Synthesis and Characterization of Alumina-Chitosan-Hydroxyapatite Biocomposites for Load Bearing Application

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Abstract:

This study investigated the influence of reinforcing hydroxyapatite (HA) with alumina (Al) and chitosan nanofiber (CH) to enhance its usefulness in load bearing application. Commercial alumina was used while chitosan fiber and hydroxyapatite were synthesized from cowry shells and limestone respectively. The developed composites were characterized with a view to assessing their suitability for use as medical implants in load bearing capacity. It was observed that the optimum compressive strength obtained was 181.73 MPa, tensile strength of 172.67 MPa, hardness value of 529.21 HV, fracture toughness of 7.42 MPa.m$^{1/2}$, elastic modulus of 8.23 GPa and bending strength of 175.51 MPa. Increasing volume fractions of Al-CH resulted in decrease in compressive strength, hardness and elastic modulus of hydroxyapatite while its tensile strength, bending strength and fracture toughness increased. The result obtained from the Fourier Transform Infrared Spectrometry revealed that the intermolecular hydrogen bond and chelate interaction between the constituents contribute to the good mechanical properties of the composite. X-Ray Fluorescence analysis result indicates that their chemical compositions contain ions commonly found in the physiological environment. The dispersion of the alumina-chitosan nano fiber in the HA matrix as revealed by the Scanning Electron Microscope micrographs result in the formation of interfaces which activates different mechanisms that improve the strength of HA. The optimum mechanical property was obtained at 20-20-60 Al-CH-HA composite. Hence, this composite will be a suitable material for load bearing application.

Keywords: Hydroxyapatite, Alumina, Chitosan nanofiber, Al-CH-HA composite, fiber (to be removed), chelate ??, Load bearing application.

Introduction:

Among the major human health challenges encountered in our day to day activity are organ and tissue failures due to an injury or disease. This may result in total loss of such organ. Consequently, urgent therapy is needed to restore the tissue, or organ that has lost its function. Organ or tissue transplant is a standard therapy to treat affected patients. This is however, limited due to the shortage of donor and adverse immunological response. Other therapies include drug therapy, surgical reconstruction, synthetic prostheses and medical devices are also fraught with diverse limitations which include the need for lifelong immune suppressant, inability of device to replace all the functions of a damaged or lost organ, stress shielding, etc. Tissue engineering has emerged as a promising alternative approach in the treatment of malfunctioning or lost organs.

In tissue engineering, a scaffold is required to serve as an adhesive substrate for the implanted cells and a physical support to guide the formation of the new organs. In addition to facilitating cell adhesion, promoting cell growth and allowing the retention of differentiated cell functions, the scaffold should be mechanically competent. For example, the strength of the scaffolds should be sufficient to provide mechanical stability in load bearing sites prior to regeneration of new tissue. In bone regeneration a suitable scaffold is expected to be bioactive, that is, allow the adsorption, consequently, adhesion and proliferation of bone cell. (???) Hydroxyapatite is a typical example of bioactive materials (added the reference).

Hydroxyapatite

Hydroxyapatite (HA) is one of the major mineral components of bones, it has excellent biocompatibility. It is one of the few materials that are classed as bioactive materials because of its excellent osteoinductive and osteoconductive attributes (reference??). Subsequently, it will enhance bone in growth and osseointegration when used in orthopedic, dental and maxillofacial applications (reference??).

It has been known for a long time that various natural biocomposites exist. They are made from organic matrix and inorganic fraction and fulfill the mechanical properties required in their function as the skeleton, teeth or shells of organisms (reference??). For example, organic collagen combined with inorganic hydroxyapatite [HA: $Ca_{10}(PO_4)_6(OH)_2$] in bone and chitin is associated with calcium carbonate in crab shell (reference??). These constitutions have very good mechanical properties and can withstand tremendous pressures (Wang *et al.*, 2001). Hydroxyapatite which has been proved to have good properties of hardness, biocompatibility, osteoconductivity, a certain degree of bioactivity, and high resistance to moisture has been used in a variety of oral and maxillofacial applications (Sono, 2003). The versatility of HA as part of biocomposites enables materials engineers to combine materials with various degrees of biocompatibility to produce satisfactory biomaterials for a certain application in the body. It is available in dense blocks, porous blocks, and granules. However each of these forms has its own drawbacks: Dense HA is difficult to machine without causing large-scale fracture, granules tend to migrate, and the macroporous material leaves a rugged surface finish.

Limestone is an industrial mineral and rock with a chemical composition of $CaCO_3$. It belongs to the carbonate minerals based on the CO_3^{2-} ions. Limestone is rich in calcium which is one of the major constituents of hydroxyapatite. Most limestone is formed from the calcareous skeletons of organisms such as corals, mollusks and foraminifera.

Generally, dense hydroxyapatite does not have the mechanical strength required to enable it succeed in long term load bearing applications; consequently, the need to improve its strength to enhance its load bearing application. One of the approaches to accomplish this is by incorporating alumina and chitosan nanofiber into its matrix. The purpose of creating alumina chitosan composite is to improve the mechanical properties of hydroxyapatite.

Alumina (Al)

Biocompatible ceramics with mechanical properties comparable to metals are preferred in parts of the body that have high wear risk. An inert ceramic, alumina is used in load bearing hip prosthesis and dental implants in dense and pure state because of its excellent corrosion resistance, high strength and high wear resistance. Alumina’s long term use in orthopedic surgery has been motivated by its excellent biocompatibility and very thin capsule formation which permits cement less fixation of prostheses as well as its very low coefficients of friction and wear. The exceptional tribologic properties of alumina are due to small grain sizes less than 4 microns and narrow grain size distribution which lead to very low surface roughness. Rapid wear of bearing surfaces takes place in the case of large grain presence owing to grain pull out due to local dry friction.

As a mechanically strong ceramic alumina, is also used as a reinforcing material in biocomposites. Strength, fatigue resistance and fracture toughness of polycrystalline alpha alumina are functions of grain size and purity. Good flexural strength, excellent resistance to dynamic and impact fatigue, resistance to subcritical crack growth and excellent compressive strength are obtained with average grain sizes < 4 microns and purity > 99.7 % (Ratner *et al*. 2004). Clinical applications of alumina include knee prostheses, bone and dental screws, alveolar ridge and maxillofacial reconstruction, ossicular bone substitutes, corneal replacements and segmental bone replacements.

