0.9 and showed a high-quality hysteresis loop (Fig. 4(a and b)). The usual pore size distributions and surface area of the composite samples are shown in Fig. 4(c and d). It is clearly noticed that with increasing the weight% of h-BN, the average pore size and specific surface area of the nanocomposite samples 10B90Z, 60B40Z, and 90B10Z also increased from 1.21 nm to 1.66 nm and 21.011 m2 g−1 to 65.66 m2 g−1, respectively. It is noticed that sample 90B10Z has the highest surface area of around 65.666 m2 g−1 but has an average pore size of 1.66 nm, whereas the sample 60B40Z has a surface area of about 31.07 m2 g−1 but it has an average pore size of 1.64 nm. Even though the average pore size is more in sample 90B10Z than sample 60B40Z, the number of pores present in sample 90B10Z or the porosity of it is more than that of sample 60B40Z and hence, sample 90B10Z has more surface area. h-BN has a highly porous nature so the pore size and surface area both increase with increasing amount of BN. Fig. 4(d) depicts that the synthesized composites can have high BET specific surface area. Therefore, large surface area and established microporous architecture can play a pivotal role in cell growth. Fig. 8(d and e) represents the comparison of the surface area as well as average pore size for the individual nanocomposite samples.
4.6. Scanning electron microscopic (SEM) analysis

The morphological features of the nanocomposites were studied using SEM. Fig. 5(a–e) depicts the SEM images of few selected samples (a) 10B90Z, (b) 20B80Z, (c) 40B60Z, (d) 60B40Z, and (e) 90B10Z, respectively. The interrelated grains and their boundaries were observed that gave rise to the crystalline nature of the prepared materials. The fine grains of ZrO2 and h-BN sheets are represented by yellow and light green coloured arrows, respectively. The SEM images of the composite 10B90Z (Fig. 5(a)) divulged the development of small grains of ZrO2 dispersed in the large, interconnected, and stacked h-BN sheets and 20B80Z showed mixed and uniform grains of h-BN and ZrO2 (Fig. 5(b)). However, the image Fig. 5(c) for 40B60Z clearly depicts the porous and layered structure of h-BN with ZrO2 grains. The images Fig. 5(d and e) for the samples 60B40Z and 90B10Z showed fully developed and randomly oriented h-BN nanosheets of thickness ~100–200 nm and side ~0.5–2 μm. The ZrO2 nanoparticles were hardly seen due to two main factors: one is that the weight percentage of h-BN is larger than that of the ZrO2 and the second one is that the density of h-BN (2.1 g cm⁻³) is comparatively lesser than that of ZrO2 (5.68 g cm⁻³), so h-BN nanosheets covered most of the volume in the samples 60B40Z and 90B10Z. Moreover, the substitution of h-BN into ZrO2 reduced the brittleness of ZrO2 and induced ductility in the sample that agreed with the stress–strain results (Fig. 7(b)). The composition obtained by addition of a suitable amount of h-BN into ZrO2 was strongly expected to be analogous to the natural bone composites. The nanocomposite sample 10B90Z withstands a maximum load of up to 227.33 MPa. It is accomplished from the SEM results that the reinforcement of h-BN into ZrO2 amazingly improved the stability of the ZrO2 with remarkable enhancement in the surface properties.

4.7. Transmission electron microscopic (TEM) analysis

Apart from the surface morphological studies, the TEM study was also carried out to analyse the more magnified view of the nanocomposite 10B90Z at the nanoscale level (Fig. 6(a–f)). Similar to SEM investigations, the mixed h-BN nanosheets and ZrO2 nanoparticles are clearly observed in the TEM images also. In the images, the red coloured arrows indicate the laterally oriented h-BN nanosheets whereas the white coloured arrows show the presence of ZrO2 nanoparticles. The
average particle size (side of h-BN) of the h-BN nanosheets was found to be in the range of 500–1000 nm, which again has a good agreement with the SEM results. However, the particle size of nano ZrO2 was found to be in the range of 5–10 nm. The fine ZrO2 nanoparticles are well dispersed with large h-BN nanosheets and appeared darker than individual h-BN nanosheets. The high resolution (HR-TEM) image (Fig. 6(e)) exhibited the symmetrically arranged layered structure of h-BN nanosheets separated by a distance of 76 nm. The SAED pattern showed various diffraction rings associated with various lattice planes (000), (002), (101), (004), and (110) of h-BN (Fig. 6(f)).

