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High strength bioinspired calcium phosphate-based material for bone repair applications

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Highlights

- A simple method of synthesising hydroxyapatite composites for biomedical applications is presented.
- Titanium-reinforced composites with calcium phosphate content close to natural bone were developed.
- Waste seashells were used for extraction and recovery of valuable calcium oxide compounds.
- Hydroxyapatite-based calcium oxide composites with high mechanical performance were synthesised.

Abstract

Owing to the increasing demand for bone repair strategies, several biomaterials have been developed. Among the materials available for this purpose, hydroxyapatite stands out for its osteoinduction capacity, since it possesses a chemical composition similar to that of inorganic bone constituents. In comparison to bones, the mechanical properties of substitute structures incorporating hydroxyapatite still remain a great challenge for scientists. This study thus presents the synthesis of hydroxyapatite incorporated with a natural bioceramic and a metallic phase of excellent biocompatibility to obtain dense biomaterials with improved mechanical strength. The mechanical responses of the synthesized biomaterials are presented and discussed. The results obtained indicate that the hydroxyapatite-natural ceramic systems fulfils the general mechanical property requirements for some bone repair applications. Separately, the synthesis of titanium-based systems was shown to be much more challenging, but promising. Therefore, recommendations for suppressing the issues with the metal-ceramic interfacial bonding strength were provided.

Keywords: hydroxyapatite; seashell; biocompatible material; biomedical applications; sustainability

1. Introduction

Regenerative medicine using autologous bone grafts is still today the golden standard for treating critical-size bone defects arising from diseases, trauma or accidents [1,2]. This is because human bones possess a unique ability to regenerate and remodel to provide mechanical stability for support, locomotion and protection of soft tissues [3,4]. However, the availability of grafts and the risk of damaging the donor tissues including the tendency for bacterial and viral infections require consideration [5]. Hence, the demand for new technologies

for bone implant manufacturing is largely focused on the use of synthetic materials that are easily available, reliable, and reproducible [6].

Natural bone is composed of both organic and inorganic components [7,8]. Calcium and phosphorus in the form of hydroxyapatite crystals are the main inorganic components endowing mechanical rigidity and load-bearing strength to bone [9]. Synthetic hydroxyapatites are recognised to be biocompatible, bioactive and nontoxic, allowing osseointegration between bones and implants [10]. Possessing similar characteristics to synthetic hydroxyapatites, although more interesting, natural hydroxyapatites can be extracted from natural resources such as calcium-rich marine waste materials. As a matter of fact, this is a sustainable approach as it provides an innovation to produce a new valuable product from waste materials [11]. However, both synthetic and natural hydroxyapatites present inferior mechanical properties to natural bones [12,13].

Metallic implants such as those made from stainless steel and titanium alloys have been extensively used in medical applications [14]. Despite being biocompatible, they cannot biodegrade and hence are retained as permanent implants *in vivo*. However, for permanent applications, alloys typically have a possible toxic effect resulting from releasing chemical compounds and elements into the surrounding tissue. Moreover, metallic implants lack bioactivity and can only bond with tissue through mechanical interlocking [15,16]. Among all metallic materials, pure titanium is widely regarded as the ultimate choice for biomedical applications due to its good mechanical properties and high biocompatibility, making it the most suitable material for developing biocomposite implants [17,18].

Hard-tissue implants containing hydroxyapatite have more flexibility in biocompatibility and greater osseointegration potential than metallic implants [19]. However, due to its brittleness and mechanical performance, hydroxyapatite alone is unsuitable for high strength bone applications [20]. Hence, hydroxyapatite composites are potential candidates as load-bearing materials, but it is very important to design them with properties similar to biological bone. The synthesis of hydroxyapatite-titanium composites is promising as titanium (the reinforcing phase) allows composites to reach special properties that meet the requirements for bone implants [21]. However, the synthesis routes and treatment techniques of hydroxyapatite-titanium composites affect the interface morphology and interfacial reactions due to their respective processing temperature and thermal diffusion characteristics [22]. Therefore, it is desirable to obtain high interfacial bonding so that any active stress can be transmitted from the hydroxyapatite to the titanium phase [23]. For this reason, it is necessary to construct synergistic mechanisms of strengthening to control and regulate the composites microstructure in order to improve their biological activity [24,25] and properties such as hardness [26,27], compressive [20,28] and flexural strength [26,29]. Unfortunately, the current literature data on the mechanical strength of hydroxyapatite composites for bone repair applications is limited and very broad [30–39]. Therefore, this work concentrates on the development of high strength hydroxyapatite-based materials from commercial hydroxyapatite and seashell waste. Additionally, it also aims at contributing to a more thorough assessment of the current issues around the synthesis of titanium-reinforced hydroxyapatites.

2. Materials and Methods

Commercial titanium powder (99.5% pure) and commercial hydroxyapatite powder $Ca_5(PO_4)_3(OH)$ were purchased from Fisher Scientific and Merck. The calcium oxide powder was prepared from seashells collected from a local beach in Dublin, Ireland. First, the seashells were thoroughly washed to eliminate contaminates and dried in an oven. Then, they were subjected to heat treatment in an electric furnace at 1000 °C for 1 h. Lastly, the calcinated seashells were crushed into a fine powder and sieved. The morphology of the titanium, hydroxyapatite and seashell powders is show in Figure 1.