Chitosan (CH)

Chitosan is a deacetylation product of chitin which is a functional and basic polysaccharide composed of b-1, 4-linked glucosamine, that can be easily isolated from exoskeletons of shrimps and crabs (Park *et al.*, 2001). Owing to its special properties such as nontoxicity, biodegradability, biocompatibility and antimicrobial activity, this polycationic biopolymer is receiving a great deal of attention for biosensing, medical and pharmaceutical applications (Suzuki *et al*., 1998; Vande Vord *et al*,. 2002; Luo *et al*., 2005). Also, it is the most commonly used natural polymer in regenerative medicine and tissue engineering (Langer *et al.*, 2004). Chitosan nanofibers have also been widely accepted as biomedical scaffolding materials to restore, maintain or improve the functions of various tissues (Nam *et al*., 2010; Jayachandran and Kim, 2010).

Chitosan is produced from chitin, which is a natural polysaccharide found in crab, shrimp, cowry shell, lobster, coral, jellyfish, butterfly, ladybug, mushroom and fungi. However, marine crustacean shells are widely used as primary sources for the production of chitosan (Madhavan and Nair, 1974; Shahidi and Abuzaytoun, 2005). Cowry shells are among the important marine species of great commercial importance in the tropical and subtropical waters of the Pacific, Atlantic and Indian Oceans. Calcium is the most abundant mineral present in the perforated cowry shells. The high content of calcium confirms its medicinal role in bone formation. It was reported that the cement of the cowry shell can be used as possible cement for bone formation (Fish and Fish, 1996) and are used as calcium supplement. Iron, aluminum and sodium are found in reasonable amount. The wastes from cowry shells have recently become a serious issue in coastal areas. Selective isolation of bioactive material from these wastes is the simplest way to decrease the pollution. It not only reduces the environmental pollution because of the disposal of this under utilized by-products of cowries but also increases the potential applications of chitosan. Moreover, the chemical hydrolysis and enzymatic methods widely used for the isolation of chitosan from marine crustacean shells are quite inexpensive.

Chitosan composites

In recent years, considerable attention has been given to chitosan composite materials and their applications in the field of bone tissue engineering. This may be ascribed to its minimal foreign body reactions, intrinsic antibacterial nature, biocompatibility, biodegradability and the ability to be molded into various geometries and forms (reference??). Such form includes porous structures, suitable for cell in growth and osteoconduction. Composite of chitosan and other material are becoming popular because of their biocompatibility and biodegradability. Chitosan composites are thus emerging as potential materials for artificial bone and bone regeneration in tissue engineering (Langer *et al.*, 2004).

Research on chitosan composites for hard tissue applications have been on for about 4 to 5 years. Although bioresorbable composite devices other than chitosan have been in clinical use for above 16 years and even this is not a very long time. This is a promising area because the use of bioresorbable polymer ceramic composites has many advantages. (Yokogawa *et al*., 2002,) introduced some biomimetic methods to grow calcium phosphate on chitin scaffolds, phosphorylated chitin fibers or chitosan films. However, no significant mechanical strength improvement has been achieved owing to poor affinity of these organic materials and the heterogeneous distribution of inorganic components (Wang *et al.*, 2001).

Research is concentrated on the preparation of composites due to the limitations originating from the poor mechanical properties of phase pure dense HA ceramics. In the last recent years, many reinforcements, including particles (Loku *et al*. 1990), platelets(Gautier *et al*. 1999), whiskers, short fibers, metal fibers and dispersoids (De with and Corbijn,1989) and nano-particles (Ahn *et al*., 2001) have been used to improve the reliability of HA ceramics. The highest reported fracture toughness values were achieved by using 20-30 % Fe-Cr alloy fibers with KIC values of 6.0 - 7.4 $MPa.m^{1/2}$ (Suchanek and Yoshimura, 1998). Biomaterials with improved mechanical properties have been synthesized by the incorporation of hydroxyapatite in composites as a ceramic matrix (Abere *et al*., 2017).

It's a fact that there is an authentic need for the preparation of highly biocompatible materials which can be used in load bearing applications such as artificial tooth roots or artificial bones. A new approach for the preparation of such a material is the use of completely biocompatible non-toxic chitosan/alumina as reinforcements in the HA matrix. Metals generally possess good mechanical properties but show poor biocompatibility, cause stress shielding and release of harmful metal ions causing eventual failure and removal of implant. Ceramics generally have good biocompatibility but poor fracture toughness and tend to be brittle. Composite materials with engineered interfaces resulting in combination of biocompatibility, mechanical strength and toughness, is the focus of extensive research and this study.

Materials and Methods

The materials used for this research are commercial alumina, synthetic chitosan from cowry shells which were purchased from Agbado Oja, Agbado, Ogun State and synthetic hydroxyapatite from limestone. The reagents used in this research are: $(NH_4)H_2PO_4$ powder (99 % MERCK), Na_2CO_3 powder (99 % ALDRICH), potassium hydroxide pellet (99 % ALDRICH), hydrogen peroxide, oxalic acid powder (99 % ALDRICH), Acetone (Sigma-Aldrich Laborchemickalien GMBH, Seelze, Germany), HCl and PEG.

Pretreatment operation

The cowry shells were boiled in an aqueous solution containing 30 volume percent of reagents grade hydrogen peroxide, H_2O_2 for 1 hour to remove the organic matter impurities in the shell. It was then pulverized to obtain a fine powder of particle size less than 100 µm.

Demineralization

Demineralization was carried out to remove the mineral matter content of the shell, i.e. $CaCO_3$. The powder was soaked in 0.5 M of HCI aqueous solution at 25 °C with a solution to solid ratio of 10 ml/g for 24 hours to remove the minerals matter. The solution was filtered and the residue was washed with deionized water until a pH of 7 was obtained. The chitin was oven dried at 105 °C for 12 hours (Madhavan, 1992).

Deproteinization

The deproteinization of chitin was carried out by dissolving 1 gram of chitin in 10 ml of 0.5 M potassium hydroxide (KOH). This results in decomposition of the albumen into water soluble amino acids. The solution was filtered while the residue was washed with deionized water until a pH of 7 was obtained. The product obtained was subsequently sterilized in hot ethanol and oven dried at 105 °C for 12 hours (Madhavan, 1992).

