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Synthesis and Characterization of Chitosan/Gum Arabic Nanoparticles for Bone Regeneration

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Abstract Chitosan /gum arabic nanoparticles (C/G)have been prepared by ionic gelation method. This was with a view to enhance the mechanical properties and its application as bone graft scaffold. The cowry shells were washed, dried, pulverized and subsequently sieved with mesh No. 60, size 250 µm. It was deproteinized, Chitin was isolated from the synthesis by demineralising in 0.5 M Hydrochloric acid, and subsequently deacetylated by the addition of 40% (W/V) of Sodium hydroxide to synthesize chitosan. The raw chitosan was purified using 2% (v/v) acetic acid solution. The synthesized chitosan and gum arabic, a product of Acacia tree, were used to prepare chitosan/gum arabic nanoparticles by ionic gelation method. Mechanical characterization was carried out on the synthesized material using universal testing machine. Analysis of the chemical composition was carried out using Fourier transform infrared spectrometer (FTIR) and X-Ray fluorescence, (XRF). Furthermore, the morphology of the materials were studied using scanning electron microscopy, SEM and the dimension of the nanoparticles were characterized using transmission electron microscopy (TEM). Finally, an attempt was made to ascertain its suitability for bone regeneration. The FTIR spectra result confirmed that the nanoparticle was actually a derivative of chitosan by the observed shift in the peak 3462 to 3404cm-^{1}. There is presence of a new peak at 1636 cm^{-1} and 1473 cm^{-1}. Peak observed at 1080 cm^{-1}, 860cm^{-1} and 712 cm^{-1} on C/G nanoparticles spectrum were similar to the native chitosan spectrum which shows that there was no change in the main backbone of chitosan structure. The scanning electron microscopy (SEM) study revealed chitosan as polymeric rods, while the chitosan /gum arabic nanoparticles in aggregate. The TEM was to confirm nanoparticles of average size of 200nm. The ultimate compressive strength was found to have increased by 78.21%, the Young Modulus by 54.4 % and percentage elongation by 7%. In overall assessment, mechanical properties of the chitosan/gum arabic nanoparticles were better than native chitosan. The study concluded that crosslinking of chitosan with gum arabic to form its nanoparticles derivative improved the mechanical properties of chitosan and consequently its application as a bone graft substitute for bone regeneration.

Keywords: *Chitosan, gum arabic, nanoparticles; bone graft scaffold, bone regeneration.*

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1. Introduction

Most often, bone disease and injuries such as osteoarthritis, rheumatoid arthritis, osteoporosis and bone fracture, dramatically affect the quality of life of patients and limit their ability to perfume basic tasks such as walking, running and standing. The high occurrence of these conditions are evidence by more than 2.2 million bone graft used in orthopaedic annually worldwide [1]. Bone grafts are used to augments or stimulates the formation of new bone, in cases such as the healing of the skeletal fractures, or between bones across a diseased joint; to replace and regenerate lost bone as a result of trauma, infection, or disease or to improve bone healing response and regeneration of tissues around surgical implanted devices. The three main types of bone grafts are autograft, allograft and xenograft. The tissue regeneration of these bone graft is measured in terms of their osteogenic, osteoconductive and osteoinductive potential [2]. Autografts are considered the gold standard for bone repair; however, such as bone donor site morbidity ([1,3]). Another alternative is the allograft, which is bone tissue from cadaver or living donors, eliminates donor site morbidity, but has the potential risk of adverse immunological response and also lacks osteogenic capacity of autograft [4]. Xenograft, which is bone graft from a species other than human such as bovine, constitutes another alternative for bone repair. This has its limitation in that it elicits cytotoxine effects which may lead to adverse immunological response [5]. The limitations associated with the utilization of these bone grafts have prompted the search for other alternatives, which is referred to as bone graft substitutes.

Bone graft substitute is based on the concept of bone tissue engineering, which aims at improving the

osteoconductive, osteogenic and osteoinductive potential by incorporating bone progenitor cells and growth factors to scaffold made of various natural and synthetic materials, or combination, in order to mimic the bone microenvironment [3]. Tissue engineering is a helpful alternative strategy for conventional treatment in medicine. It was officially coined at a National Science Foundation Workshop in 1988 to mean "the application of principles and methods of engineering and life science toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve the tissue function or the whole organs" [6]. The field relies on the use of three-dimensional scaffolds to provide appropriate environment for the regeneration of tissues and organs [1,6].

Scaffolds provide three dimensional surfaces for cell seeding, proliferation and filling of bone defects. It also provides mechanical competence during bone regeneration. The selection of the most suitable material for the production of bone regeneration scaffold is a determinant step, since its properties will determine the final attributes of the regenerated bone. Osteoconductivity, porosity and biodegradability are some of the required properties needed for scaffolds to be successful in bone tissue engineering. These properties will enhance bone formation, angiogenesis, support the attachment and proliferation of osteoblasts cells on the scaffold [7].