4.8. Density analysis

The densities of all the sintered pellets were determined from the geometrical dimensions and their respective masses. Fig. 7(a) shows the density values of the prepared nanocomposites of h-BN–ZrO2. It is obvious from Fig. 7(a) that the density decreases with successive increments in the amount of h-BN or decrease in the amount of ZrO2 due to the inferior density of h-BN (2.1 g cm\(^{-3}\)) in comparison to the density of pure ZrO2 (5.68 g cm\(^{-3}\)). Moreover, the determined values of density vary from 2.67 g cm\(^{-3}\) to 3.12 g cm\(^{-3}\) for all composites, viz., 3.12, 3.04, 2.82, 2.79, 2.70, and 2.67 g cm\(^{-3}\) for 10B90Z, 20B80Z, 40B60Z, 60B40Z, 80B20Z, and 90B10Z, respectively (Table 1).
Fig. 6 Transmission electron microscopy of the nanostructured composite sample 10B90Z. (a) Low magnification TEM image showing ZrO$_2$ nanoparticles embedded in h-BN sheets. (b and c) High magnification TEM images showing interconnected large nanorods of hBN–ZrO$_2$ nanoparticles. (d) High magnification bright field TEM image showing interconnected large h-BN sheets consisting of ZrO$_2$ nanoparticles. (e) High resolution TEM image clearly showing the very fine arrangement of large nanorods. (f) Selected area electron diffraction pattern (SAED) showing spotty (ZrO$_2$) and crystalline pattern of h-BN.
4.9. Mechanical behaviour

The stress–strain graphs for pure ZrO$_2$, 10B90Z, 20B80Z, 40B60Z, 60B40Z, 80B20Z, and 90B10Z nanocomposites are given in Fig. 7(b). It is noticed from Fig. 7(b) that on increasing the amount of h-BN into ZrO$_2$, the mechanical strength, Young’s modulus, and toughness decrease (Table 1). Moreover, due to less density of h-BN and its highly lubricating nature, it provides the flexibility to the synthesized composites that inhibit the brittleness of the composites. However, the nanocomposite 10B90Z achieved the highest compressive strength of $\approx 227$ MPa along with adequate values of Young’s modulus and fracture toughness 10.10 GPa and 2.56 MJ m$^{-3}$, respectively. This composite also has a density of 3.12 g cm$^{-3}$, which is very close to that of natural teeth. It is well known that ZrO$_2$ has superior mechanical properties due to its dense microstructure. When ZrO$_2$ is added with h-BN and sintered at 1000 °C, they are strongly bonded with each other and provide better strength to the final product. The fine spherical particles linked on the h-BN surface are mostly the minor phase of ZrB$_2$ and ZrB. Because these two kinds of particles have enormous modulus and mechanical strength, they contribute to the reinforcement of the hBN–ZrO$_2$ composites. Moreover, the typical SEM image (Fig. 5(a)) of this composite sample shows a unique morphology, i.e., the formation of stacked h-BN grains during the sintering process. When the compressive load is applied, the stacked h-BN grains prevent the crack propagation and hence, the composite shows its maximum strength. Thus, these findings fulfilled the mechanical criteria for this nanocomposite as a capable alternative to artificial teeth and dental implants. The hBN–ZrO$_2$ composites expressed trivial deformability, which occurs due to the ductile nature of h-BN that prevents disastrous failures. Thus, the addition of h-BN into ZrO$_2$ paves the way to reduce the brittle nature of pure ZrO$_2$ that could stand better than natural bone. The Young’s modulus and toughness decreased with increasing concentration of h-BN in ZrO$_2$ (Fig. 7(c–d)). The comparative analysis of density, porosity, Young’s modulus, and compressive strength of the various composite samples obtained via different synthetic routes, as reported in the literature, with the synthesized composite sample 10B90Z is given in Table 3 along with suitable references. Among all the synthesized nanocomposite composites, 10B90Z was found to be the best and superior in comparison to the reported biomaterials such as porous titanium (pTi), nHAp, La$_2$O$_3$-Hap, tricalcium phosphate, and 3D graphene.