Deacetylation

About 1.0 gram of chitin was dissolved in 10 ml of 0.50 M KOH and boiled for 2 hours on a hot plate at 100 °C to deacetylate the chitin. The product was cooled to room temperature for 1 hour. It was then filtered and washed with deionized water at 60 °C to retain the solid matter which is chitosan. The prepared chitosan was oven dried at 110 °C for 24 hours (Madhavan, 1992).

Figure 1: Cowry shells.

Preparation of chitosan nanofiber

The chitosan was suspended in acetic acid: ethyl acetate: water (40:30:30) by stirring overnight with the magnetic stirrer at 4 °C to obtain a 12 % (w.v^{-1}) chitosan suspension. The solution was loaded into a 5 ml syringe with a 21 G needle and electrospun at 0.1 ml.h^{-1}, under a high electrostatic field (22 kV) onto 12 mm diameter cover glasses attached on aluminum foil wrapped on a rotating cylinder collector, at 400 rpm, placed at a distance of 120 mm from the needle tip. The procedure was carried out repeatedly. The resulting fiber was dried overnight to remove any solvent left on its surface and then collected from the collector.

Synthesis of hydroxyapatite from limestone

Limestone was pulverized to obtain a fine powder of particle size less than 100 µm. 750 g of limestone powder was dissolved in 0.5 M of dilute HCl until the mixture ceased to produce carbon dioxide. Insoluble material such as silica was filtered out with filter paper. Oxalic acid was added to the residue to form calcium oxalate as a solid precipitate. The precipitate was washed with deionized water and transferred into a beaker. The calcium oxalate was converted to calcium chloride solution through the addition of dilute HCl. Concentrated sodium carbonate was added to the calcium chloride solution to obtain calcium carbonate precipitate. The precipitate was filtered off and the residue was washed with deionized water. The calcium carbonate was then dried in an ovum at 110 °C overnight and subjected to heating at 100 °C to produce calcium oxide (CaO).

An analytical weighing scale was used to accurately weigh CaO powder. 1.42 mol (79.55g) CaO powder was added to 500 ml of deionizer water in a 1000 ml beaker and vigorously stirred at 1000 rpm at the 20 °C for 24 hours to react and form a suspension of $Ca(OH)_2$ in an excess of deionizer water. The beaker was covered in order to avoid possible contamination through contact with atmospheric conditions. The temperature of the reaction (20 °C) was maintained by a thermostat-controlled water bath. An analytical weighing scale was used to accurately weigh the required quantity of orthophosphoric acid. 97.32 g of 85 % H_3PO_4 was added to $Ca(OH)_2$ solution at a rate of 1.5 ml/min. During the course of the acid addition, the pH of the solution was monitored via a handheld pH meter with an accuracy of ± 0.2. The reactants were stirred for further 24 hours to aid the maturation stage, under continuous stirring conditions at 1000 rpm, held at the respective reaction temperature of 200 °C. 0.28 mol (9.94 g) NH_4OH, was added to the HA slurry after 24 hours ripening period to stabilize the pH of the super saturation solution to above 9. Assay samples were taken for analysis of the composition of mixture in the barrel. A small crucible was filled with a sample of the mixture in the mixing barrel and dried in a drying oven for 1 hour at 110 °C. The dried samples were then placed in a furnace and sintered at 1200 °C for 1 hour. When the assays were cooled, they were removed from the furnace and ground to powder with the aid of a motor and pestle.

Preparation of composite of alumina/chitosan nanofiber/nanohydroxyapatite

Different volume fractions of alumina, chitosan nanofiber and nanohydroxyapatite composites were prepared as shown in **Table 1**.

Mechanical characterization of composite

Different test specimens were prepared to analyze the mechanical properties of the different volume fractions of the composite in order to evaluate the effects of the chitosan nanofiber and alumina on the strength of hydroxyapatite and to determine the volume fraction with the optimum mechanical properties. The mechanical properties investigated include the compressive strength, tensile strength, hardness, fracture toughness, modulus of elasticity, and bending strength.

Table 1: Composite volume fractions (%).

Samples	Al (volume fraction %)	CH (volume fraction %)	HA (volume fraction %)
1	40	30	30
2	30	40	30
3	20	50	30
4	50	20	30
5	30	30	40
6	20	30	50

7	30	20	50
8	20	20	60
9	30	10	60
10	10	30	60
11	10	20	70
12	20	10	70
13	25	5	70

Compression test

The compression test was carried out to determine the behavior of the composite materials under a compressive load. It was conducted by loading the test specimen between two plates, and then applying a force to the specimen by moving the crossheads together. During the test, the specimen was compressed, and deformation versus the applied load was recorded. The compression test was used to determine the compressive strength.

Tensile test

This was carried out to investigate the behavior of the composite materials under axial tensile loading. The tests were conducted by fixturing the specimen into the test apparatus and then applying a force to the specimen by separating the testing machine crossheads. The crosshead speed was varied to control the rate of strain in the test specimen. Data from the test were used to determine tensile strength, modulus of elasticity and bending strength.

Measurement of hardness and fracture toughness

The hardness and fracture toughness were obtained from the Vickers micro hardness test. The indenter employed in the Vickers test was a square-based pyramid whose opposite sides met at the apex at an angle of 136 º. 50 g load was applied for each indentation with a dwell time of 10 seconds. Five indentations were made for each sample but the average was determined and recorded. The size of the impression was measured with the aid of a calibrated microscope. The Vickers number (HV) was calculated using the following formula: $HV = 1.854 (F/D^2)$, with F being the applied load (measured in kilograms-force) and D^2 the area of the indentation (measured in square millimeters).

Elemental analysis of composite

To characterize the composite's chemical interactions; composite with optimum mechanical properties, 20-20-60 (Al-CH-HA) composite was used for the Fourier Transform Infrared Spectroscopy (FTIR) and the X-Ray Fluorescence (XRF) analyses. The identification of functional groups in the composite samples as well as the interfacial modification (i.e., nature of bonding between particles and the polymeric matrix) were analyzed by FTIR analysis (Model NEXUS870, FTIR, Thermo Nicolet, USA) within the scanning range 4000–500 cm^{-1}. The ED-XRF was carried out to investigate the chemical composition of the composite.