Polymeric biomaterials, which are biocompatible with the physiological system, have found wide applications in regenerative medicine and tissue engineering [1]. Biodegradable polymers are appropriate substrates for cells to attach, grow and maintain a differentiated phenotype [7]. They have also attracted significant interest because of their flexibility, in terms of chemical manipulation, and their biodegradability [3]. Natural materials, due to their bioactive have been observed to influence cell morphology, modulation, and differentiation [1]. These properties tend to have better interaction with cells. This allows them to have better performance in service. Natural polymer can be classified as proteins such as silk, collagen, fibrinogen, elastin and myosin, polysaccharides such as cellulose, amylose, dextran, chitin, chitosan and glycosaminoglycan, or polynucleotides, deoxyribonucleic acid, DNA, and ribonucleic acid, RNA [8].

Chitosan is a co-polymer of N-acetylglucosamine and N-glucosamine units, which are produced by alkaline deacetylation of naturally occurring chitin. It presents excellent biological properties such as biocompatibility, biodegradability and immunogenicity antibacterial activity, wound healing properties and bio-adhesive character. These attributes enhanced its biomedical applications [1,9]. It is isolated from exoskeleton of insects, arthropods and crustaceans (crabs lobsters, and shrimps). Internal shells of cephalopods, radulae of molluscs, scales of fish and lissamphibians are alternative sources of chitin [10]. Chitosan is a natural biological polymer possessing reactive amine and hydroxyl group that promotes osteoblast growth and in-vivo bone formation [9]. The molecular weight of chitosan may range from 300 to 1000KDa, depending on its origin and the method of preparation. Chitosan is semi crystalline polymer and its crystallization depends on the degree of deacetylation [1] The solubility, biodegradability, reactivity, and absorption of its substrates depend on the amount of protonated amino groups in the polymer chain [1,9] The cationic nature of chitosan allows electrostatic interaction with anionic glycosaminoglycan and proteoglycans [1].

Chitosan has found diverse applications in regenerative medicine, orthopedics, periodontology, drug delivery systems, wound healing and tissue engineering [9,10]. However, chitosan low mechanical resistance limits its application in tissue engineering. In other to optimize resistance and elasticity, crosslinking agents such polyethyleneglycol, dialdehydes (such as glutaraldehyde and glyoxal), and starch are used [9]. Chitosan as natural polymer finds immense application in various fields. Despite the intensive research on the transformation of chitosan to novel biomaterial, still research for suitable cross linkers to have high mechanical strength chitosan based scaffold material is ongoing [11]. The work, therefore exploit the dual role of gum arabic for the preparation of chitosan based scaffold with enhanced mechanical properties.

Gum arabic (GA) is a natural complex mixture of hydrophilic carbohydrate and hydrophobic protein component obtained from the stems and branches of Acacia Senegal [12,13]. GA consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid. It is highly soluble in water to form a solution of pH _ 4.5 and insoluble in alcohol [12,14]. Gum arabic has diverse industrial applications such as acting as a stabilizer, thickening agent and emulsifier in food industry as well as textile, lithography, cosmetics and pharmaceutical industries [14]. GA has also found wide applications in nanotechnology, where it has been used as a crosslinking agent to produce chitosan/gum arabic nanoparticles for sustained drug release [15].

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 1-1000 nm [16,17]. The physical and biological properties of nanomaterials are unique and differ depending on their corresponding bulk material, making these entities an intense focus of recent studies. Nanostructured surfaces play major role in advanced biomedical implant design because of their enhanced bioactive properties, as well as their incompatible behavior toward bacterial colonization [18]. Several attempts have been made to synthesize chitosan nanoparticles from different chitosan sources. Different methods, such as the microemulsion method, reverse micellar method, self-assembling method and ionic gelation have been used to prepare chitosan nanoparticles [16,19].

Ionotropic gelation method involves the formation of chitosan nanoparticles based on the electrostatic interaction between the amine group of chitosan and negatively charged group of polyanions [19]. The mechanism of chitosan nanoparticle formation is based on the electrostatic interaction between amine group of chitosan and negatively charged group of polyanion such as tripolyphosphate. This technique offers a simple and mild preparation method in the aqueous environment [16,19]. Consequently, this method was adopted in this work to synthesize cowry shell based chitosan /gum arabic nanoparticles for bone regeneration.

2. Materials and Methods

2.1. Materials

Materials used for this work were cowry shells and gum arabic were gotten from Il-Ife Market in Nigeria, and reagent and chemical such as hydrochloric acid, acetic acid, sodium hydroxide and acetone are from BDH Chemicals Ltd Poole, England and Sigma-Aldrich Laborchemickalien GMBH, Seelze, Germany. Distilled water was the solvent used throughout the work.

