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Suitability of Bovine-Bone as an Alternative for Dental Filler

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Abstract*: Tooth decay is one of the most common teeth diseases, which usually leads to cavities unless prevented. Dental cavities are holes in the teeth that are formed when acid in the mouth erodes the enamel. This is a problem that has plagued man for a really long time. Several researchers have proposed different solutions to mitigate the problem with various biocompatibility and other challenges associated with each available remedy. For an instance, the use of bio ceramics like zirconia as a dental filler was widely accepted due to its inertness as well as high hardness value. However, high brittleness and poor re-sorption limits its usage. Therefore, to the best of researchers' knowledge, investigation of materials properties for suitability and consequently selection for dental filler is an open gap. In this regard, this study examined the suitability of bovine bone as dental fillers. Ten Bovine bone sample was ground to 300 µm and calcined at temperatures from 600°C-900°C at 100°C intervals. The different samples were held in the oven for two hours and four hours at each temperature. Comparative analyses of the samples were carried out using Atomic Absorption Spectroscopy (AAS), to examine the chemical content of the control sample, i.e., Bovine bone before calcination, and compared with Bovine bone after calcination. AAS showed an increase in Na and Ca with an average percentage increase of 5%. The samples were also placed in Simulated Body Fluid (Saliva) for two weeks and four weeks respectively, to study how the sample would behave in the mouth. The results did not show any sample decomposition and the resulting pH value was indicative of a resorption of hydroxyapatite. Scanning Electron Microscope also revealed an increase in the size of the particles which is a case for the increase in calcium content. These results point towards the viability of Bovine bone as a suitable material for dental fillers.*

Keywords: *Dental filler, Bovine bone, simulated body fluid, calcination.*

1. INTRODUCTION

One of the most common tooth diseases is tooth decay. It is estimated that approximately 80% of the population in developed countries like US, Australia, Denmark, Sweden, etc., have experienced the problem [1,]. When the decay is not prevented, it leads to cavities [2]. To avoid pain and eventual tooth loss, it is necessary to fill the affected hole and reinstate the cavity by filling with a similar bio-material. Also, the material should be able to resist and work perfectly in the buccal environment. Ideal buccal environment contains saliva which has a normal pH range of 6.2 -7.6, with 6.7 being the average pH. A pH of 7.0 indicates a healthy dental situation. A pH below 7.0 indicates acidemia, while pH above 7.0 indicates alkalinity. Excessive alkalinity can bring about the same condition as acidemia [3]

Different materials (artificial, natural, stable and bio-resorbable) have been investigated and used to produce dental filler. The most extensively considered of these materials is bioceramic materials [4]. The tissue responses of ceramics are generally, better compared to polymers and metals [5]. Some of the bioceramics, such as hydroxyapatite (HA), biphasic calcium phosphate (BCP), Tricalcium phosphate (TCP) and alumina, are stable materials, thus they do not discharge their contents into the human body and usually do not create foreign body reactions. In other words, if aimed as absorbable biomaterials (e.g., most bioactive glasses) with various resorption kinetics (from days to months), their ion dissolution outcome (typically Calcium, Silicon, phosphorus and Sodium ions) can be produced through normal metabolism [6] or used to bring a desired healing effect, such as promotion of angiogenesis and antibacterial properties [7].

Restoration of dental cavity have a limited lifespan and once a cavity has been filled, it is very likely that the filling will be changed many times throughout the lifetime of the patient [8]. There are many choices of materials used for fillings. The main reasons for the application of these numerous materials are biocompatibility, moderate degradation, and toxicity.

There are various reasons for dental restoration and they may be as a result of the tooth type and the restorative material used in a prior application. Once used, various factors can cause restorative material to fail at different rates; This can be due

to the progressive decay of the tooth at surrounding of the filling material. Also, the failure of the material used in filling, effect of modifiers related to operator's skill, patient's dental features or history and the overall environs of the affected tooth [9]. The decision to fill a dental cavity may also be due to factors such as the dentist's understanding of the restoration's condition, the criteria used to define failure or in some cases, it may be the patient's demand.