Microstructure characterization

The surface roughness of the composite samples was investigated by scanning electron microscopy (SEM) (Model JSM-5800, JEOL, scanning electron microscope, Japan).

Results and Discussion

Added on introduction of this party regrouping the results obtained in the tables 2, 3… and the figures or plats???

Table 2: Compressive Strength of the composites

S/N	Al-CH-HA Composite volume fractions	I	II	III	IV	V	Average Compressive Strength (MPa)
1	40-30-30	148.50	156.20	161.10	165.40	151.62	156.56
2	30-40-30	164.05	158.32	153.43	160.20	159.31	159.06
3	20-50-30	163.84	167.06	161.34	168.51	160.62	164.27
4	50-20-30	158.84	169.70	170.13	162.84	172.38	166.78
5	30-30-40	173.34	162.25	165.51	169.08	167.21	167.48
6	20-30-50	175.68	171.52	168.40	162.23	170.89	169.74
7	30-20-50	176.23	169.40	177.30	170.06	168.92	172.38
8	20-20-60	174.05	178.51	187.21	183.58	185.30	181.73
9	30-10-60	173.83	170.08	178.41	180.21	175.45	175.60
10	10-30-60	169.85	178.31	172.51	176.40	175.44	174.50
11	10-20-70	171.41	173.48	168.20	178.31	170.05	172.29
12	20-10-70	168.48	171.21	165.30	169.43	172.34	169.35
13	25-5-70	156.61	168.32	163.38	160.04	159.52	161.57

Table 3: Tensile Strength of the composites.

S/N	Al-CH-HA Composite volume fractions	I	II	III	IV	V	Average Tensile Strength (MPa)
1	40-30-30	121.48	134.32	125.40	129.31	120.03	126.11
2	30-40-30	124.05	122.43	136.54	128.07	127.59	127.74
3	20-50-30	125.58	130.08	138.20	135.46	128.51	131.57
4	50-20-30	131.42	128.50	133.49	137.08	133.05	132.71
5	30-30-40	129.06	135.34	133.94	138.07	130.41	133.36
6	20-30-50	134.08	141.20	138.43	132.58	144.33	138.12
7	30-20-50	157.21	164.78	177.08	175.38	165.40	167.97
8	20-20-60	168.52	171.41	183.04	175.03	165.35	172.67
9	30-10-60	172.02	163.41	167.52	170.56	173.40	169.38

10	10-30-60	168.58	171.35	165.52	163.42	160.08	165.79
11	10-20-70	157.34	161.09	167.84	158.55	165.09	161.98
12	20-10-70	160.51	162.33	156.57	157.73	160.54	159.54
13	25-5-70	145.55	151.30	143.84	154.07	140.32	147.02

Table 4: Hardness value of the composites.

S/N	Al-CH-HA Composite volume fractions	I	II	III	IV	V	Average Hardness Value
1	40-30-30	449.08	473.58	455.08	483.21	462.29	464.65
2	30-40-30	450.06	455.29	485.52	462.23	473.05	465.23
3	20-50-30	501.24	496.30	472.18	461.47	505.48	487.33
4	50-20-30	514.09	481.51	495.29	503.15	489.58	496.72
5	30-30-40	492.53	518.04	503.24	512.30	484.50	502.12
6	20-30-50	495.26	501.10	485.58	511.00	508.04	500.21
7	30-20-50	480.20	545.35	508.10	467.51	510.31	502.30
8	20-20-60	508.51	535.50	510.05	541.81	550.17	529.21
9	30-10-60	508.31	513.25	503.20	515.30	506.09	509.23
10	10-30-60	479.51	483.25	508.21	512.04	518.30	500.26
11	10-20-70	485.31	471.01	493.48	505.06	482.51	487.47
12	20-10-70	461.08	459.85	468.05	492.51	483.22	472.94
13	25-5-70	409.25	421.31	406.50	434.04	441.32	422.48

Table 5: Fracture toughness of the composites.

S/N	Al-CH-HA Composite volume fractions	I	II	III	IV	V	Average Fracture Toughness (MPa.m$^{1/2}$)
1	40-30-30	4.50	4.13	4.03	3.84	3.92	4.08
2	30-40-30	4.65	4.37	4.62	4.01	3.72	4.27
3	20-50-30	6.08	5.94	6.21	6.04	6.01	6.06
4	50-20-30	6.64	6.28	5.96	5.84	6.08	6.16
5	30-30-40	6.20	5.96	6.84	6.72	6.09	6.36
6	20-30-50	6.85	6.21	7.03	6.69	7.18	6.79
7	30-20-50	7.81	7.02	7.31	7.05	7.10	7.26
8	20-20-60	7.51	7.20	7.09	7.94	7.38	7.42
9	30-10-60	6.85	6.09	7.02	7.30	7.12	6.88
10	10-30-60	5.91	5.28	4.94	5.33	5.21	5.33
11	10-20-70	3.94	3.15	4.20	4.08	4.01	3.88
12	20-10-70	4.51	3.27	3.04	4.13	3.92	3.77

13	25-5-70	3.88	3.51	2.96	3.04	2.61	3.20

Table 6: Elastic modulus of the composites.

S/N	Al-CH-HA (composite volume fractions)	Average elastic modulus (GPa)
1	40-30-30	2.95
2	30-40-30	3.54
3	20-50-30	3.85
4	50-20-30	4.21
5	30-30-40	5.55
6	20-30-50	5.99
7	30-20-50	6.75
8	20-20-60	7.62
9	25-15-60	8.23
10	15-25-60	6.89
11	10-20-70	6.01
12	20-10-70	5.73
13	25-5-70	3.33

Table 7: Bending strength of the composites.