4.10. Tribological analysis of the composite samples

The tribological characteristics of the few selected samples 10B90Z, 60B40Z, and 90B10Z sintered at 1000 °C for 3 h were...
tested to analyze their wear and coefficient of friction behaviors. Fig. 8(a–e) shows the variation of wear and coefficient of frictional force with sliding time for the above composite samples. The weight loss of the materials at 15 N load is found to be significantly lesser than that at 5 N and 10 N loads, whereas weight loss is maximum for 10 N load (Fig. 8(a–c)). Fig. 8(e) reveals the higher weight loss at load 15 N and minimum at load 5 N. As the concentration of h-BN increases and the percentage of ZrO₂ decreases at higher load (15 N), the weight loss increases appreciably. However, in all the above mentioned nanocomposites, a moderate wear property was observed for 5 N loads. Thus, these composites may be recommended for wear resistance materials for low loads. It is noticed that the variation of coefficient of friction for all the compositions is fluctuating in nature and within the range of 0.1 to 0.4 (Fig. 8(b, d and f)). In the present investigations, at higher load, the coefficient of friction is higher than that at a lower load of 5 N. Therefore, the optimum value of all compositions occurs at 5 N load. Eventually, the present developed nanocomposite is suitable for 5 N load. On increasing the reinforcement of h-BN in ZrO₂, the composite becomes ductile in nature, and owing to the ductile and soft nature of h-BN, the sufficient weight loss was found to increase beyond a certain limit of h-BN concentration. Therefore, in view of the tribological properties, the composite

Fig. 8  Variations of wear against sliding time and coefficient of friction against sliding time at different loads of the composite samples (a and b) 10B90Z, (c and d) 60B40Z, and (e and f) 90B10Z sintered at 1000 °C for 3 h.
10B90Z sample was found to be very compatible with teeth implant applications.

5. Biological activities

5.1. Life cycle analysis

Nanocomposites may induce defects in the developmental cycle of the fly. But in our case, we did not observe any delay in developmental cycle. From pupal count of third day after appearance of pupa, we observed that the composite 10B90Z (Fig. 9(A)) induced negative impact on the pupal number at higher concentrations such as 1 μg mL⁻¹ and 10 μg mL⁻¹, whose values were 9.000 ± 1.000 and 36.50 ± 1.500, respectively. However, at higher concentrations such as 25 μg mL⁻¹, 50 μg mL⁻¹, and 100 μg mL⁻¹, no such significant changes were observed, which was confirmed by the values 48.00 ± 5.000, 37.00 ± 4.000, and 51.00 ± 7.000, respectively. The control value for pupal count was 50.00 ± 0.0. This result suggests that nanocomposite 90B10Z induces damage to pupal development at lower concentrations. However, the nanocomposite 10B90Z has no impact on the pupal count, which revealed the superiority of 10B90Z over 90B10Z.

5.2. Larva crawling assay

The larva crawling assay was done to check any neuronal defects in the larva due to ingestion of nanocomposites 10B90Z and 90B10Z through food. The wild type larvae or the control ones moved almost in a straight line from the centre of the petri dish towards the periphery, whilst many treated larvae traveled confused and curvy paths, and some traveled almost negligible distances. The larvae treated with different concentrations of the sample 10B90Z also showed some confused movements in the crawling plates, which can be observed from the traced path (Fig. 10(A)). At concentrations such as 1 μg mL⁻¹, 50 μg mL⁻¹, and 100 μg mL⁻¹, the path traveled by the larvae is not straight and was confusing. Some larvae took a lot more time to reach the periphery as compared to the control larvae. More than half of the larvae treated with the highest concentration of 10B90Z, i.e., 100 μg mL⁻¹, didn’t travel a significant path. This suggests neuronal damage of the larvae. Mostly, the damage of the larvae is seen at higher concentrations.

