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*Research Article*

# An In Vitro Assessment of the Acid Resistance Characteristics of Nanohydroxyapatite/Silica Biocomposite Synthesized Using Mechanochemistry

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This paper reports on the in vitro assessment of the acid resistance characteristics of mesoporous silica/nanohydroxyapatite (MSN@nHAp) biocomposite synthesized through the mechanochemical method. Bovine enamel models were used to study the acid resistance characteristics of the composite ($n = 5$). X-ray diffraction and Fourier transform infrared spectroscopy were used to characterize the surface morphology of the MSN@nHAp. The XRD and FTIR results confirmed the successful syntheses and surface modification of nanohydroxyapatite with silica. The MSN@nHAp exhibits superior acid resistance characteristics. The salient aspect of this study suggests that mechanochemistry is a useful technique in the synthesis and surface modification of valuable biomaterials. The study concludes that the MSN@nHAp composite could be utilised in toothpaste formulation for oral healthcare management due to its acid resistance properties.

## 1. Introduction

Dentin hypersensitivity (DH) is a noticeable dental problem with a negative consequence on the individual's quality of life [1]. From an epidemiological context, Zeola et al. [2] revealed that discomfort from dentin hypersensitivity is a common finding in the adult population, ranging between 11.5 and 35.5%. While different dentin hypersensitivity toothpastes are commercially available in the market, their overall effectiveness is limited in an acidic environment [3]. Owing to this, a novel approach in the treatment and management of DH is the use of various nanomaterials [4, 5]. Among these materials, mesoporous silica (MSN) and nanohydroxyapatite (nHAp) had gained enormous research interest due to their antibacterial action; physical, mechanical, and biological characteristics; and distinctive particle size [6, 7].

Previous studies reported that nHAp could repair tooth enamel [8, 9], which suggests that it might be useful to treat DH. Besides, nHAps could easily penetrate the dentinal tubules, which may improve their dentin occlusion properties [10]. On the other hand, MSNs had strong osteoconductivity and bioactivity properties [11], which render them compatible with other bioactive materials [12]. In addition, MSNs have been widely used as a dentin tubule occluding material in combination with other inorganic nanoparticles. For example, Tian et al. [12] showed that a new MSN-based biocomposite successfully occludes dentin tubules. Yu et al. [6] reported that the mesoporous silica/hydroxyapatite composite was effective against acidic substances in occluding dentin tubules.

While MSN@nHAp biocomposites are widely synthesized in the laboratory through the precipitation process [13], nevertheless, the chemical method of synthesizing the composite may be cumbersome and time-consuming and, in most times, requires the use of toxic chemicals [14]. Consequently, there is a need for an alternative

environmentally friendly technique for synthesizing the biocomposite using the mechanochemical method. The application of the mechanochemical method in biocomposite material preparation has gained widespread interest among researchers, due to its simplicity and environmental friendliness [15]. The technique utilises mechanical force to affect chemical reactions as well as structural changes in a material [16]. Despite the enormous potential of the mechanochemical method, there is limited evidence in its use for the preparation of mesoporous silica and nanohydroxyapatite biocomposites (MSN@nHAp). In this present study, we reported on the use of a mechanochemical method for synthesizing MSN@nHAp. The technique entails wet-milling calcined eggshell waste and sodium triphosphate to form nHAp. Thereafter, the synthesized nHAp was modified with MSN silica by ball milling both materials together using the mechanochemical method. This study was aimed at assessing in vitro the acid resistance characteristics of MSN@nHAp biocomposite synthesized through the mechanochemical method for its potential application in the management of DH.