An ideal dental composition contains a monomer and initiator that can be polymerized, also, an inorganic/organic filler and it is required to have adequate mechanical strength and hardness [10]. To serve as a replacement for natural teeth, other desirable requirement for dental fillers include resistance to wear against occlusion or teeth in any oral activity, good surface smoothness, color matching with natural teeth, transparency etc. In addition, the ideal dental composition must have good handling properties. It could come in form of paste, in this case, it is expected to have proper fluidity and high forming property. Especially, a kind of constitution to be filled directly in a cavity must have a viscosity low enough to be filled directly from a syringe. These desired properties are affected by the constituent materials, shape, and particle size of the filler used. It is also influenced by the composition of the filler.

Several studies have been carried out to investigate the effect of different dental fillers in teeth decay. [11] evaluated the influence of filling material and depth of filling on the strength and deformation of teeth. They modeled the teeth of a 28-year-old-man using 3D. Stress analysis was carried out on the model in three different states; normal tooth, amalgam filled tooth, and composite filled tooth. From their study, it was found that amalgam performed better as a dental filling material. Bovine bone replacements have been used mainly in maxillary sinus lifting and implant procedures because of their high stability and low immunogenicity. In some cases, a step-by-step annealing process treatment is carried on Bovine bone and then chemical treatment using NaOH. This is to form a permeable hydroxyapatite material made up of only the inorganic constituents of bovine bone. The formed permeable structure can provide required mechanical properties in a similar way to that of human bone and enhance bone healing [12]. In this paper, the suitability of bovine-bone as an alternative for dental filler was investigated

2. MATERIALS AND METHODS

The raw materials used for this research were sourced locally. Bovine bone was obtained from an abattoir located at Sabo, Lagos State, Nigeria. Water was boiled to 100°C and the bones were soaked for three days to allow dried flesh that remains on the bone to soften and be easily removed. Also, to remove all the dirt and oils from the bone. This cycle was repeated twice to ensure a thorough washing. The bones were properly scrubbed afterwards and were sun-dried for two weeks. This was the time when there was no moisture on the bone and constant weight was observed, which indicates that the drying is complete. They were then crushed and milled into powders using a sieving machine, the size of the powders was 300 μm or less. Preheating was performed at 100°C for two hours to remove all forms of moisture from the crushed bones. Subsequently, firing was from 600°C – 900°C and the holding time varied from two hours to four hours. These parameters were chosen after conducting a pre-test before the experiment.

2.1 Preparation of Simulated Body Fluid (Saliva)

The following chemical reagents were used to prepare the Simulated Body Fluid (Saliva),

a) Methyl Paraben ($C_8H_8O_3$)
b) Potassium Chloride (KCl)
c) Magnesium Chloride ($MgCl_2$)
d) Potassium Phosphate (K_3PO_4)
e) Sodium Fluoride (NaF)
f) Dextrose ($C_6H_{12}O_6$)
g) Albumin
h) Methyl cellulose

These chemicals were mixed in the proportions, as shown in Table 1, to achieve the desired result (artificial saliva) and a pH of 7. Preparation of SBF was adopted from the protocol developed by [13].

A 100 ml plastic container was used to store 90 ml of distilled water at room temperature. All the seven reagents were dissolved in the distilled water one after the other. The last reagent was dissolved to adjust the pH of the solution to 7.6. After pH adjustments, the solution was allowed to settle. Glass containers were avoided because apatite nucleation can be induced at the surface of a glass container of the edge of scratches. A plastic container with smooth surface was therefore chosen.

The solution was then poured into another plastic container with 20 ml, 5 g of bovine powder was then immersed into the solution and left for two and four weeks respectively. Since the pH value is dependent on the solubility of resorbability of the hydroxyapatite. When the pH decreases as the solubility increases, the change in pH value can be used as an indicator of the change in the solubility of hydroxyapatite.