2.2. Synthesis of Chitosan from Cowry Shell

The cowry shells were washed with distilled water, dried, pulverized and subsequently sieved with standard mesh No. 60, size 250 µm. The pulverized cowry shells were put in a conical flask and 0.1M NaOH was added. The mixture was boiled and stirred at 100°C for 2 hours in a water bath. The product was then filtered and washed with distilled water. Red litmus paper was used to check whether the filtrate pH was completely neutral. It was then dried in an oven at a temperature of 80°C. The deproteinized product was decolorized by soaking in pure acetone for 24 hours and was subsequently washed and dried in an oven at 80°C. Then, 2250 ml of hydrochloric acid (0.5 M) was added to the deproteinized cowry shell (500 g) to leach out $CaCO_3$ and the mixture was boiled and stirred for 45 minutes at 100°C in a water bath and subsequent washing and drying were done. The residue, chitin was dried in an oven at 100°C for 2 hours. Furthermore, the chitin was boiled in 40% (W/V) of sodium hydroxide, at 100°C for 3 hours in water bath and it was cooled for 30minutes at room temperature to convert the chitin to chitosan. The product was filtered and the filtrate, chitosan, was washed and tested with litmus paper until the pH was neutral. The chitosan was subsequently dried overnight in an oven at a low temperature 25°C. Furthermore, the raw chitosan was dissolved in 2 % (v/v) acetic acid solution and filtered to remove the residue of insoluble particles. Afterwards 2 M of NaOH was added to the filtrate to obtain chitosan in form of white precipitate. The precipitated chitosan was washed thoroughly using distilled water, and dried at 40°C for 48 hours [20].

2.3. Preparation of Nanoparticles

Chitosan nanoparticles were prepared using the ionotropic gelation method used by [19,20] with these steps. The use of acetic acid to dissolve chitosan was avoided due to the fact that chitosan can protonate in presence of gum arabic. Purified chitosan (1.5% w/v) was dispersed in distilled water. The gum arabic (1% w/v) was dissolving in distilled water to obtain a known concentration under magnetic stirring at room temperature. Then 5 ml of gum arabic solution (1% w/v) was added into the chitosan (5 ml) through an insulin syringe needle at the speed of 60 ml/h under magnetic stirring at room temperature. Equation (1)

$$\begin{aligned} &RNH_2 + R\grave{}COOH \rightarrow RN^{+}H_3 + R\grave{}COO- \\ &\rightarrow RNH_3\ OCR' + H_2O. \end{aligned} \quad (1)$$

The resulting chitosan nanoparticle suspension was centrifuged at 3000 rpm for 20 minutes. Supernatant was discarded and nanoparticles were resuspended with distilled water. The samples were dried by air flow at 25°C.

2.4. Characterization of Chitosan and Chitosan/gum Arabic Nanoparticles Synthesized

The compressive tests were performed on test samples. The particles were compressed to round shaped test samples of diameter and thickness 5.69mm and 13.00mm respectively with an aluminum mold. Compressive tests were carried out on the test samples with the aid of a Computerized Mechanical Testing Machine (Instron machine series 3369). The samples were subjected to axial compressive load at a crosshead speed of 2mm/min. Load and displacement data were used to determine the compressive strength, relative deformation, modulus of elasticity and yield strength for each sample.

Chemical analyses of the nanoparticles were carried out using FTIR (FTIR 410® Jasco Colchester, United Kingdom) and XRF techniques .was carried out on chitosan extracted from cowry shell, gum arabic and synthesized chitosan/gum arabic nanoparticles. The surface of chitosan/gum arabic nanoparticles, chitosan and gum arabic were analyzed to determine their chemical composition. About 0.5 g of the test samples were pelletized each to obtain cylindrical pellets suitable for the XRF machine model HERZOG PW1606. Each of the pellets were mounted on the sample holder and each sample were irradiated for 20 minutes at a fixed tube operating condition of 25 kVA and 6 mA. The test result was displayed on the computer connected to the XRF and the percentage of the elements present were analyzed and recorded.

The chemical interaction between the chitosan and gum arabic molecules were established using FTIR spectrometry. Five milligrams of the analyst were mixed thoroughly with about 100 mg of dry KBr and appropriate amounts of the disk were prepared by compression to form KBr pellets for the test. All samples were scanned in the wave number region from 4000 and 400 cm^{-1}. The absorbance of A_{1660} cm^{-1} and A_{3450} cm^{-1} were obtained from chitosan spectrum and used to calculate the degree of deacetylation as shown in equation (2).

$$DD = 100 - \left[\frac{\left(A1660cm-1/\ A3450cm-1\right)\times 100}{1.33}\right](\%) \quad (2)$$

Where DD is deacetylation degree; A_{1660} cm^{-1} and A_{3450} cm^{-1} are absorbance at 1655 cm^{-1} and 3460 cm^{-1} respectively.

The morphological properties of dried nanoparticles were studied using scanning electron microscope (SEM). The SEM having a magnification range of 20000-30000 and accelerating voltage of 15 KV were used for characterization of prepared chitosan and chitosan/gum arabic nanoparticles. All the samples were coated with gold before SEM testing. Similarly, the dimension of the synthesized nanoparticles was measured using Transmission Electron microscopy (TEM) (Philips 400®, 80 KV, The

Netherlands). The samples were immobilized on copper grids and stained with phosphate tungsten acid and examined by TEM.