## 2. Material and Methods

*2.1. Preparation Nanohydroxyapatite and Silica Composite (MSN@nHAp).* Locally sourced chicken eggshells were prepared according to the method described by Onwubu et al. [17]. The oven-dried eggshells were calcined by heating them in a furnace at 800°C for 3 hours at a heating rate of 3°C/min. Thereafter, nanohydroxyapatite was synthesized and subsequently modified with mesoporous silica following two steps: step 1: nHAp synthesis through wet-milling. In this step, 20 g of the calcined eggshell powder was wet-milled together with 13.4 g of sodium triphosphate in 100 mL of deionized water using a planetary ball mill (Retsch® PM 100) at 500 rpm for 5 hours. After milling, the mixture was centrifuged at speed of 1000 rpm for 30 min and thereafter oven-dried at 40°C for 5 days. Next is step 2: synthesis of MSN@nHAp through dry milling. Using the planetary ball mill in step 1, mesoporous silica (0.9 $\mu$m; Sigma-Aldrich) was used to modify the synthesized nHAp at 500 rpm for 40 min in a ratio of 1 : 10 (1 g of MSN to 10 g of nHAp). The parameters used for the milling process in the two steps include 30 stainless steel balls of 10 mm diameter in a 250 mL bow. The powder obtained after milling was characterized to establish the formation of nHAp in the first step and the subsequent modification with silica in the second step. The particle size and shape of the prepared nHAp are reported in another study [18]. The pH of the samples was measured using a pH meter. 1 g of each sample was dissolved in 30 mL deionized water, and the solution agitated at a low speed of 500 rpm for 1 min. The pH reading measured for the MSN was 2.5, nHAp was 13.94, and MSN@nHAp was 13.07.

*2.1.1. Fourier Transform Infrared Spectroscopy Analysis.* A Perkin Elmer Universal ATR was used to study the chemical composition and functional group present in nHAp and MSN@nHAp. An initial background check was performed before scanning the samples. Thereafter, few quantities of the samples were placed in the sample holder for FTIR analysis. The scanning range was 400-4500 $cm^{-1}$ at a resolution of 4 $cm^{-1}$.

*2.1.2. X-Ray Diffraction Analysis.* The changes in the crystallinity of nHAp, MSN@nHAp, and MSN were studied using an XRD diffractometer (D8 Advance, BRUKER AXS instrument, Germany instrument; Cu-Ka radiation (lKa1 = 1.5406 Å). The samples were analysed between 0 and 90 (2 theta) while the voltage, current, and pass time were kept at 40 Kv, 40 mA, and 0.5 s, respectively.

*2.2. Acid Resistance Test on Tooth Enamel.* In line with the procedure demonstrated by Onwubu et al. [18], bovine tooth enamel was used to assess the acid resistance characteristics of the samples. The tooth enamel specimens were assigned randomly into five groups ($n = 5$). The tooth enamel was placed in a 4% citric acid solution containing each of the sample powders for 2 min. The images of the specimens before and after acidic exposure were studied using a scanning electron microscope (Field Emission-Carl Zeiss). Energy-dispersive spectroscopy was further used to quantify the elemental loss in the samples after acidic exposure. Each tooth sample was tested at four separate locations on the tooth surface, and the mean elemental loss calculated as a percentage of the total weight of all elements found after acidic exposure.

## 3. Results and Discussion

*3.1. Characterization of the Prepared Composite.* The FTIR spectra of the prepared nHAp and MSN@nHAp are given in Figure 1. In Figure 1(a), the spectrum observed around ~1000 $cm^{-1}$ is attributed to the P–O asymmetrical in the hydroxyapatite [19]. The broad spectrum observed around ~3450 $cm^{-1}$ and 3000 $cm^{-1}$ and a sharp peak at 1600 $cm^{-1}$ was attributed to water molecules [7, 18, 20]. The sharp spectrum around 3750 $cm^{-1}$ is attributed to the OH stretching [19]. The absorption bands of carbonates ($CO_3^{2-}$ ions) in the hydroxyapatite structure were observed around 1450 $cm^{-1}$ [7, 18]. On the contrary, the image in Figure 1(c) reveals the presence of Si-O-Si stretching. The presence of Si-O-Si vibration suggests the formation of nHAp crystals within the MSN structure and agrees with other studies [6, 21]. Although Yu et al. [6] in their study observed OH bending in the MSN@nHAp structure, the image in Figure 1(c), however, reveals the absence of OH bending. The plausible explanation for this may be attributed to the dry milling process used in the modification of MSN and nHAp as against the wet-chemical process reported by [6].