Powder mixtures to be used in the synthesis of the matrix samples (hydroxyapatite 50 wt% + seashell 50 wt%) and of the composite samples (hydroxyapatite 10 wt% + seashell 10 wt% + titanium 80 wt%) were developed

via ball milling. These powder mixtures were then uniaxially pressed at 160 MPa into pellets (20 mm in diameter and 6 mm in length) and bars (50 x 12 x 3 mm^3). Lastly, the green compacts were sintered in a moist argon atmosphere at 1300 °C for 2 h with a heating rate of 5 °C/min. Images of the sintered samples are available in Figure 1.

The density of the sintered samples was measured with a Micromeritics AccuPyc 1330 helium pycnometer. The Vickers hardness was measured using a Leitz microhardness tester and the measurements were performed according to ASTM E92 standard [40]. A Zeiss EVO LS-15 scanning electron microscope equipped with a Xplore 15 detector from Oxford Instruments was utilised for microstructural and elemental analysis. The phases presented in the samples were determined using a triple-axis Jordan Valley Bede D1 high resolution x-ray diffraction system with a copper (λ = 1.5405 Å) radiation source operated at 45 kV and 40 mA [41]. Structural changes also were examined by Fourier transform infrared spectroscopy using a Perkin Elmer Spectrum Two spectrometer. The three-point bending and compression tests were performed using a Zwick Z050 (Zwick/Roell GmbH, Germany) fitted with Zwick TestXpert software and a 50 kN load cell [42]. The support span length of the three-point outer jaws was 48 mm. The testing was carried out with a crosshead speed of 0.5 mm/min.

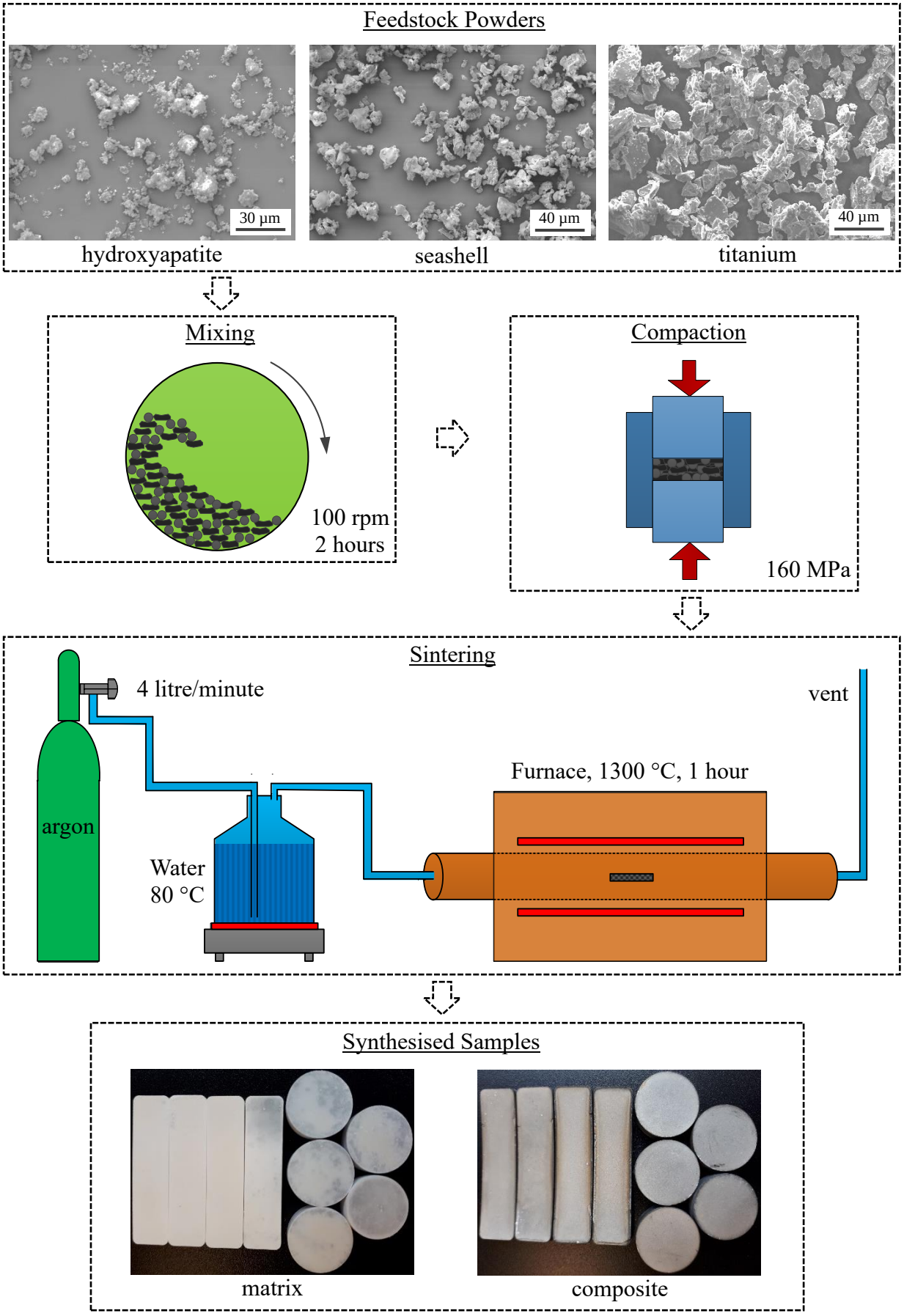


Figure 1 Feedstock powders and schematic of the synthesis process.