S/N	Al-CH-HA (composite volume fractions)	Average bending strength (MPa)
1	40-30-30	155.80
2	30-40-30	157.05
3	20-50-30	158.43
4	50-20-30	165.31
5	30-30-40	166.82
6	20-30-50	167.00
7	30-20-50	169.93
8	20-20-60	171.20
9	25-15-60	175.51
10	15-25-60	160.35
11	10-20-70	156.56
12	20-10-70	154.71
13	25-5-70	152.55

Table 8: X-Ray Fluorescence analysis of the composite

Elements	Ca^{2+}	P	O	K^{+}	Na^{+}	CO_3^{2-}	Cl^{-}	F^{-}	P_2O_7	Mg^{2+}	Al^{2+}	Fe^{2+}
Composition wt (%)	40.33	20.72	29.83	0.02	0.3	3.53	0.01	0.02	0.03	0.04	0.13	0.34

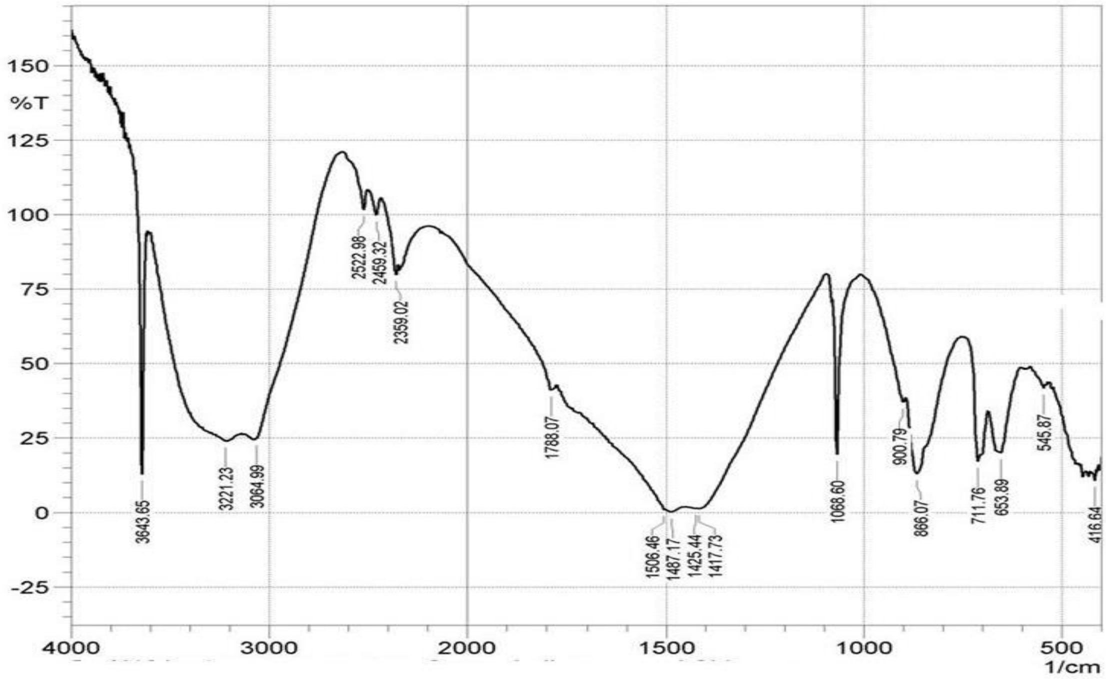
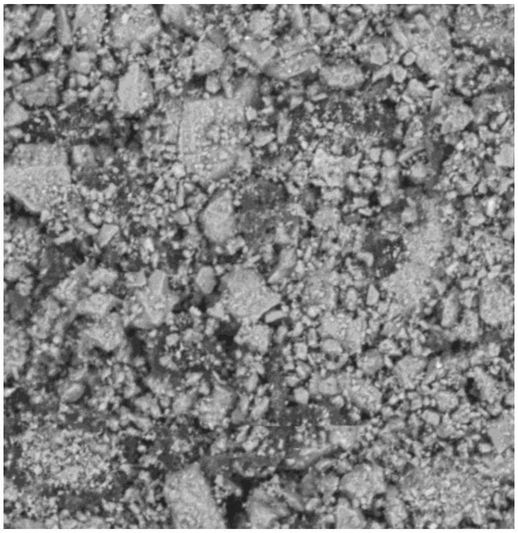


Figure 2: FTIR pattern of the composite.



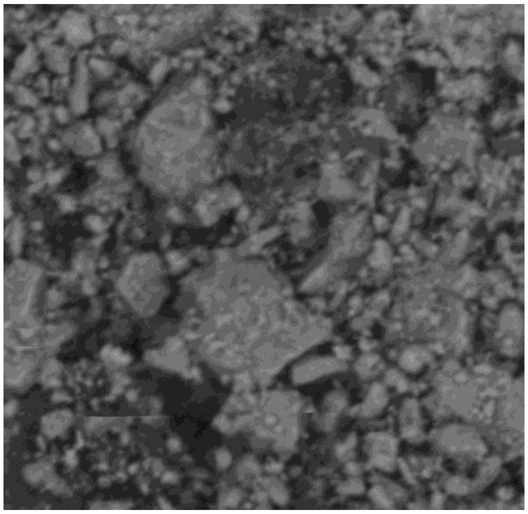


Plate 2: SEM image at 1000 magnification**.**

Plate1: SEM image at 750 magnification.

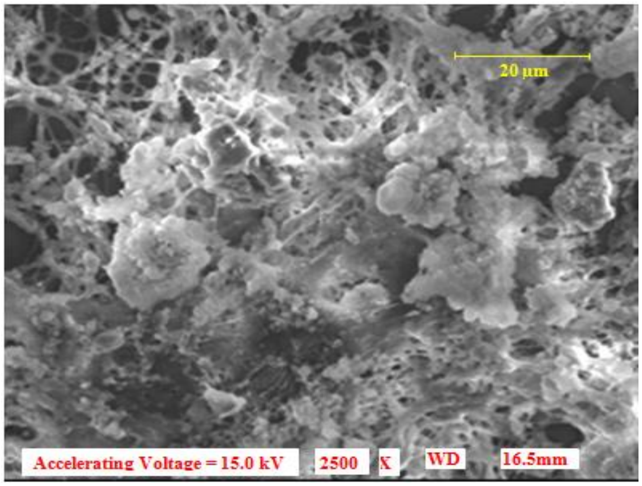


Plate 3: SEM image at 2500 magnification.