Figure 2 depicts the XRD patterns of the prepared nHAp, MSN@nHAp, and MSN. In Figure 2(a) (A), the pattern with (111), (112), (042), and (260) corresponds to the hydroxyapatite peak. This was further confirmed by the international standard (JCP2-76-0694). For the MSN image in Figure 2(a) (C), noncrystalline scattering observed between 10 and 35° is indicative that the material is amorphous [6]. The MSN@nHAp displays peaks, which are

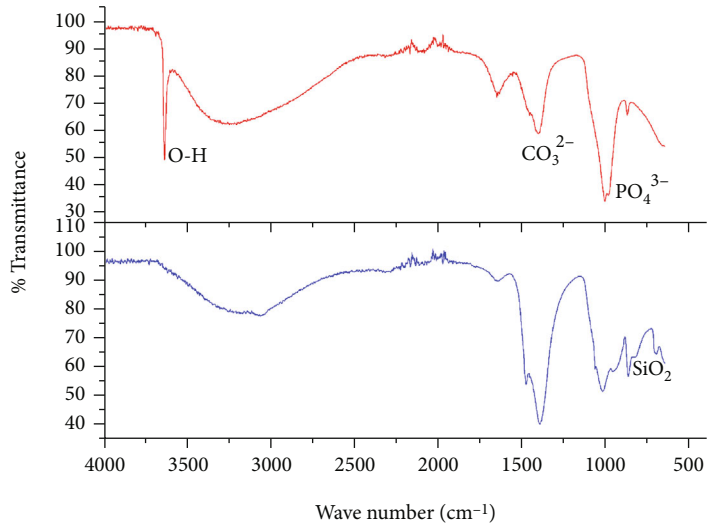


Figure 1: FTIR spectra of (a) nHAp and (b) MSN@nHAp.

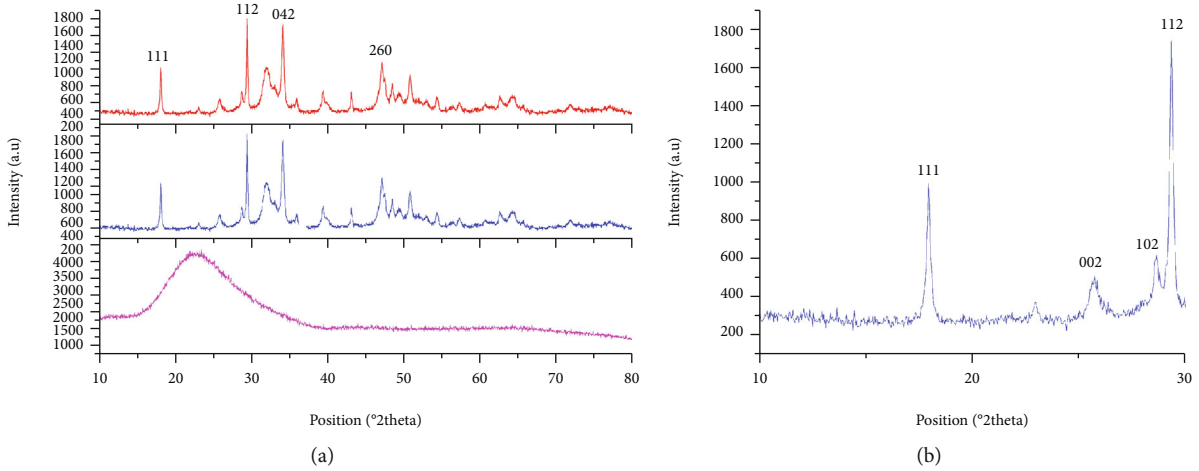


Figure 2: XRD pattern of (a): (A) nHAp, (B) MSN@nHAp, and (C) MSN; (b) extended MSN showing the amorphous region observed between 10 and 15°.

characteristics of the standard diffraction peaks of the nHAp phase and are consistent with the image in Figure 2(a). Moreover, the MSN@nHAp composite shows an amorphous peak which corresponds to the silica peak between 10 to 15° (Figure 2(b)).