3. Results and Discussion

3.1 Microstructure

The set of micrographs in Figure 2 show the microstructure of the sintered samples. The surface morphology of the matrix sample, Figure 2a, reveals a smooth densely packed microstructure, displaying 1-10 µm in size closed and interconnected pores distributed in a homogeneous fashion. Regarding to the mechanical performance of implants, pore homogeneity and morphology play important roles [43]. Additionally, porous microstructures such as that of the matrix sample are promising for bioimplant products, as they intensify cell ingrowth into the implants and supply minerals [44,45]. Figure 2b shows the microstructure of the composite samples. It is observed that the titanium particles were uniformly and dispersedly distributed. However, the phases did not appear to be strongly fused together. Interfacial chemical reactions between hydroxyapatite and titanium are enhanced if the surface of the titanium particles are rich in oxide [46]. This then facilitates the diffusion of hydroxyapatite ions into the titanium to form titanium phosphide and calcium titanate interfaces [47,48]. Additionally, it appears that the matrix experienced dehydration, hence inducing volume and structural changes. Despite the sintering been conducted under a moist argon atmosphere these issues were not suppressed. Similar challenges with the synthesisation of metallic composites containing high volumes of hydroxyapatite were also demonstrated in previous studies [33,49,34,39].

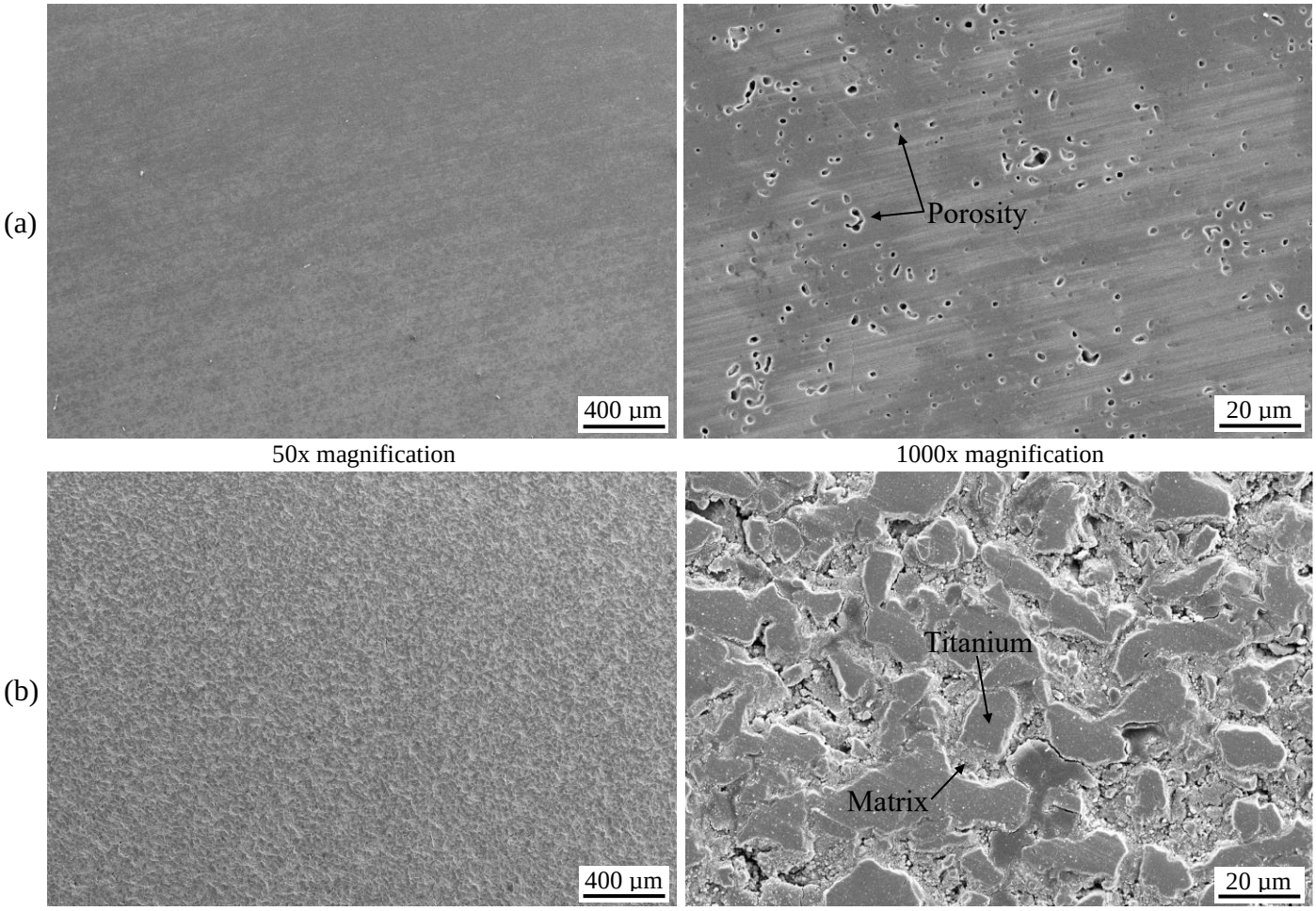


Figure 2 Low and high magnification micrographs of the microstructure of the synthesised (a) matrix and (b) composite samples.