In Fig. 10(B), we can see that the path traced by the larvae treated with 10 μg mL⁻¹ was very curvy and it fails to reach the periphery. Also, for those treated with 25 μg mL⁻¹ concentration, the path travelled to reach their destination was very long and curvy, which shows confused behaviour of the larvae. This suggests that some motor neurons of the larvae were affected by the nanocomposite 90B10Z at lower concentrations. This data shows similarity with literatures where nanoparticles induced toxicity affects the neuronal health of the fly. To validate the crawling assay, we checked the speed of the larva treated with nanocomposite 10B90Z. In Fig. 10(C), the control crawling speed was found to be 1.544 ± 0.1075. We did not find any significant changes in the speed at 10 μg mL⁻¹ and 25 μg mL⁻¹ treated larvae, whose speeds were found to be 1.206 ± 0.1181 and 1.310 ± 0.1089, respectively. On the other hand, in the case of 1 μg mL⁻¹, 50
In order to check the dead cells inside the gut of the larvae, trypan blue test was also performed. It differentiates the dead cells from the live cells by staining the dead cells blue while the live cells are unaffected. In Fig. 11(A), only mild gut damage was seen in the third instar larvae at 100 μg mL⁻¹ concentration of the nanocomposite 10B90Z. All the larvae from other treated concentrations of the nanocomposite 10B90Z gave negative results to the trypan blue test. This suggests that this has no toxic effect on the gut of larva.

Fig. 11(B) suggests gut damage at lower concentrations such as 1 μg mL⁻¹, 10 μg mL⁻¹, and 25 μg mL⁻¹ while the larva with high concentrations shows a negative result to the test. This may be due to agglomeration of nanoparticles at higher concentrations. Mild damage in the gut was seen at 1 μg mL⁻¹ concentration larva while severe gut damage was seen in the third instar larvae of 10 μg mL⁻¹ and 25 μg mL⁻¹ concentrations of the nanocomposite 90B10Z. From this result we can confirm that the nanocomposite 90B10Z causes damage to the gut tissues when employed at lower concentration.
5.4. NBT reduction assay for ROS production

The verification of quantity of ROS generated in the haemolymph of third instar larvae after up taking nanoparticles was done through NBT assay. The amount of ROS generation is directly proportional to the absorbance. For nanocomposite 10B90Z, (Fig. 11(C)) absorbance for ROS of control was found to be 0.09067 ± 0.007535. At 1 μg mL⁻¹ and 50 μg mL⁻¹, the value was decreased to 0.04133 ± 0.004096 and 0.05033 ± 0.009333, respectively, showing no increase in ROS generation. Also, we did not find a significant change in the absorbance at 10 μg mL⁻¹ and 100 μg mL⁻¹, which was 0.1030 ± 0.003215 and 0.09133 ± 0.001764, respectively, which is equivalent to the control showing no increase in ROS generation but a significant increase was observed in 25 μg mL⁻¹, which was 0.1287 ± 0.004256. In each case, the experiment was repeated three times. Herein, most of the concentrations show negative effect to increased ROS generation. Only at 25 μg mL⁻¹ toxicity was seen. Hence, we can infer that this nanoparticle is less toxic when applied in all these concentrations.

For the nanocomposite 90B10Z, (Fig. 11(D)) the absorbance for ROS of the control was found to be 0.08333 ± 0.003333. At 1 μg mL⁻¹ and 100 μg mL⁻¹, the absorbance was found to be 0.0170 ± 0.003215 and 0.06567 ± 0.002667, respectively, which was less than that of the control, while no such significant changes were observed in the case of 50 μg mL⁻¹, which was 0.0700 ± 0.003464. Interestingly, at concentrations of 10 μg mL⁻¹ and 25 μg mL⁻¹, a large increase in the ROS production was observed and the values were 0.2007 ± 0.01170 and 0.2117 ± 0.002848, respectively. The literatures suggest that the nanoparticles induce stress in the fly, resulting in oxidative damage. So, ROS production will be more. Lower concentrations of the nanocomposite 90B10Z were mostly affected by the nanoparticles. At higher concentration, the nanoparticle...
size may become larger by agglomeration, so they cannot travel through all the tissues. This may be one of the reasons of less toxicity at higher concentration of the nanoparticles.