The tooth enamel is mainly made up of hydroxyapatite in the form of calcium ($Ca^{2+}$) and phosphate ions ($PO_4^{3-}$). It has been suggested in the literature that the loss of $Ca^{2+}$ and $PO_4^{3-}$ ions is indicative of tooth demineralization [22]. The elemental analysis of the samples in Table 1 suggests that the samples exposed to citric acid alone had the most elemental loss of calcium (33.55%) and phosphorus (14.33%) while the nHAp and MSN@nHAp group the lowest elemental losses, respectively. Arguably, it could be assumed that the lower elemental loss of $Ca^{2+}$ and $PO_4^{3-}$ ions in the MSN@nHAp group suggests resistance to acidic attacks while the higher loss of the elements is indicative of enamel demineralization. This is in agreement with Shellis et al. [22] that the exposure of tooth enamel to acidic attacks causes the enamel to release $Ca^{2+}$ and $PO_4^{3-}$ ions to the oral environment to attain a new state of equilibrium.

The FESEM images of the bovine enamel before exposure, exposed to acid alone, MSN, nHAp, and MSN@nHAp, are illustrated in Figure 3. There was visible evidence of the destruction of the prismatic enamel structure in the samples exposed to citric acid alone (Figure 3(b)). This supports the assertion that dietary acids such as citric acid result in enamel demineralization [23]. In Figure 3(c), the MSN, to some extent, offers protection against the erosive challenge. This may be attributed to the acid-resistant stability of MSN, which offers strong acid resistance properties [6]. Furthermore, the FESEM images in Figure 3(d) suggest that nHAp offers effective protection against acid dissolution. There was minimal effect on the enamel surface, which

Table 1: Elemental analysis of mineral loss in tooth enamel samples.

| Element (weight in %) | Sample groups | | | | |
|---|---|---|---|---|---|
| | Unexposed tooth | Exposed to citric acid alone | MSS | nHAp | MSN@nHAp |
| Carbon (C) | 27.48% | 8.54% | 13.61% | 32.08 | 28.93 |
| Calcium (Ca) | 20.95% | 33.55% | 30.48% | 21.91 | 21.77 |
| Oxygen (O) | 39.42% | 42.59% | 40.38% | 34.41% | 35.35 |
| Phosphorus (P) | 11.20% | 14.33% | 14.26% | 10.29% | 10.71% |