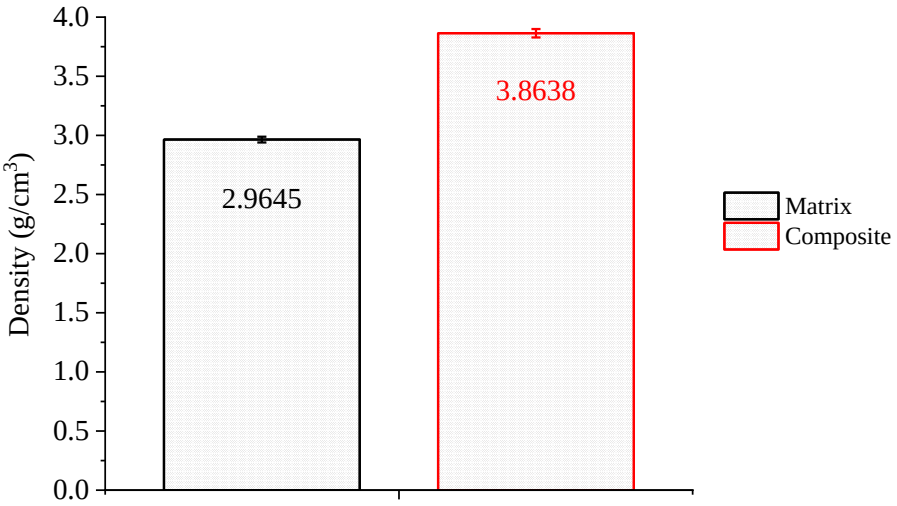
3.2 Density, X-ray Diffraction and Fourier Transform Infrared Analyses

Results of pycnometric density measurements of the sintered samples are shown in Figure 3a. By taking the theoretical density of hydroxyapatite and titanium as 3.18 and 4.51 g/cm^3 the percent porosity of the matrix and composite samples were estimated to be 7 and 9 %, respectively. In this regard, dehydration-induced shrinkage of the matrix is accountable for the greater porosity percentage in the composite sample. The aforementioned porosity in this sample refers to complex voids resulting from a prevented sintering condition, as it is not possible to fuse loose particles (particles that are not in contact with each other) [50].

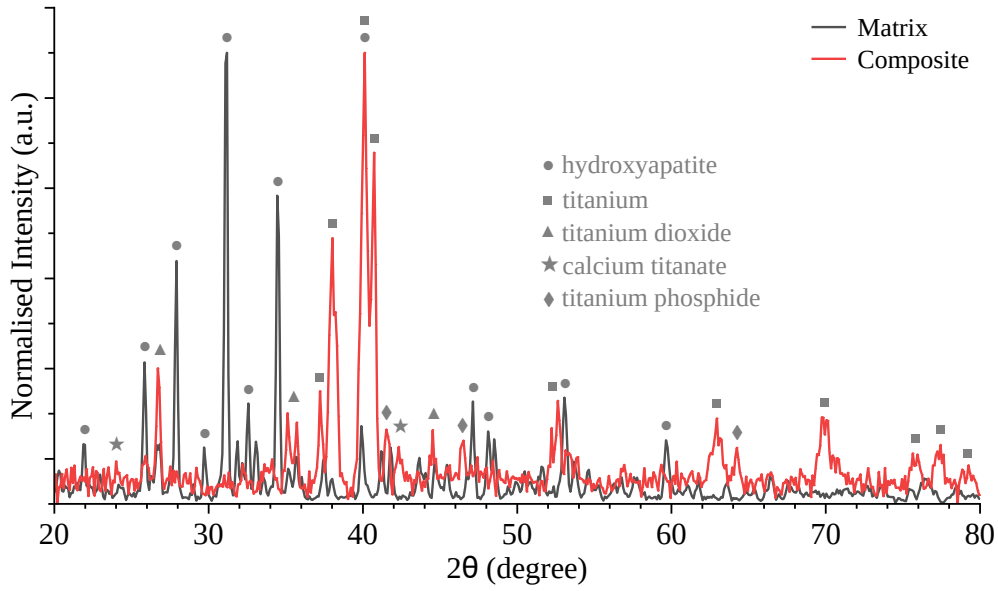
The x-ray diffraction patterns obtained from the sintered samples are presented in Figure 3b. The spectrum of the matrix is in good agreement with the phase-pure hydroxyapatite [51,52]. The composite sample, which contained 80 wt% titanium, had a hexagonal close-packed structure. The peaks at 26.1°, 35.7°, and 44.4° diffraction angles identified titanium oxide phases. Titanium particles of the starting powder were naturally covered with an oxide film of anatase. Also, some moisture may have been trapped inside the pores of the green compact, therefore crystalline rutile could have formed during the sintering process.

Prior and post sintering Fourier transform infrared spectrum of the samples are displayed in Figure 3c. Peaks belonging to the phosphate group PO_4^{3-} of hydroxyapatite at 1090, 1040, 1030, 960, 600, 561 and 472 cm^{-1} were observed. Dicalcium phosphate dehydrate and octa-calcium phosphate phases belonging to the acidic phosphate group HPO_4^{2-} were confirmed by the weak 886 and 535 cm^{-1} peaks [53,54]. The peak at 1450 cm^{-1} originated from carbonate ions CO_3^{2-} due to impurities in the raw materials and or atmosphere. In the sintered samples, the OH^- peak disappeared, hence indicating the occurrence and development of dihydroxylation resulting from the employed sintering temperature.