Table 1: Composition of reagents used for SBF

S/N	Name of Chemical	Amount (%)	Amount (g)
1	Methyl Paraben	0.2	0.4
2	Potassium Chloride	0.062	0.124
3	Magnesium Chloride	0.005	0.01
4	Potassium Phosphate	0.034	0.068
5	Sodium Fluoride	0.01	0.02
6	Dextrose	4.69	9.38
7	Albumin	0.1	0.2
8	Methyl Cellulose	0.25	0.5

3. RESULTS AND DISCUSSION

3.1 XRF Result

The result of the chemical analysis for the Bovine powder sample carried out using X-ray fluorescence (XRF) machine is shown in Table 2 and acted as control sample.

Table 2: XRF result for the bovine bone- control sample

Element	Concentration (%)	Peak (Cps/Ma)	Background (Cps/Ma)
CaO	42.152	53903	< 7354
K_2O	<0.020	183	160
FeO	0.2416	411	20
Zn	0.0268	73	< 3
MnO	< 2.000	382	< 18
TiO_2	0.081	7	4
SiO_2	10.89	113	38
P_2O_5	< 60.00	1259	92
S	0.169	127	56
Al_2O_3	< 1.0	49	55
MgO	1.1	5	26
Na_2O	0.0072	5	3

The elemental composition of the Bovine Bone sample before calcination. The test was carried out to reveal the elements (oxides) present and their respective quantity present in the sample. As indicated in the result above, CaO is the most abundant compound (42.15%) in the sample, followed by P_2O_5. Other notable compounds to look out for are Na_2O (0.0072), MgO (1.1), and FeO (0.2416).

3.2 AAS Result

The result of the chemical analysis of the Bovine Bone sample (after calcination) and on the control sample (before calcination) were carried out on the Atomic Absorption Spectrometric machine (AAS as shown in Table 3). This test was carried out to determine the elemental composition of the sample and to examine the effect of calcination on the control sample. The calcium content of the sample increased after calcination and the highest Calcium content being evident in Sample 1 (600ºC at 2hrs) and Sample 6 (800ºC at 2hrs). Sodium increased in some cases and reduced in some. Magnesium

also increased after calcination, with sample 5 (700ºC at 4hrs) being the highest. Iron and Copper show very little variation after calcination.

AAS was also carried out on a composite Resin to compare the composition of what is used in dental clinics with the bovine bone sample. There is an obvious disparity, Sodium was as high as 4.35 ppm compared to Sample 4 (the highest Sodium concentration), which is only 0.77 ppm.

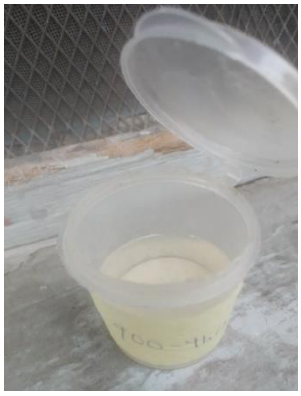
Table 3: AAS Result for Bovine Bone Sample

S/N	Sample	Na (ppm)	Mg (ppm)	Fe (ppm)	Cu (ppm)	Ca (ppm)
1	Control	0.4	1.57	0.01	0.02	0.16
2	600ºC 2hrs	0.41	1.47	0.01	0.02	0.49
3	600ºC 4hrs	0.554	1.82	0.02	0.02	0.39
4	700ºC 2hrs	0.77	2.13	0.01	0.02	0.46
5	700ºC 4hrs	0.41	2.17	0.02	0.05	0.37
6	800ºC 2hrs	0.36	1.66	0.03	0.04	0.49
7	800ºC 4hrs	0.65	1.6	0.04	0.05	0.45
8	900ºC 2hrs	0.28	1.61	0.05	0.13	0.35
9	900ºC 4hrs	0.32	1.8	0.03	0.14	0.29
10	Composite Resin	4.35	0.62	0.01	0	0.11

3.3 Simulated Body Fluid (Saliva) Test

Simulated Body Fluid test was carried out to simulate how the sample would behave in the mouth. The samples were soaked in the artificial saliva for two weeks and four weeks (Figure 1 (a) – (h)). The control sample was also soaked in SBF and it can be seen from Figure 1 (i) that the sample started decay after two weeks. This can be due to the oxides or the oils present in the sample.