Mechanical properties of biomaterials

Several types of metals and alloys such as stainless steel, titanium, nickel, magnesium, Co–Cr alloys, and Ti alloys; ceramics like zirconia, bioglass, alumina, hydroxyapatite; and polymers such as acrylic, nylon, silicone, polyurethane, polycaprolactone, polyanhydrides are currently being used for load bearing applications (Katti, 2004). The applications include dental replacement and bone joining or replacement for medical and clinical application. Thus, the mechanical properties of biomaterials are very significant. Hydroxyapatite is the most widely studied bioactive and biocompatible material among them. Hydroxyapatite, though a candidate material for bone regeneration is, however, brittle and therefore not suitable for load bearing application. The incorporation of alumina and nanofiber of chitosan into the HA matrix has been found to have pronounced effects on the mechanical properties of HA (Li *et al*., 2002). Increasing the volume fraction of alumina and chitosan (Tables 2-7) resulted in a corresponding effect on mechanical properties of the composite.

Compressive strength of Al-CH-HA composite

The compressive strength of dense HA is between 500 – 1000 MPa (Wang *et al*., (1995;) and Silva *et al*., (2000). Increasing volume fraction of chitosan nanofiber in HA matrix had been reported to result in decreasing compressive strength of HA (Abere *et al*., 2017; Li *et al*., 2002 and 2005). Hence, the result of this analysis is also observed to follow this trend as increasing volume fraction of Al-CH was found to result in corresponding decrease in compressive strength of HA (**Table 2**). However, at an optimum volume fraction 20-20-60 Al-CH-HA, the compressive strength obtained was 181.73 MPa; after which it was observed that the compressive strength decreased (**Table 2**). This might be attributed to the fact that increasing Al-CH volume fraction may have affected the incorporation of the fiber into the matrix resulting in lower adhesion between the matrix and the reinforcement (Abere *et al*., 2017). The compressive strength of the composite obtained was found to be within the range of compact human bone which is 170 – 193 MPa (Silva *et al*., 2000).

Tensile strength

Increasing volume fraction of chitosan nanofiber has been found to increase the tensile strength of the composite (**Table 3**). The tensile strength of dense HA was found to be within the range of 40 – 100 MPa (Silva *et al*., 2000). As the volume fraction of the composite was increased a corresponding increase in tensile strength was observed until an optimum composition was attained (**Table 3**). After the optimum volume fraction of 20-20-60, a decrease in strength was however observed. This might be as a result of decrease in the force of adhesion between the matrix and reinforcement. The optimum volume fraction 20-20-60 was found to have a tensile strength of 172.67 MPa, which is in the range of the tensile strength of cortical bone, within the range of 50 – 151 MPa (Wang *et al*., 1995; Ashman *et al*., 1984; Rho *et al.,* 1998) and tensile strength of compact human bone is 124 -174 MPa (Gibson, 1985). Thus, the composite with the optimum volume fraction will be a good load bearing material.

Hardness

The behavior of any material under different load conditions is determined by its strength and hardness. When an external force is applied in a bone or in any other material, there is an internal reaction. The strength may be assessed by checking the relation between the external force and internal reaction that takes place in the material, known as load-deformation curve. (Holtrop, 1975). Hardness is among the most significant mechanical properties of materials when considering the suitability of the materials for biomedical applications. In bone regeneration for example, it is desirable that the hardness of the implants be comparable to that of bone. However, peradventure the hardness of the implant is higher than the bone then it penetrates into the bone (Kokubo, 2008). The hardness of dense hydroxyapatite ranges between 500 to 800 HV (Silva *et al.,* 2000). It was observed that the hardness of the composite synthesized is within the range of the hardness of hydroxyapatite but the optimum hardness value obtained is 529.21 at 20-20-60 composite volume fraction (**Table 4**)

Fracture toughness

Fracture strength of materials is defined as the maximum stresses that can be endured before fracture occurs. Strength of biomaterials (bioceramics) is an important mechanical property because they are brittle in nature. In brittle material, crack easily propagates in tensile load and therefore, it is more critical than compressive load. It has been reported that high fracture toughness material improved clinical performance and reliability compare to low fracture toughness (Fischer and Marx, 2002). The fracture toughness of dense hydroxyapatite is approximately 1 MPa.m$^{1/2}$ (Silva *et al*. 2000) which is very low when compared to the fracture toughness of human cortical bone and compact bone which has value ranging from 2 – 12 MPa.m$^{1/2}$ (Kim *et al.,* 2000). Increasing Al-CH volume fraction results in a corresponding increase in the fracture toughness of the composite (**Table 5**). Composite volume fraction 20-20-60 was observed to have the optimal fracture toughness of 7.2 MPa.m$^{1/2}$. This might be due to the chemical compatibility of the composite which results in high adhesion between the matrix and reinforcement. This helps to obtain homogeneous microstructures with the formation of interfaces, which allow the activation of different mechanisms that enable loads to be transferred efficiently and prevent cracking when stressed. Thus composite with volume fraction 20-20-60 (Al-CH-HA), with high fracture toughness will enhance clinical performance and service reliability in load bearing application.

Elastic propriety

One of the major problems materials science and engineers working on the improvement of current implant materials is the fact that these biomaterials are generally much stiffer than the human cortical bone. According to the load sharing principle of the composite theory, if a stiff metal or ceramic implant is placed in the bone, the bone will be subjected to lower mechanical stresses, and consequently bone will resorb. This is known as “Wolff’s Law”, i.e., with the changing stress or strain imposed, bone will remodel so that the stress or strain is retained within specific levels.

Aseptic loosening of the prosthesis in the case of total hip replacement, due to bone resorption in the proximal femur is believed to be caused by the state of stress and strain in the femoral cortex after the metallic femoral hip replacement is implanted. Elastic characteristics of the implant play a significant role in allowing the femur to attain a physiologically acceptable stress state. In order to overcome the problem of modulus-mismatch between existing implant materials and bone and promote the formation of a secure bond between the implant and host tissue, the concept of analogue biomaterials was introduced in the 1980's (Wang *et al*. 2010). Since then, a variety of bioactive composite materials have been produced and investigated. A synthetic bone substitute should have similar strength to that of the cortical/cancellous bone being replaced (> 200 MPa). It should also have a similar modulus of elasticity to that of bone (20 GPa) in an attempt to prevent both stress shielding and fatigue fracture under cyclic loading by maintaining adequate toughness (Giannoudis *et al*. 2005). However, if less than that of bone the load is bore by bone only. The elastic propriety of hydroxyapatite is 70-120 GPa (Wang *et al*., 1995) which is too high and can cause stress shielding that result in loosening of implants in patients with osteoporosis but the incorporation of alumina chitosan nanofiber into the matrix of hydroxyapatite reduced its elastic properties. The composite of volume fraction 25-15-60 (Al-CH-HA) gave the peak value of elasticity (8.23 GPa) (**Table 6**).