(a)


Density (g/cm3)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2.9645
3.8638
Matrix
Composite

(b)


Normalised Intensity (a.u.)
Matrix
Composite
hydroxyapatite
titanium
titanium dioxide
calcium titanate
titanium phosphide
20
30
40
50
60
70
80
2θ (degree)

(c)

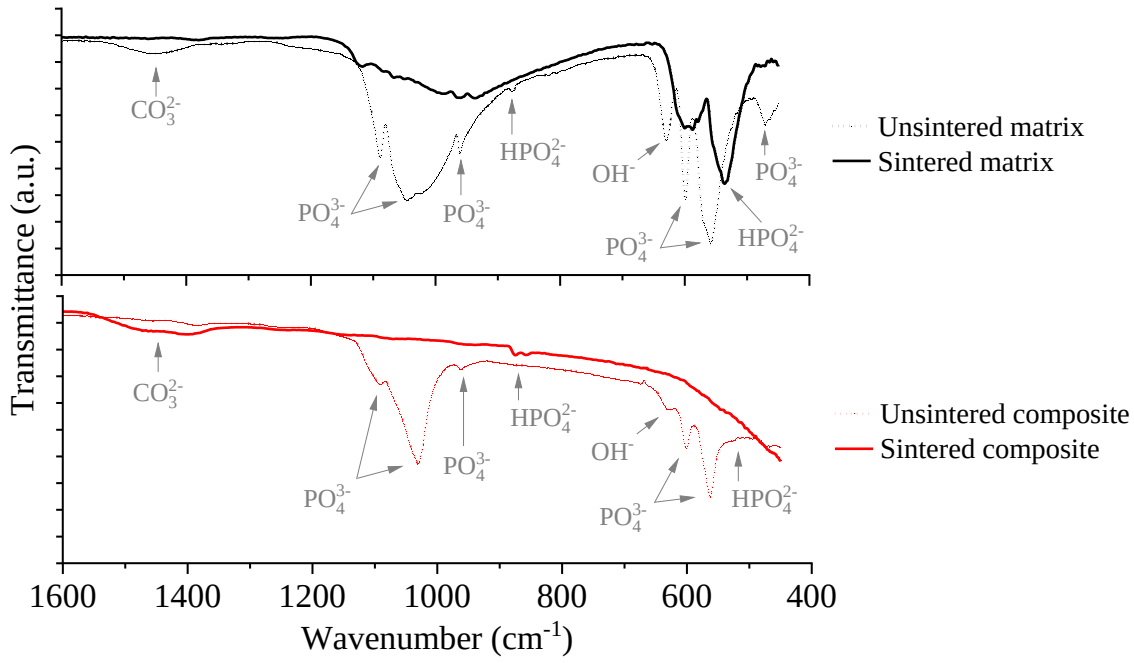

Transmittance (a.u.)
CO3 2-
PO4 3-
HPO4 2-
OH-
Unsintered matrix
Sintered matrix
Unsintered composite
Sintered composite
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)

Figure 3 Comparison of (a) the density and (b) the x-ray diffraction spectrum of the sintered samples and (c) Fourier transform infrared spectrum of the unsintered and sintered samples. The data whiskers represent the 95% confidence interval, n=15.

3.3 Mechanical Strength

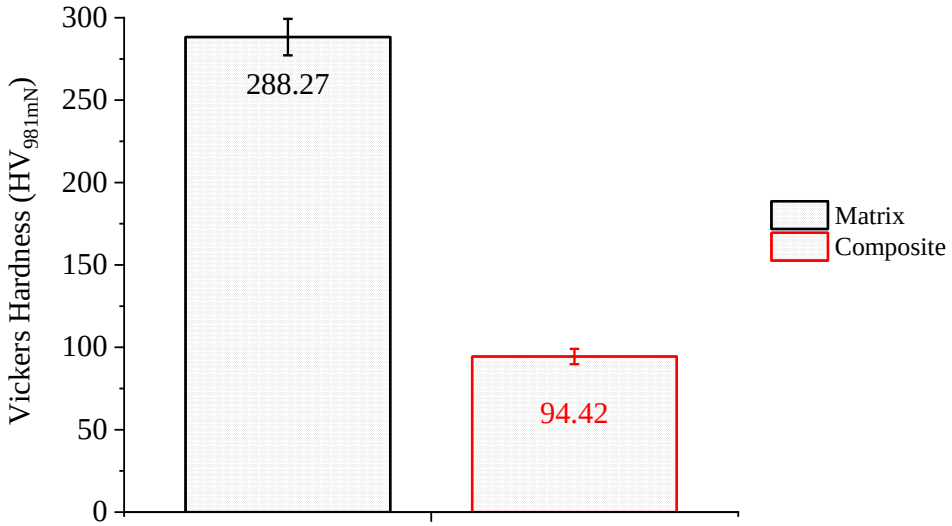
The mechanical performance of the sintered samples is contrasted in Figure 4. Despite the existing porosity, the matrix samples exhibited an average microhardness of 288.27 HV, which is higher than that of the human cortical and trabecular bone lamellae [55]. A low microhardness for the composite samples was already expected based on the uncovered microstructure in Figure 2b. Also, the microhardness obtained from these samples are not surprising as dispersing a metallic phase to hydroxyapatite is already known to lower the overall hardness of the material [56,57]. In the present study, the sintering capacity of the composites was affected by the coating of nano hydroxyapatite present onto the surface of the titanium particles (see Figure 5b), which impeded the fusion of titanium particles between themselves. From this it was speculated that if melting of the titanium particles is achieved then the liquid-phase diffusion bonding process could also enhance the titanium-hydroxyapatite diffusion [22,58,59].