The *invivo* test was carried out by acquiring and conditioning the animals for two weeks before the test, using the standard protocol for laboratory animals. Surgical defects (Mandibular defects) were created and managed post operatively by trained personnel in the procedure. Linear submandibular incision was made approximately 10 mm from the lower border of the mandible using a #15 blade mounted on a #3 cable scalpel. Blunt dissection was performed by detaching the masseter muscle and periosteum, following which the flap was retracted with fine retractors. The defect site of each rabbit was filled with the test material allocated to it and appropriately labelled with an indelible marker. For example, the mandibular defect in Rabbit labelled A was filled with gum arabic; Rabbit B was filled with chitosan while Rabbit C was filled with chitosan/gum arabic nanoparticles. The wound was sutured in layers.

The animals were sacrificed after 21 days using standard protocols. The masseter muscle and periosteum were detached from the bone. The bone tissues were processes in the laboratory and mounted on slides and stained in Haematoxylin and eosin (H and E) for histological analysis.

3. Result and Discussion

3.1. Results

The SEM micrographs obtained for both chitosan and chitosan /gum arabic nanoparticles were presented in Plate 1. Similarly, the TEM micrographs for the synthesized chitosan/gum arabic nanoparticles is shown in Plate 2. The FTIR spectra obtained for the chitosan, gum arabic and chitosan/gum arabic nanoparticles were presented in Figure 1 - Figure 3. The result of the wave number and chemical group of FTIR absorption bands and XRF results are shown in Table 1 and Table 2 respectively. The result of the compressive strength, Young's Modulus, % elongation and yield strength of the synthesized chitosan and chitosan/gum arabic nanoparticles are presented in (Figure 4 a-d). Finally, the *In-vivo* results, Plate 3, show the photomicrographs interaction of bone with gum arabic, chitosan, chitosan/gum arabic nanoparticles after 21 days of implantation.

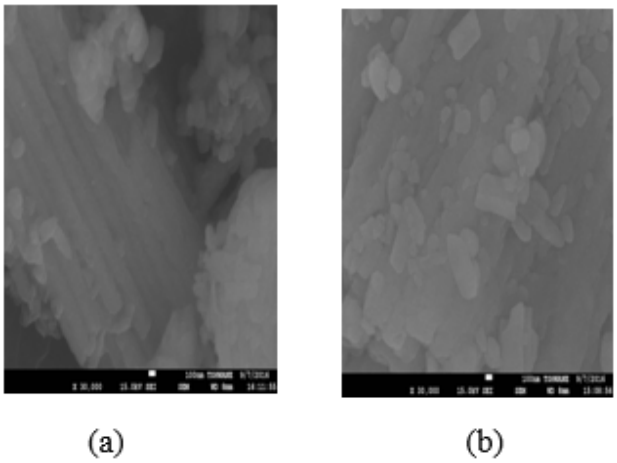


Plate 1. SEM Micrograph of (a) chitosan (b) chitosan/ gum arabic nanoparticle (Magnification x30000)