Bending strength

It has been reported that the bending strength of dense hydroxyapatite is 20 – 80 MPa (Silva, 2000) whereas the bending strength of human cortical bone is 50 – 150 MPa (Currey, 1990). The optimum bending strength value of 175.5 MPa was obtained at 25-15-60 composite volume fraction, while 171.20 MPa was obtained at 20-20-60 MPa (**Table 7**). However, the values of the bending strength of the composite synthesized are within the range of the human cortical bone and hence suitable for bone substitute to prevent stress shielding that result in loosening of implants in patients with osteoporosis. The factors responsible for the mechanical attributes of the composite might result from particle size and particle size distribution of alumina, chitosan, interfacial interactions between the alumina-chitosan nanofiber and HA; and a good distribution of nanofiber in the matrix of HA.

X-Ray Fluorescence analysis of the composite

The composites have the advantage of being compatible with the human body environment. Their biocompatibility is a direct result of their chemical compositions which contain ions commonly found in the physiological environment such as Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, Al^{2+} and of other ions showing very limited toxicity to body tissues (**Table 8**). Due to their excellent tribological properties and with their improved fracture toughness and reliability, structural ceramics such as polycrystalline alumina and toughened chitosan hydroxyapatite will find application in hard tissue replacement materials.

FT-IR analysis

Figure 2 presents the FT-IR spectrum of Al-CH-HA composite. The peak on the band 3643.65 cm^{-1} is attributed to the OH···O stretching vibration and N-H band which lies between 3221.23 cm^{-1} and 3064.99 cm^{-1} shows the intermolecular hydrogen bonding of the chitosan polymer molecules (Li *et al*, 2006; Wan *et al*, 2009). The peaks at 2522.98 cm^{-1}, 2459.32 cm^{-1}, and 2359.02 cm^{-1} can be attributed to the primary amine $–NH_2$ and secondary amine absorption band respectively (IR correlation chart). Three peaks at 1487.17 cm^{-1}, 1425.44 cm^{-1}, 1417.73 cm^{-1} assigned to the deformation of $C-CH_3$ band and the band 1506.46 cm^{-1} may be attributed to the carbonyl C=O-NHR band (Wan *et al*, 2009). At 1068.60 cm^{-1} is attributed to skeletal vibration involving C-O stretching.

Microstructure analysis

The dispersion of the reinforcement in the matrix is another crucial factor that determines the mechanical attributes of composites. The dispersion of the fiber in the matrix of the synthesized composite was studied for the 20-20-60 Al-CH-HA volume fraction, being the fraction with optimum properties. In **Plates 1** and **2**, the SEM images taken at a magnification of 750 and 1000 x respectively show the dispersion of the reinforcement, the chitosan nanofiber, in the matrix (HA). However, at a higher magnification of 2500 x as in **Plate 3**, the dispersion of the fiber in the matrix of HA becomes very visible. The chemical interactions and compatibility between the constituents of the composite help to obtain the homogeneous microstructures which results in strong bond strength between the phases. The chemical interactions between the -NH_2 group and primary and secondary –OH group of CH reinforcement fiber with the ionic bond in Ca^{2+} of HA matrix resulted in high adhesion between CH and HA, which enable the matrix to hold the CH fiber together and transfer the applying load to the reinforcement fiber, consequently, improving the strength of Al-CH-HA composite (Yamaguchi *et al*., 2001). The incorporation of CH fiber into the HA matrix results in good mechanical property of the composite which cannot be attained by any of the constituents alone.

Conclusion

Al-CH-HA composite was successfully synthesized with the 20-20-60 being the optimum volume fraction. Increasing volume fractions of alumina-chitosan nanofiber in the matrix of HA decreased the compressive strength (181.73 MPa), hardness (529.21 HV) and elastic modulus (8.23 GPa) of HA while its tensile strength (172.67 MPa), bending strength (175.51 MPa) and fracture toughness (7.42 MPa.m$^{1/2}$) increased. The mechanical properties of the composite synthesized this work is similar to that of cancellous and cortical bone. Consequently, the material will be a suitable candidate for load bearing applications. X-Ray Fluorescence analysis result indicates that their chemical compositions contain ions commonly found in the physiological environment. In addition, the (SEM) micrograph revealed the dispersion of the alumina-chitosan fiber in the matrix of hydroxyapatite. The chemical compatibility and the interactions between the constituents of the composite were revealed by the FT-IR result and may be responsible for the microstructure obtained with the formation of interfaces which enhance the activation of different mechanisms that improve the mechanical strength of hydroxyapatite. Thus, the improvement of the mechanical property of HA was achieved through the strengthening of hydroxyapatite with alumina-chitosan nanofiber with enhanced usefulness in load bearing application.

Reference

Abere D.V., Oyatogun G. M., Akinwole I.E.,, Abioye A.A., Rominiyi A.L., Igbonwelundu M. T. (2017). *Effects of increasing chitosan nanofibre volume fraction on the mechanical property of hydroxyapatite*. Amer. ican J. ournal of Mater. ials Sci. ence and Engin. eering, vol. 5 , no. (1), 6-16.

Ashman R. B., Guo A., and Hasting L. (1984). *A continuous wave technique for the measurement of the elastic points of cortical bone*.," J. Biomech. anics, 17 (5), 349-361.

Currey J.D. (1990). *Physical characteristics affecting the tensile failure property of compact bone*. J. Biomech. 23, 837-844.

De With G., Corbijn A.J., (1989). "*Metal fibre reinforced HA ceramics*. , J. ournal of Mater. ials Sci. ence, 24, pp : 3411-3415.

Fischer, H., & Marx, R. (2002). *Fracture toughness of dental ceramics: Comparison of bending* *and indentation method*. Dent. al Mater. ials, 18 (1), 12-19.

Gautier S., West J.L., Champion E., (1999*). Rheological characteristics of alumina platelet-HA composite suspensions*. , J. ournal of Europ. ean Ceram. ics Soc. iety, 19, pp: 469-477.