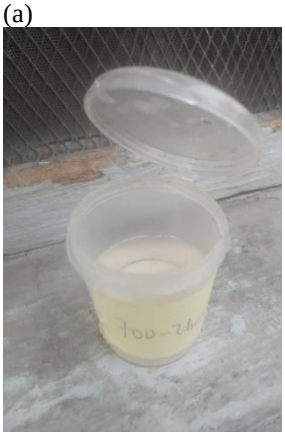
(a)



(b)

(c)

(d)



(e)

(f)

(g)

(h)

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(i)

Figure 1: (a) 900ºC 2hrs (b) 900ºC 4hrs (c) 800ºC 2hrs (d) 800ºC 4hrs (e) 700ºC 2hrs (f) 700ºC 4hrs (g) 600ºC 2hrs (h) 600ºC 4hrs (i) Control Sample

3.3 pH Value of SBF

Since the pH value is dependent on the solubility or resorbability of the hydroxyapatite, wherein the pH decreases as the solubility increases, the change in the pH value can be used as an indicator for the change in the solubility of hydroxyapatite. The change of the pH of the prepared samples was examined in SBF under room temperature using the pH meter and the results are presented in Table 4.

The pH values of our samples reduced to indicate the resorbability of hydroxyapatite. For the two weeks, the average percentage reduction is 2%, while the average percentage reduction for the 4 weeks is 1.68%.

Table 4: The Result of the pH of the prepared samples examined in SBF

Sample	pH value (2 weeks)	pH value (4 weeks)
Artificial Saliva	7.64	7.72
900ºC 2hrs	7.58	7.70
900ºC 4hrs	7.52	7.65
800ºC 2hrs	7.48	7.59
800ºC 4hrs	7.42	7.58
700ºC 2hrs	7.46	7.52
700ºC 4hrs	7.51	7.53
600ºC 2hrs	7.46	7.60
600ºC 4hrs	7.46	7.55

3.4 Micro Structural Analysis Result

SEM was used to investigate the microstructural analysis of the Bovine bone sample. Figures 1-8 represent the sample after calcination while Figure 9 is the sample before calcination. The dull appearance of Figure 9 is indicative of the oxides present in the sample. As the temperature of calcination increased, as shown from Figure 1 to Figure 8, it is apparent that the sample got clearer to indicate the effect of calcination. Comparing the sample between holding time, e.g. 600°C at two hours and four hours, it is evident that the particles increased in size and pore spaces were also reduced.