6. Adult phenotype assay

6.1. Eye phenotype

In nanocomposite 10B90Z (Fig. 12(A)), we found that at 10 μg mL\(^{-1}\) and 100 μg mL\(^{-1}\) concentrations, the structural arrangement of the eyes got disturbed. Ommatidial loss near the periphery region indicates the apoptosis of structures. At other concentrations, the eye structures were unaffected. In case of the composite sample 90B10Z (Fig. 12(B)) at lower concentrations such as 1 μg mL\(^{-1}\) and 10 μg mL\(^{-1}\), we observed some ommatidia loss in the compound eye of Drosophila. The similar kind of eye defect was observed at higher concentrations. There were no such defects observed at 25 μg mL\(^{-1}\) and 50 μg mL\(^{-1}\) concentrations.

6.2. Wing phenotype

To check the toxic effect of the nanoparticles, the adult phenotypes were taken. For the nanocomposite 10B90Z (Fig. 12(C)), no such defects appeared in the adult. This suggests that the nanocomposite 10B90Z has no negative effect on wing development in the fly. For the nanocomposite 90B10Z (Fig. 12(D)), at 10 μg mL\(^{-1}\) as well as 100 μg mL\(^{-1}\) concentration, some black spots in wings were seen that are absent in the control wings. Wing spots were observed in the wings due to genotoxicity of the nanoparticles on the fly's body. Some previous data suggest that the nanoparticles have genotoxic effects that may lead to wing spot formation. Therefore, the nanocomposite 90B10Z affects the normal development of wings at lower concentrations as well as higher concentrations.

6.3. Bristle phenotype

The genes achaete–scute complex are responsible for the proper formation of microchaetes in fly. In the case of nanocomposite 10B90Z (Fig. 13(A)), no major defects were observed. However, at 25 μg mL\(^{-1}\) concentration, some bristles were absent in the thorax regions. This indicates that the nanocomposite 10B90Z has no damaging effect on the bristle patterning in Drosophila. In the composite sample 90B10Z (Fig. 13(B)), at 1 μg mL\(^{-1}\) concentration, formation of extra bristle was seen. A number of missing bristles at their respective regions were observed at 10 μg mL\(^{-1}\). Previous reports suggest that the damage in bristle...
formation in *Drosophila* is due to nanoparticle uptake. At 25 μg mL⁻¹ and 100 μg mL⁻¹ concentrations, the thorax region was melanized. The pigmentation in the thorax region of *Drosophila* is due to some genes such as *ebony* and *dopa*. They are the key regulators of melanin synthesis in the fly’s body. Some previous studies on zirconia toxicity have shown the pigmentation at abdominal regions of the fly. The defective patterning in pigmentation of the thorax of flies suggests that the nanocomposites affect the pigmentation genes only at higher concentrations.

7. Conclusions

The various 3-D nano biocomposites of hBN-ZrO₂ of high strength were successfully synthesized via solid state reaction method. Their structural, morphological, mechanical, and
tribological properties were well correlated. A fine interconnected network was observed in the microstructural study. The zirconia nanoparticles were finely decorated into large and organized hexagonal nanosheets of boron nitride. The nanocomposite 10B90Z showed less toxicity in the in vivo study using Drosophila melanogaster, while the composite 90B10Z showed toxicity in Drosophila. Due to the less toxicity of the composite 10B90Z, its utilization in nano formulation of various drug delivery systems can be proposed. Among all the synthesized compositions, the composite 10B90Z achieved the most significant mechanical, tribological, and biological properties compared to the desired properties for artificial teeth and teeth implant materials.

Conflicts of interest
The authors declare no competing financial interest.

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