The flexural strength of the samples with and without titanium are very similar, see Figure 4b. These flexural strength values are in the range reported in previous studies for hydroxyapatite [60,61]. Even though several have developed composite materials with bone-like flexural strengths by using alternative metals and ceramics, one should consider the compatibility and long-term integrity of such composites as implants [62–65]. Titanium has the highest biocompatibility of all metals and it is promising not only to achieve a desirable flexural strength but also to add ductility to hydroxyapatite [66,67]. By tuning the composite stoichiometry and the input process parameters and processing conditions, the development of titanium-reinforced hydroxyapatite composites having mechanical properties suitable for bone implant applications is possible.

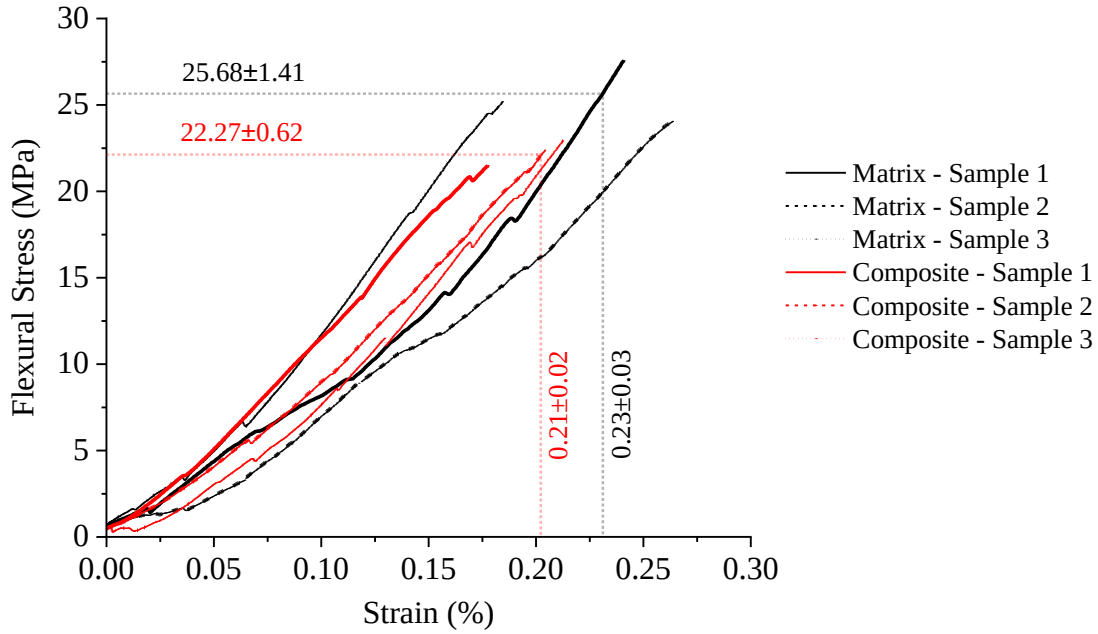
Figure 4c shows the compression curves of the sintered samples. Evidently, the matrix samples developed the best maximum compressive resistance, bearing up to 260 MPa, which is higher than that of human cortical bone [68,69]. This high compressive strength was due to the high relative density and small pore sizes. In contrast, the results shows that the added titanium significantly reduced the compressive strength of the composites. Despite such compressive strength values being still acceptable for some bone restorations, the integrity of the microstructure of these composite samples is not [70,71]. In addition to the already given recommendation, it is expected that the incorporation of lower amounts or retention of the original morphology of the hydroxyapatite particles within the powder mixture may address the issue. These will therefore enhance the bonding strength of the metal-ceramic interface and so result in the strengthening and toughening of the microstructure of sintered composites.

To successfully design materials for bone repair applications the anisotropic nature of bone and the complex loading conditions it is subjected to should be considered [72]. Also, the mechanical properties of bone tissue depend on several factors such as: water content, amount of mineral phase, gender and age of individual, as well as bone type, function and anatomy [73]. Many standards for mechanical testing and biological evaluation of implants are available [74–84]. However, there is no certified database for the mechanical and physical properties of human bones. This hampers the development of bone-like materials for human implantation and creates uncertainties regarding the structural success of implants. Therefore, researchers and clinicians should aim at establishing a comprehensive database for the mechanical and physical properties of human bones.