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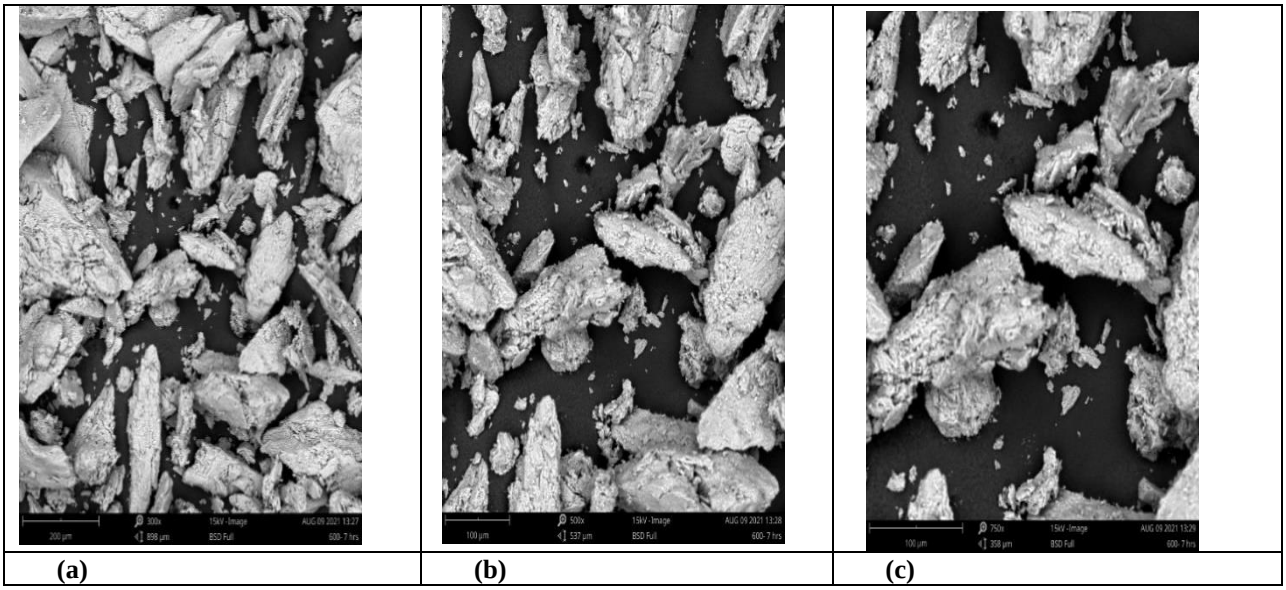
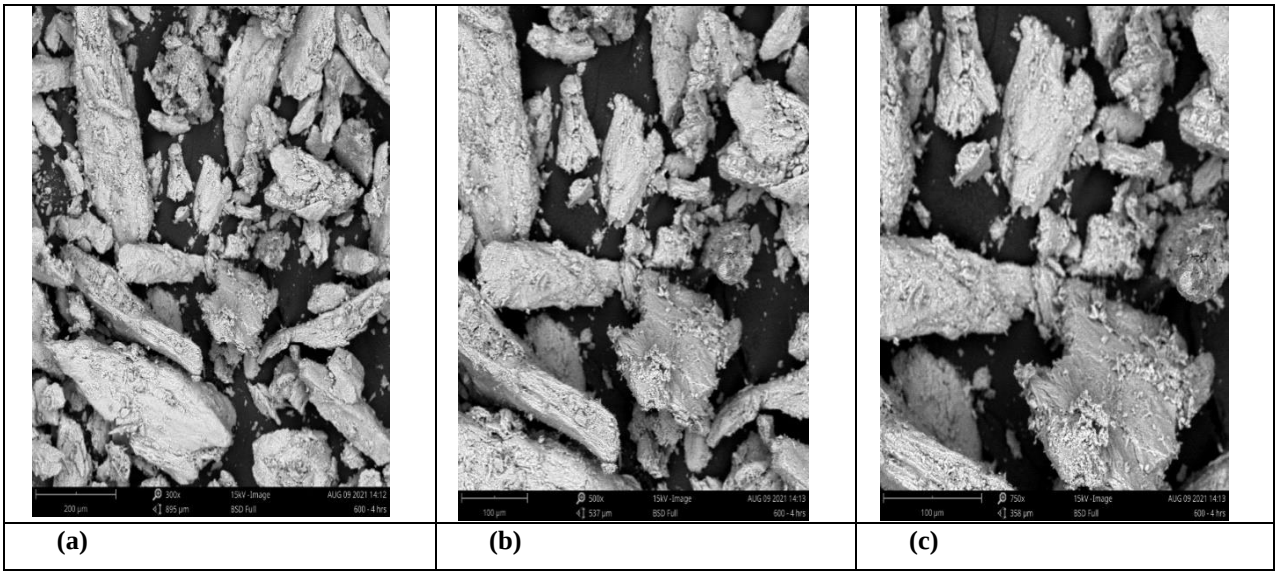