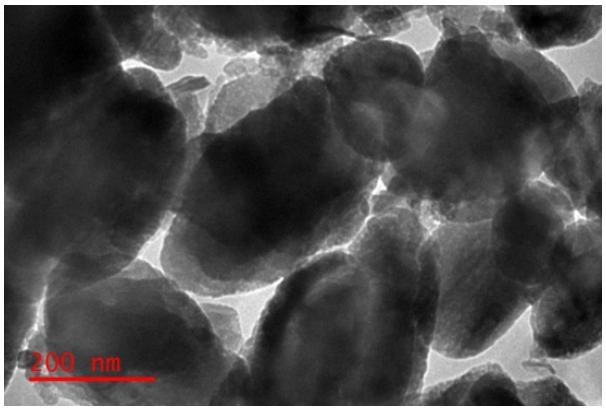


Plate 2. TEM Micrograph of chitosan/gum arabic nanoparticles

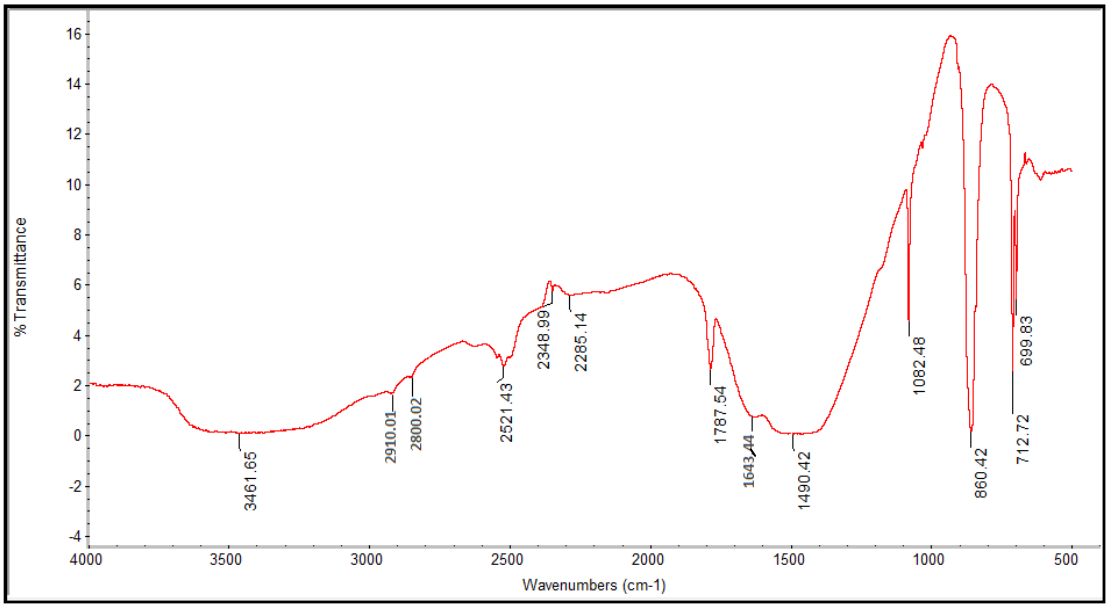


Figure 1. FTIR Spectrum of chitosan

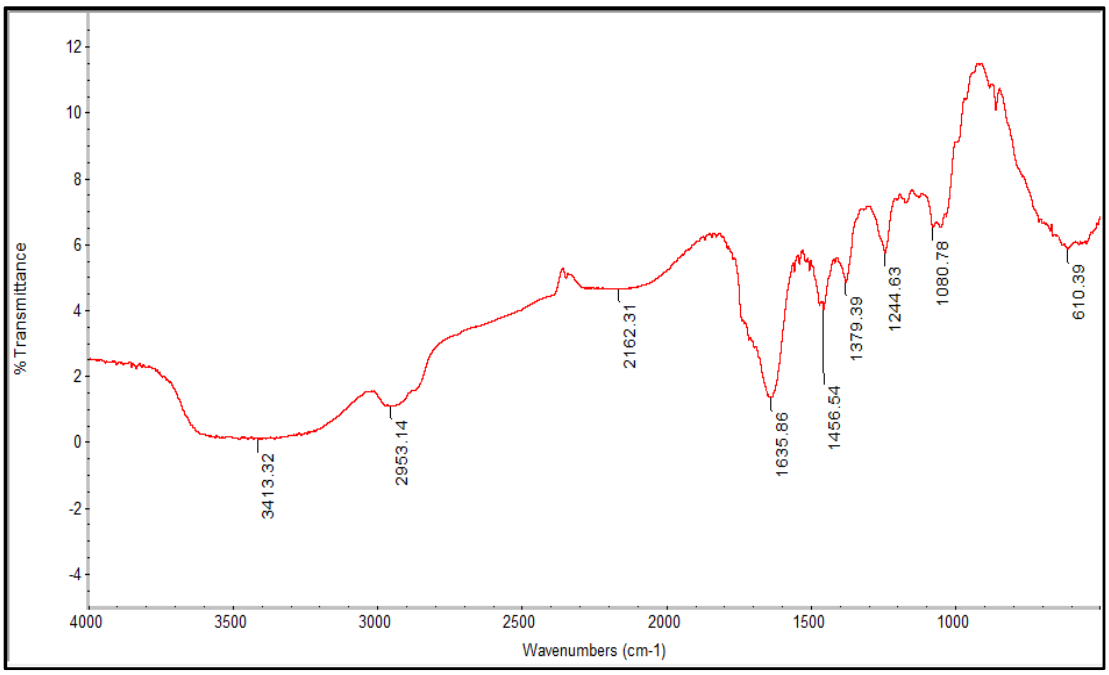


Figure 2. FTIR Spectrum of gum arabic

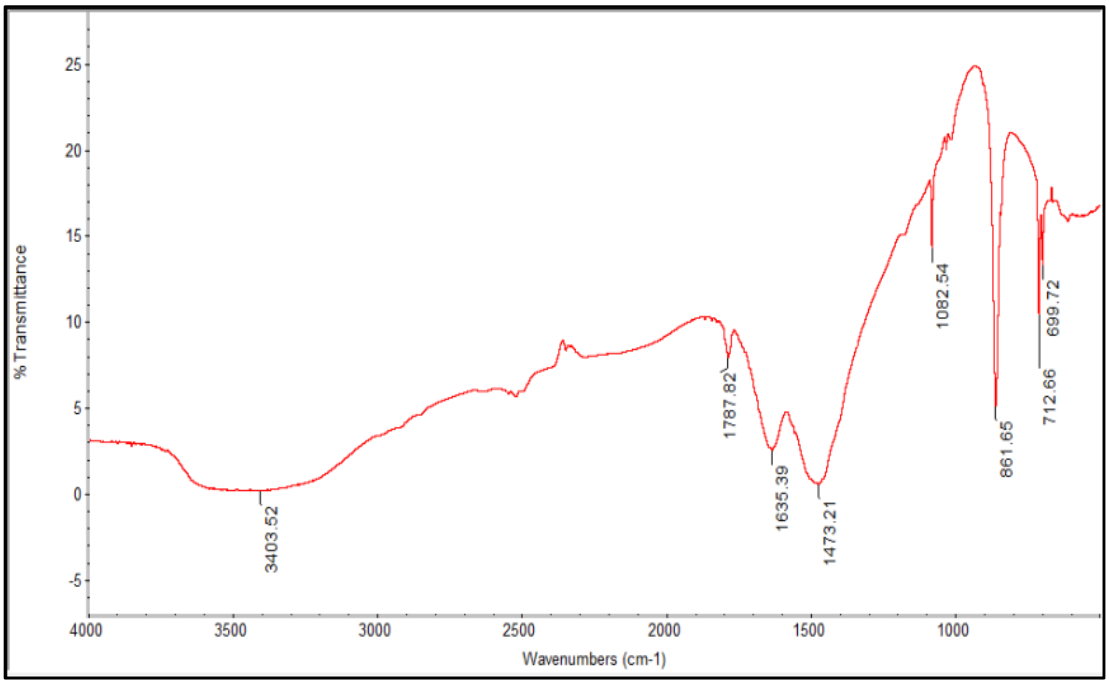
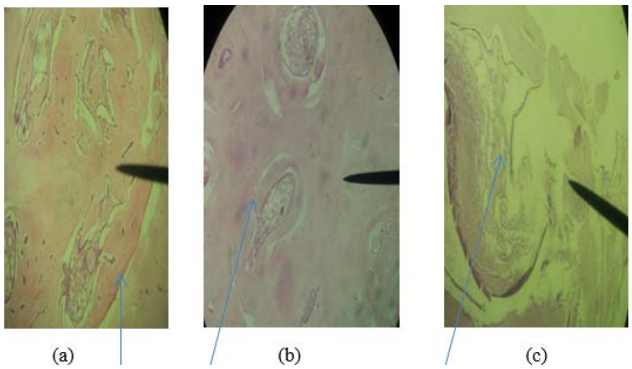


Figure 3. FTIR Spectrum for chitosan/gum arabic nanoparticles

Table 1. Characteristic absorption band of the chitosan and gum arabic

Materials	wave length (cm^{-1})	Vibration Mode/ Chemical groups
chitosan	3462-3000	$\nu(NH_2)$ assoc. in primary amine (OH)assoc. in pyranose hydroxyl group
	2910-2800	$vas(CH_2)$ inCH_2OH group ν(C-H) in CH_3 pyranose ring
	1643	Amide 1 band ν(C=O) in $NHCOCH_3$ group
	1082	ν(C-O) in secondary OH group
	860	CH_3COH group in pyranose ring vibration (Corresponding to saccharide structure)
Gum arabic		
	3413.32	O-H stretching , characteristic of glucosidic ring
	2953.14	C-H stretching
	1635.86	COO- symmetric stretching
	1456.54	COO- asymmetric stretching
	1200-900	Finger print of carbohydrates