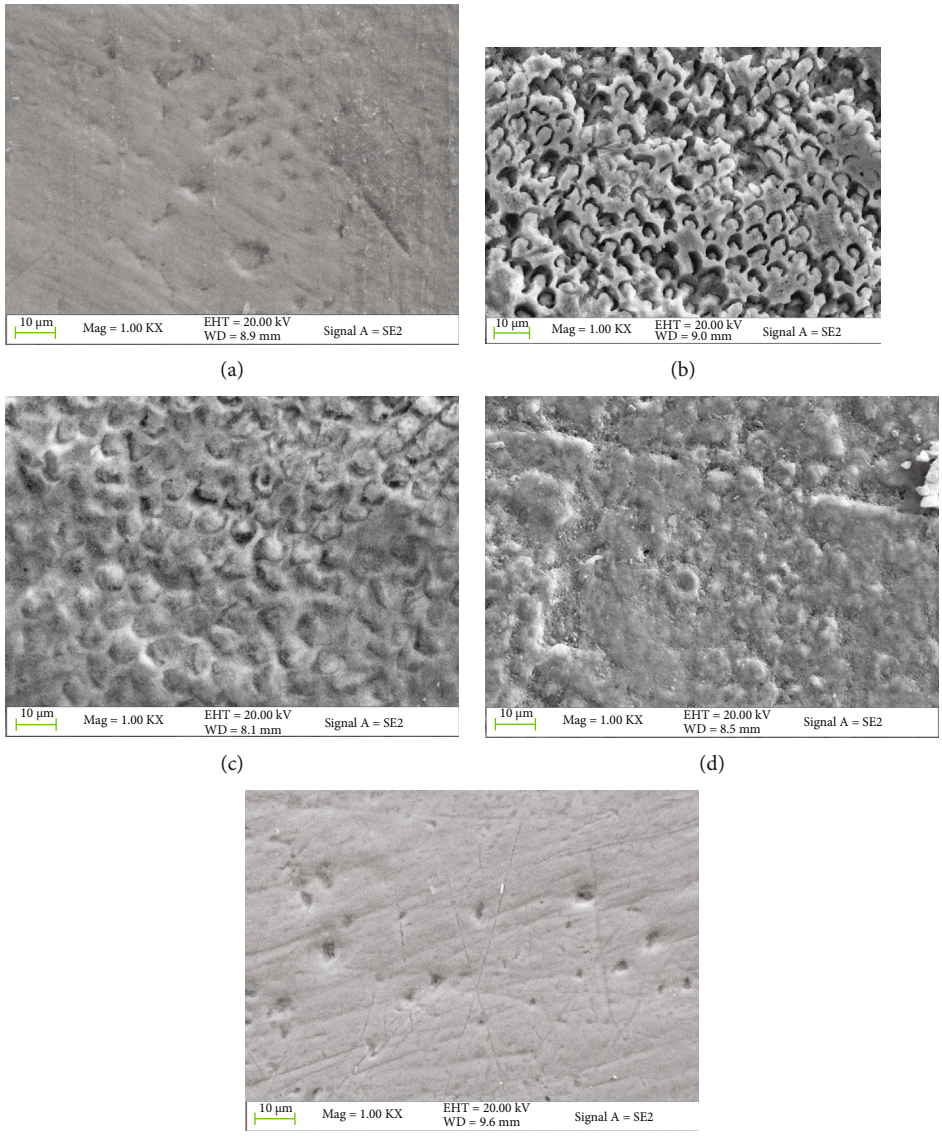


Figure 3: SEM image of tooth enamel: (a) unexposed tooth; (b) exposed alone; (c) MSS; (d) nHAp; (e) MSN@nHAp.

could be attributed to the pH of the nHAp slurry (pH = 3.94 ). Consistent with Tschoppe et al. [24], it could be assumed that the higher pH value of the nHAp slurries acts by reducing demineralization.

The FESEM image of the MSN@nHAp suggests that there was outstanding protection against erosive challenges. The image indicates that acid resistance protection of MSN@nHAp was superior when compared to the MSN and nHAp groups, as there was no visible evidence of enamel demineralization. This is in agreement with the finding of Yu et al. [6] who observed similar superior acid-resistant stability for the mesoporous silica and nanohydroxyapatite

composites. A plausible explanation for the outstanding enamel protection offered by the composite may likely be due to the capacity of the silica constituent of the composite to increase the bioavailability and slow the release of the incorporated calcium and phosphate ions [25, 26].

## 4. Conclusion

In summary, the results obtained from the study indicate that MSN@nHAp was successfully synthesized and modified using the mechanochemical method. The FTIR and XRD results confirmed the presence of both crystalline and amorphous structures in the synthesized composite. The FESEM and EDX results indicate that the modified biocomposite exhibits superior acid resistance properties which suggest that it could be useful as a biomaterial for dental application. Particularly, the biocomposite may be highly useful in toothpaste formulation for the treatment of dentin hypersensitivity as well as valuable where an acid resistance characteristic is required in oral care products. Hence, future studies will evaluate the dentin tubule occluding properties of the composite in the management of DH.

## Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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