Giannoudis, P.V., Dinopoulos, H., Tsiridis, E., (2005). *Bone substitutes: An update, injury*. , Int. J. Care Injured. , Issue 365, pp. 20-27.

Jayachandran V., & enkatesan and im Se-Kwon K., (2010). *Chitosan composites for bone tissue engineering—An overview*. J. Mar. Drugs. , 8, 2252-2266.

Katti, K.S., (2004). *Biomaterials in total joint replacement*. Colloid. s and Surf. aces B: Biointerf. aces, 39 (3), 133-142.

Kim H.M., Sasaki Y., Suzuki S. (2000). *Mechanical property of bioactive ceramics and cancellous bones*. J. ournal of Bioceram. ics, 6, 45-48.

Kokubo, T. (2008). *Bioceramics and their clinical applications*. Bioceram. ics, 13, 227-230.

Langer R., Tirrell D.A., Kumar M. N., (2004). *Toughening of alumina/zirconia ceramic composites with silver particles*. J. Eur. Ceram. Soc., 22, 2165- 2168.

Li, B., ; Hu, Q.,; Qian, X.,; Fang, Z.,; Shen, J., (2002). *Bioabsorbable chitosan/hydroxyapatite composite rod for internal fixation of bone fracture prepared by in situ precipitation*. Acta. Polym. Sin., 6, 828-833.

Li, Q., ; Chen, Z., ; Darvell, B., ; Zeng, Q., ; Li, G., ; Ou, G., ; Wu, M., (2006). *Biomimetic synthesis of the composites of hydroxyapatite and chitosan–phosphorylated chitosan polyelectrolyte complex*. Mater. Lett., 60, 3533-3536.

Li, Q., ; Wu, M., ; Tang, L., (2008). *Bioactivity of a novel nano composite of hydroxyapatite and chitosan phosphorylated chitosan polyelectrolyte complex*. J. Bioact. Compat. Polym., 23, 520-xx.

Li, Z., ; Yubao, L., ; Aiping, Y., ; Xuelin, P., ; Xuejiang, W., ; Xiang, Z., (2005*). Preparation and in vitro investigation of chitosan/nano-hydroxyapatite composite used as bone substitute materials*. J. Mater. Sci. Mater. Med., 16, 213-219.

Loku K., Yoshimura M., Somia S., (1990). *Microstructure and mechanical properties of HA ceramics with zirconia dispersion prepared by post-sintering*. Biomater. ials 11, pp: 57-61.

Luo X. L., Xu J. J., Wang J. L., Chen H. Y., (2005). *Chitosan composite*“ . Chem. Commun., 21, 69-72.

Madhavan P.X., (1992). *Scaffolds for tissue fabrication: Chitin chitosan and their novel applications*. Madhavan editor. Science Lecture Series, CIFT, Kochi.

Madhavan, P., ; Nair, K., (1974). *Utilization of prawn waste: Isolation of chitin and its conversion to chitosan*. Fish. Technol., 11, 50-53. 9.

Park S.B., You J.O., Park H.Y., Haam S.J., Kim W.S., (2001). *Biomaterials*. Park ed. 22. France.

Ratner, B.D., Hoffman, A.S., Schoen, F.J., Lemons, J.E., (2004). *Biomaterials science, an introduction to materials in medicine*, 2nd edition”, (Elsevier Academic Press, San Diego,) pp.162.

Rho J.Y., Kuhn-Spearing, and Zioupos P., (1998). *Mechanical property and the hierarchical structure of bone*, Med. Eng. Phys., 20, 99-102.

Shahidi, F.; Abuzaytoun, R. (2005). *Chitin, chitosan, and co products: Chemistry, production, applications, and health effects*. Adv. Food Nutr. Res., 49, 93-135.

Silva, V.V., Lameiras, F.S., Domingues, R.Z. (2000). *Microstructural and mechanical properties of bioceramics*. J. ournal of Biomed. ical Mater. ials Res. earch, 54, 139-148.

Sono A., (2003). *Synthesis of nano-hydroxyapatite*. J. ournal of Biomed. Res., 3, 34-39.

Suchanek, W., & Yoshimura, M., (1997). *Processing and properties of hydroxyapatite- based* *biomaterials for use as hard tissue replacement implants*., J. ournal of Mater. ials Res. earch, Volume 13, Issue (1), pp.94-117.

Suzuki T., Matsumoto T., Hagino Y., (1998). *Science and technology of polymers and advanced materials (*Eds: P. N. Prasad, J. E. Mark, S. H. VandeVord P. J., Matthew H. W., De Silva S.P., Mayton L. Wu B., Wooley P. H., (2002). *Property and Applications of Chitosan*". J. Biomed. Mater. Res., 59, 585.

Wang H., Li Y., Zuo Y., Li J., Ma S., Cheng L., (2010). *Biocompatibility and osteogenesis of biomimetic nano-hydroxyapatite/polyamide composite scaffolds for bone tissue engineering*". Biomater.ials; 28: 3338-3348.

Wang, R.Z., Cui, F.Z., Lu, H.B., Wen, H.B., Ma, C.L., and Li, H.D., (1995). *Synthesis of nanophase hydroxyapatite/collagen composite*. J. ournal of Mater. ials Sci. ence Lett. ers. , 14 (7), 490-492.

Wang, X., Ma, J., Wang, Y., He, B., (2001*)*. *Structural characterization of phosphorylated chitosan and their applications as effective additives of calcium phosphate cements*. Biomater. ials, 22, pp. 2247-2255.

Wang, X.,; Tan, Y.,; Zhang, B.,; Gu, Z.,; Li, X., (2009). "Synthesis and evaluation of collagen chitosanhydroxyapatite nanocomposites for bone grafting". *J. Biomed. Mater. Res. Part A*, 89, 1079-1087.

Yamaguchi, I.,; Tokuchi, K.,; Fukuzaki, H.,; Koyama, Y.,; Takakuda, K.,; Monma, H.,; Tanaka, J., (2001). *Preparation and microstructure analysis of chitosan/hydroxyapatite nanocomposites*". J. Biomed. Mater. Res. 55, 20-27.

Yokoyama, A., Yamamoto, S., Kawasaki, T., Kohgo, T., Nakasu, M., (2002). *Development of calcium phosphate cements using chitosan and citric acid for bone substitute materials*". Biomater. ials, **Vol.** 23, pp.1091-1101.