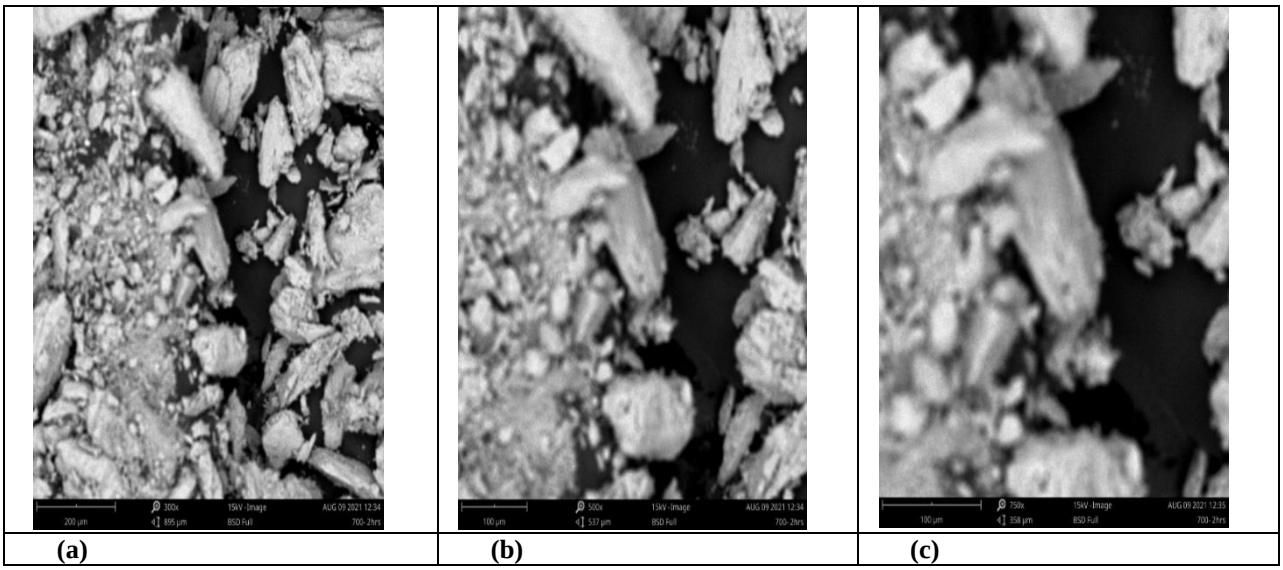
(a) **(b)** **(c)**

Figure 1: Microstructure of Bovine bone sample at 600°C. Holding time-2hrs: (a) 300X (b) 500X (c) 750X



(a) **(b)** **(c)**

Figure 2: Microstructure of Bovine bone sample at 600°C. Holding time-4hrs: (a) 300X (b) 500X (c) 750X



(a) **(b)** **(c)**

Figure 3: Microstructure of Bovine bone sample at 700°C. Holding time-2hrs: (a) 300X (b) 500X (c) 750X