(a)


Vickers Hardness (HV981mN)
288.27
94.42
Matrix
Composite

(b)


Flexural Stress (MPa)
25.68±1.41
22.27±0.62
0.21±0.02
0.23±0.03
Strain (%)
Matrix - Sample 1
Matrix - Sample 2
Matrix - Sample 3
Composite - Sample 1
Composite - Sample 2
Composite - Sample 3

(c)

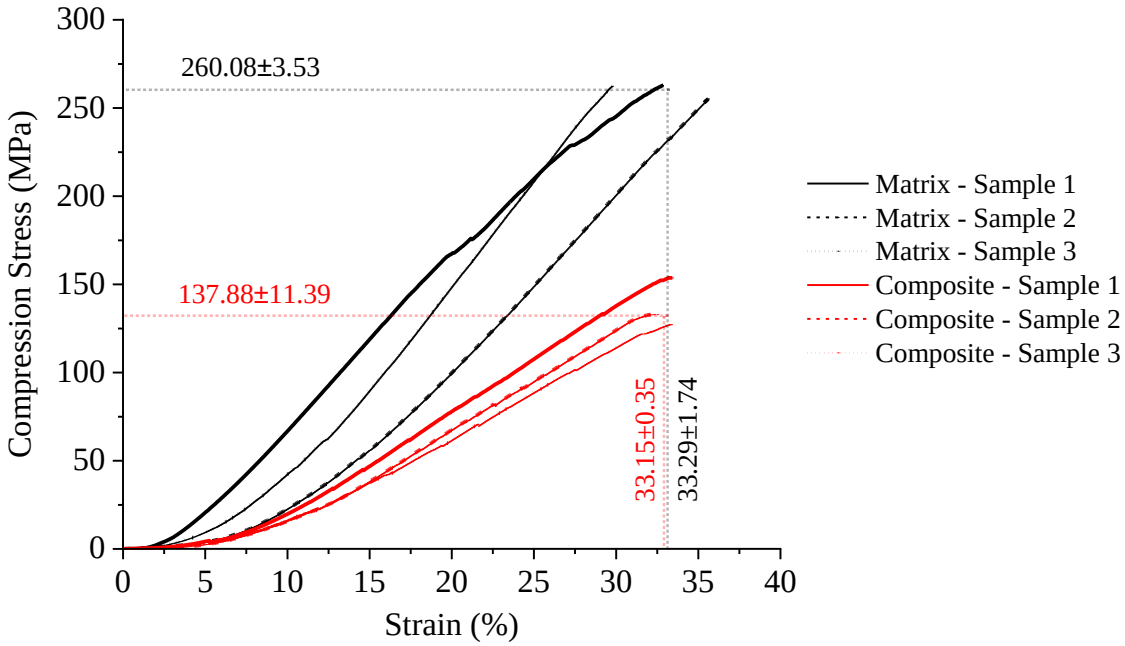

Compression Stress (MPa)
260.08±3.53
137.88±11.39
33.15±0.35
33.29±1.74
Strain (%)
Matrix - Sample 1
Matrix - Sample 2
Matrix - Sample 3
Composite - Sample 1
Composite - Sample 2
Composite - Sample 3

Figure 4 Mechanical performance of the sample: (a) microhardness, (b) flexural strength and (c) compressive strength. The data whiskers represent the 95% confidence interval, n=30.

4. Conclusions

To achieve the next generation of bone tissue engineering materials with improved mechanical properties and high bioactivity, composite biomaterials were synthesised and their properties investigated. Considering the development of biodegradable materials for bone repair and hard tissue reconstruction, hydroxyapatite-based composites are good candidates. In this context, this study combined calcium oxide extracted from a natural waste source to hydroxyapatite, bringing sustainability and biological flair into the material system. The synthesised samples formed a highly dense microstructure composed of a uniformly distributed porosity and holding very attractive mechanical properties for hard biomaterials engineering. Instead, the incorporation of titanium particles intended to further enhance the mechanical strength and to add ductility to the biocomposites resulted in sinterability issues, which caused deterioration of the mechanical performance of the samples. To mitigate these issues and to maximise the metal-ceramic interfacial bonding strength future studies should address the recommendations provided in this article.

Acknowledgments

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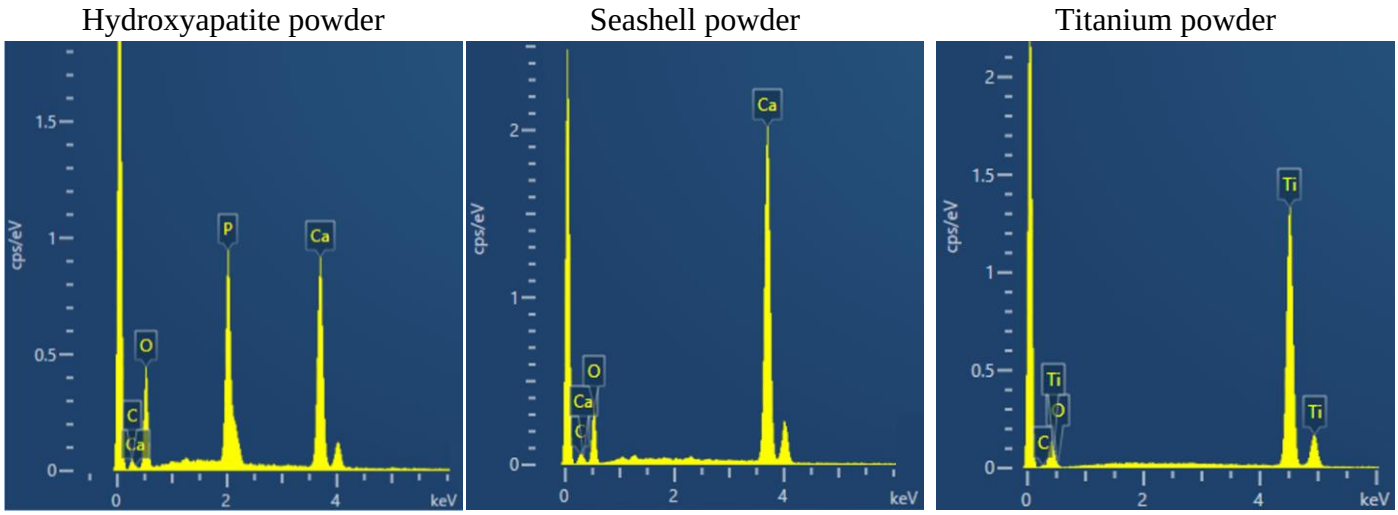
Appendix