Plates 3. Photomicrographs of bone at 21 days after implantation of (a)gum arabic; attempts at bone formation(b) chitosan new bone enclosing (c) chitosan/gum arabic nanoparticles (c) bone formation with osteocytes in lacunae present above and beside the test material

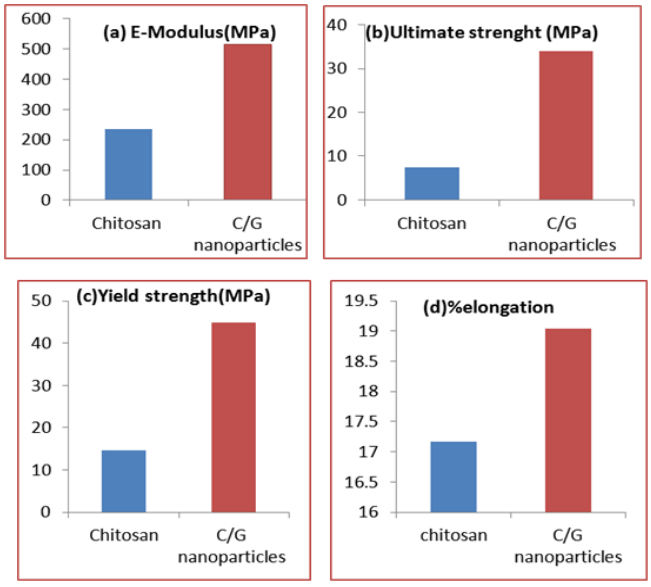


Figure 4. (a-d) The mechanical properties of chitosan and chitosan/gum arabic nanoparticles

Table 2. X-Ray Fluorescence results of chitosan, gum arabic and chitosan/gum arabic nanoparticles

element	Chitosan (Wt. %)	Gum arabic (Wt. %)	Chitosan /gum arabic nanoparticles
P	1.7673	5.5235	1.0798
Cl	1.2200	3.2972	0.9725
K	1.7494	8.6066	1.7445
Ca	35.7397	18.2917	36.6864
V	0.0213	0.0808	0.0185
Mn	0.0210	0.3314	0.0113
Fe	0.1142	1.9781	0.1343
Cu	0.0156	0.5176	0.0142
Zn	0.0498	0.2196	0.0204
Ga	0.0091	0.0781	0.0089
Se	0.0043	0.0909	0.0048
Sr	0.0349	0.0686	0.0412
Ti	0.0120	0.2355	0.0191
Mo	0.0033	0.0916	0.0025
Cr	0.0039	0.1568	0.0024
Ni	0.0261	0.4866	0.0295
As	0.0024	0.0308	0.0036
Bi	0.0087	0.2028	0.0087
W	0.0108	0.4078	0.0106
Pb	0.0006	0.0444	0.0010
Nb	0.0034	0.0886	0.0039