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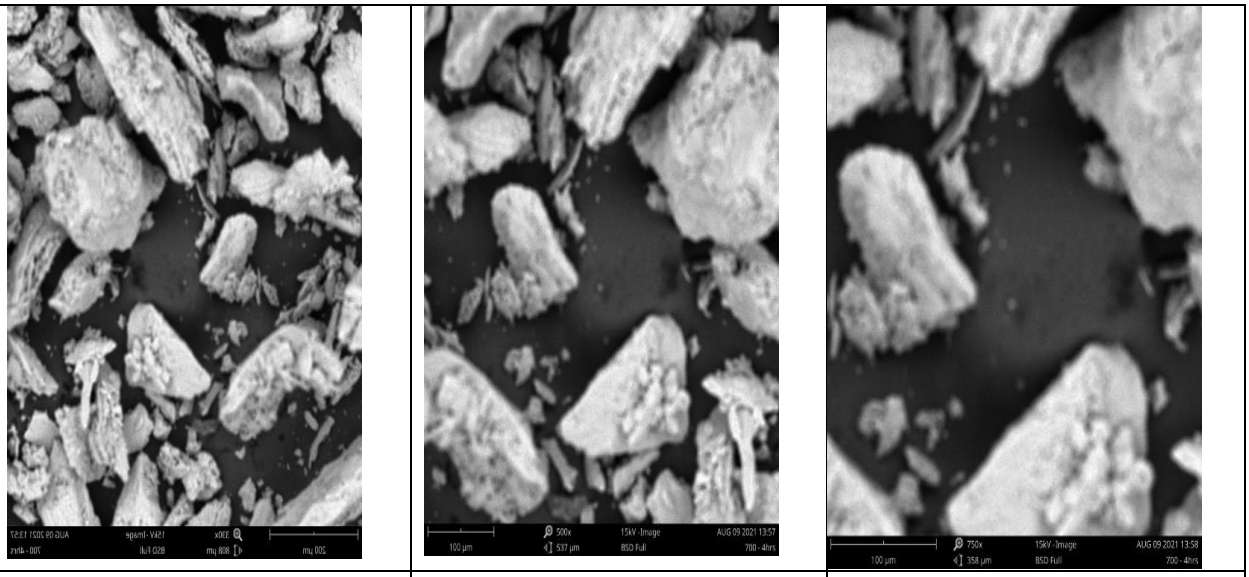
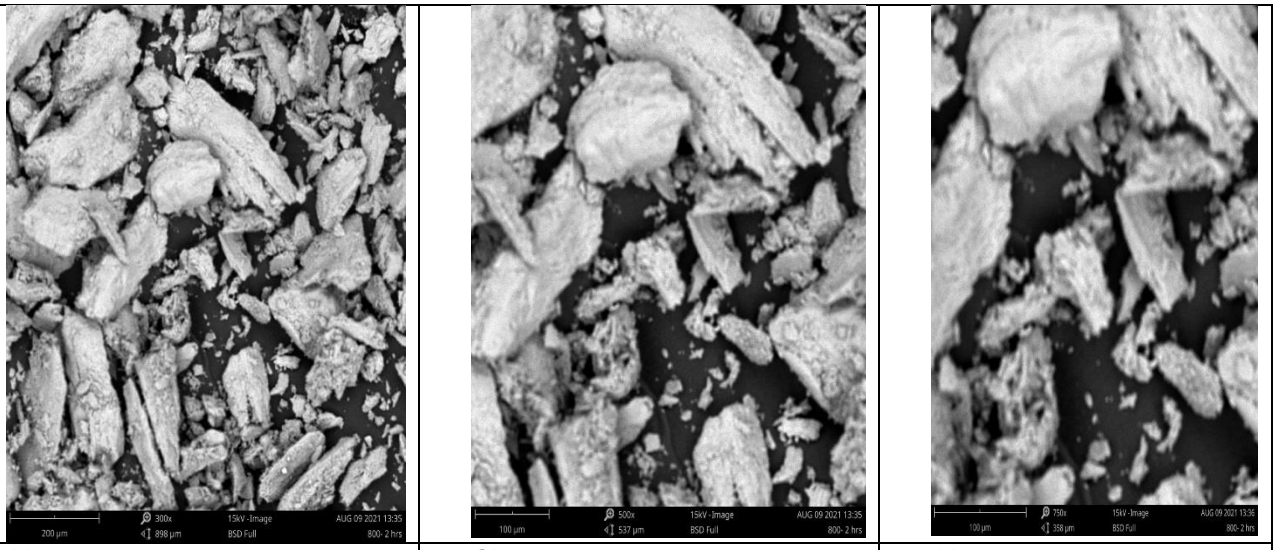


(a) **(b)** **(c)**

Figure 4: Microstructure of Bovine bone sample at 700°C. Holding time-4hrs: (a) 300X (b) 500X (c) 750X



(a) (b) (c)

Figure 5: Microstructure of Bovine bone sample at 800°C. Holding time-2hrs: (a) 300X (b) 500X (c) 750X

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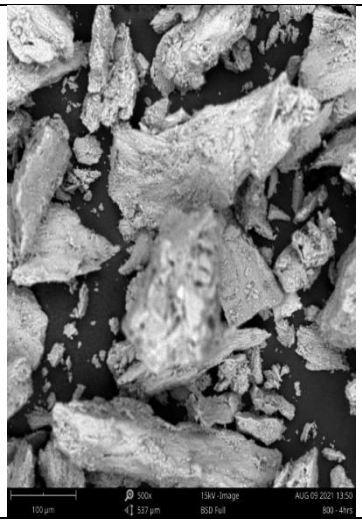
(a)