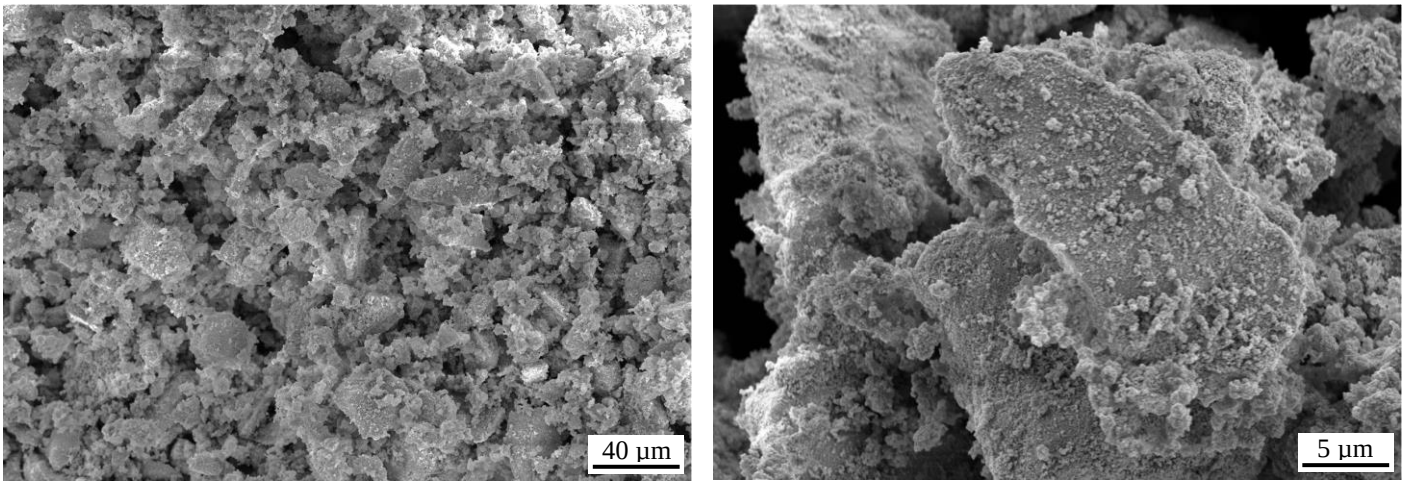
(a)



wt% | Ca $_{39.05}$, P $_{18.01}$, O $_{42.94}$ | wt% | Ca $_{64.59}$, O $_{35.41}$ | wt% | Ti $_{97.01}$, O $_{2.99}$

(b)

The developed hydroxyapatite (10 wt%) + seashell (10 wt%) + titanium (80 wt%) feedstock



(c)

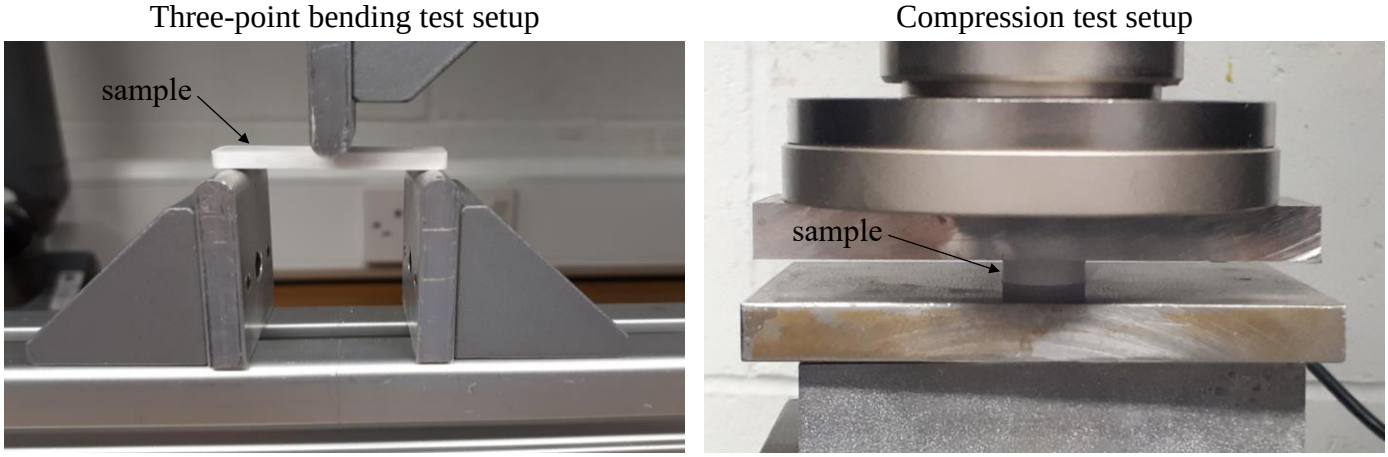


Figure 5 Supplementary material: (a) energy dispersive x-ray spectrum of the powders, (b) characteristics of the developed titanium-containing powder and (c) used setup for the bending and compression tests.

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