3.2. Discussion

3.2.1. Mechanical Properties

Mechanical properties of a scaffold used for tissue engineering are very important due to the need for the structural stability to oppose the various stresses incurred during culture, in-vitro or implantation, in-vivo [24]. Bone tissues undergo compression and due to that compression test was used for evaluation. The result of this study has shown that The Young Modulus (compression) for chitosan and chitosan/gum arabic nanoparticles were 235 and 515 MPa respectively. The result strongly indicated that the Young Modulus (compression) chitosan/gum arabic nanoparticles is higher than native chitosan. Similarly, compressive strength, % elongation and yields strength shown in Figures 3 indicate that chitosan/gum arabic nanoparticles have a better mechanical properties than chitosan The observed increased in the mechanical strength is ascribed to cross linking of amine group of chitosan with carboxylic acidic of the gum arabic leading to enhance mechanical properties of the chitosan/gum arabic nanoparticles. The Young's Modulus (compression) and the ultimate's strength of bone are at the range of 1-20GPa and 1-200 MPa respectively [27]. The chitosan/gum arabic nanoparticles synthesized has Young's Modulus (compression) and the ultimate compressive strength of 515 MPa and 34 MPa which are nearer and within the range of human bone respectively. This shows that it will function well as bone graft substitute.

3.2.2. Morphology Characterisation

Plate 1 show SEM of chitosan and chitosan/gum arabic nanoparticles. The morphology of chitosan presented a typical of polymeric rods, with smooth surface and they agree with the previous observation by [23], where it was discovered that chitosan conformations depend on molecular weight. When the molecular weights are as high as 223 kDa, chitosan will acquire a random coil shape. While, when chitosan molecular weights are low such as below 148kDa, chitosan will form a rod shape structure. Similarly, when the chitosan crossing linking reaction occurred through the addition of gum arabic, aggregated particulates were formed as noted by [24]. Higher hydrogen ion concentration favours rearrangement of protonated chitosan free chains, leading to formation of semi spherical shapes and homogeneous population of nanoparticles. This might be responsible for observed enhanced mechanical properties of chitosan/gum arabic nanoparticles. The TEM analysis confirms that the synthesized chitosan/gum arabic nanoparticles have a mean particle size of 200 nm which is in the range of biological molecules and much smaller than cells [25] and this will favours bone cells adsorption.

3.2.3. X RF of the Synthesized Materials

The result X RF of the analysis obtained for chitosan, gum arabic and chitosan/ gum arabic nanoparticles (Table 2) show the purity of the synthesized chitosan and chitosan/gum arabic nanoparticles. It can be noted that the amount of trace elements present in the synthesized materials are very low. This confirms that the synthesized

products are pure chitosan and chitosan/gum arabic nanoparticles. This implies that the synthesized materials will be suitable for bone regeneration. Evaluation of element with atomic number (Z) less than 11 is however, not possible with this technique. Hence, major elements such as C, O, H and N were not visible. However, the FTIR results show some of the major functional group present in the synthesized materials.

3.2.4. FTIR Spectra of the Synthesized Materials

The FTIR spectrum of chitosan/gum arabic nanoparticles, in Figure 3, shows that few significant changes was occurs in chitosan/gum arabic nanoparticles compared to the native chitosan (Figure 1).

FTIR spectrum of chitosan shows a peak at 3461 cm^{-1} which indicates symmetric stretching vibration of O-H bond (in alcohol) and N-H amine while the absorptions bands observed at 2910 cm^{-1}, 2800 cm^{-1}, 1643 cm^{-1} and 1082 cm^{-1} are respectively attributed to the stretching vibration of CH (in methylene), CH (in methyl), C=O in (amide 1) and C-O-C in (glucosamine ring). This peaks are similar to the one observed by [26,27]. In the chitosan/gum arabic nanoparticles, the tip of the peak 3461 cm^{-1} on chitosan, has shifted to 3403 cm^{-1} and becomes wider. This resulted from superimposed OH and NH_3^+ stretching band [11]. The absorption in 1635 cm^{-1} and 1473 cm^{-1} correspond to the presence of asymmetric N-H ($-NH_3^+$) band and asymmetric –COO- stretching respectively. Peaks at 1080 cm^{-1}, 860 cm^{-1} and 712 cm^{-1} in chitosan/gum arabic nanoparticles spectrum were similar to the native chitosan spectrum, which indicates no change in the main backbone of chitosan structure [28]. These resulted ionic interaction and multiple intermolecular hydrogen bonding between the $-NH_2$ groups of chitosan and consequently the crosslinking that resulted in the observed improved mechanical properties [11,28].

3.2.5. Evaluation of Degree of Deacetylation

Degree of deacetylation (DD) is an important parameter affecting solubility, chemical reactivity and biodegradability. It depends on the source of the chitosan and procedure used to synthesize it. DD may range from 30-95%. It is rare that the production of chitosan with 100% degree of deacetylation is achievable. Therefore, commercial chitosan with various degree of deacetylation in the range of 75-85% are commonly found [29]. From the FTIR results, using absorbance A_{3450} cm^{-1} and A_{1660} cm^{-1}, the degree of deacetylation (DD) was found to be 70%. This is good anticipated good enough for bone regeneration, as higher DD shows a higher range of cells attachment and higher cellular activities than the lower degree of deacetylation [30].

3.2.6. In-vivo Analysis

The results of the *in-vivo* analysis of the synthesized chitosan/gum arabic nanoparticles, gum arabic and chitosan showed evidence of new bone formation after 21 days post implantation in defect created in the mandibular rami of rabbits (Plates 1-3). All the surgical sites healed with no observed postoperative complications or clinical signs of reaction to any of the materials used in these experiments. Histological analysis of the defect site showed that the defect site implanted with the chitosan /gum arabic nanoparticles displayed some new bone formation after 21 days post implantation (Plates 3c). However, the defect implanted with chitosan particles alone exhibited more bone formation compared to defects treated with chitosan/gum arabic nanoparticles (Plates 3c). In defects treated with gum arabic alone, the bone edges were separated by soft tissue and there was little or no evidence of new bone formation (Plates 3a).

The result of this study is in agreement with previous studies [31,32]. Klokkevold *et al*. [33] also reported that chitosan increases the activity of osteoblasts and helps bone formation. [34] reported that spongy chitosan supports the proliferation of osteoblastic cells. Several studies have investigated various effects of chitosan on bone healing and raised some hypotheses on its mechanisms [35,36,37]. For instance [38], observerd that chitosan increases the vascularization of blood vessels and stimulates budding tissue (tissue comprising of budding capillaries and fibroblasts) [6,38] noted that the mechanical strength of scaffold decreases with porosity. This might have led to longer healing time of the chitosan/gum arabic nanoparticle in comparison with chitosan. Also, it is important that a scaffold should possess adequate mechanical properties to function effectively at the site of implantation and should be consistent with the anatomical site into which it is implanted. According to Wolfs law, once a new bone is formed, remodeling occur to produce a functional and durable bone tissue such that it stimulates osteoblast and osteoclasts to remodeling bone structure to a better resistance to strain [22,39]. With inadequate strain, induce mode remodeling will result into bone removal. Thus adequate mechanical strains must be present to maintain the newly regenerated bone [39]. Therefore, there is need to balance between mechanical properties and porosity of a scaffold to allow cell infiltration and vascularization.

Furthermore, chitosan/gum arabic nanoparticles (Plate 3a), show the presence of osteoblast, osteocyst and new bone matrix, which indicates progressive new bone formation [40]. Osteoblasts are responsible for synthesizing the organic components of the bone matrix and enzyme alkaline phosphatase, which is needed locally for the mineralization of osteoid, while osteocytes are actively involved with the maintenance of the bony matrix [41]. During bone remodeling, osteoblasts deposit a layer of osteoid seam (approximately 10μm thick) on the surface of the resisting bone, which then begin to mineralize in approximately 20 days. This interval is known as the mineralization lag time [41]. The failure for complete healing of the bone defect within 21 days, is attributed to bone healing been out of the mineralization lag time. Therefore the chitosan/gum arabic nanoparticles has a better mechanical and remodeling ability and subsequently good for bone regeneration.

4. Conclusion

Chitosan from cowry shells and chitosan/gum arabic nanoparticles were successfully synthesized for bone regeneration. Mechanical, chemical and microscopic analyses were done to know the suitability of the chitosan

and chitosan/gum arabic nanoparticles as a bone graft substitute for bone regeneration. SEM micrographs present chitosan and chitosan/gum arabic nanoparticles as a polymeric rods and aggregates respectively while TEM confirms size of the chitosan/gum arabic nanoparticles as 200 nm. FTIR results showed the functional groups in chitosan and chitosan/gum arabic nanoparticles while XRF was able to show some of the elements present in the synthesis products. The compressive strength, Young Modulus, yield strength and % elongation were enhance by 79.3%, 54.4%, 67.5 % and 7% respectively. *In-vivo* results were able to show bone regeneration properties of chitosan and chitosan/gum arabic nanoparticles. Chitosan/gum arabic nanoparticles has bone regeneration properties with better mechanical properties. Hence chitosan application as a bone graft substitute is enhanced.

5. Recommendation

Based on the findings of the study, it is recommended that longer term histological and histomorphometric studies be carried out to understand better the healing times and the nature of remodeling bone formed by chitosan/gum arabic nanoparticles and chitosan.

Contribution to Knowledge

Chitosan/gum arabic nanoparticles with mechanical properties comparable to human bone have been developed with potential to serve as bone regeneration scaffold.

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