(b)

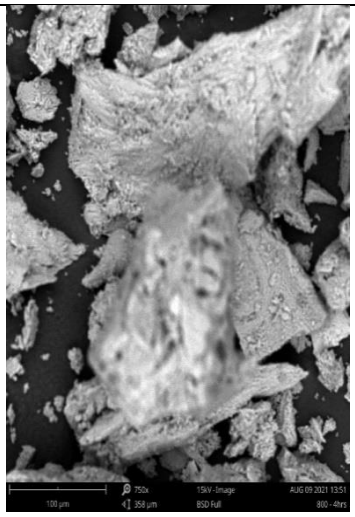

(c)

Figure 6: Microstructure of Bovine bone sample at 800°C. Holding time-4hrs: (a) 300X (b) 500X (c) 750X


(a)


(b)

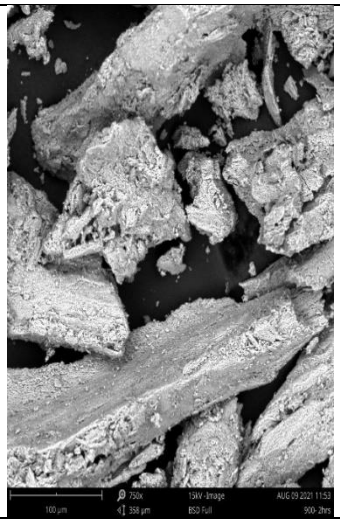
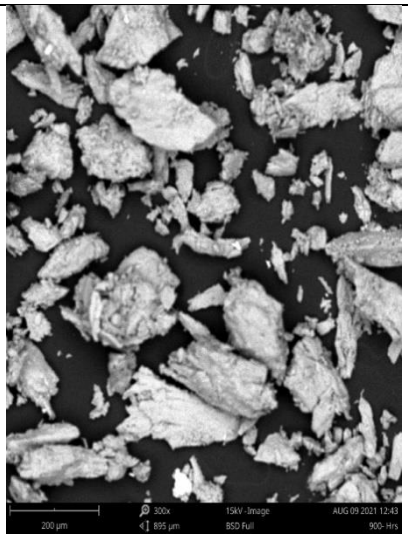
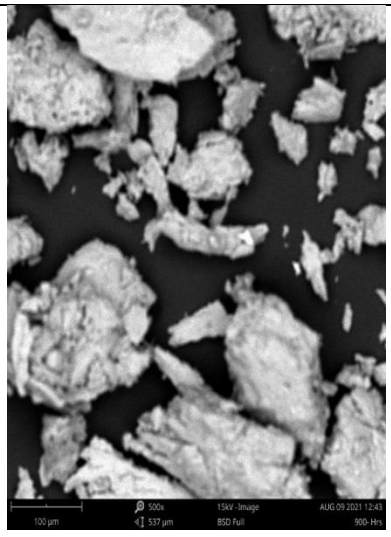

(c)

Figure 7: Microstructure of Bovine bone sample at 900°C. Holding time-2hrs: (a) 300X (b) 500X (c) 750X


(a)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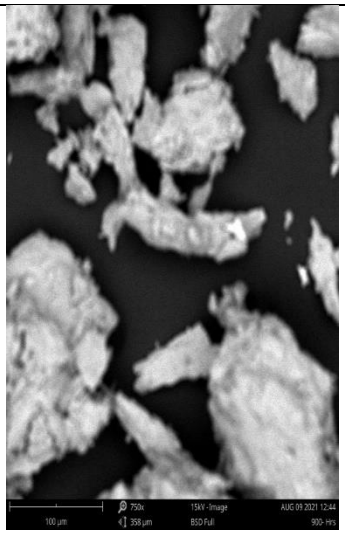
(c)

Figure 8: Microstructure of Bovine bone sample at 900°C. Holding time-4hrs: (a) 300X (b) 500X (c) 750X

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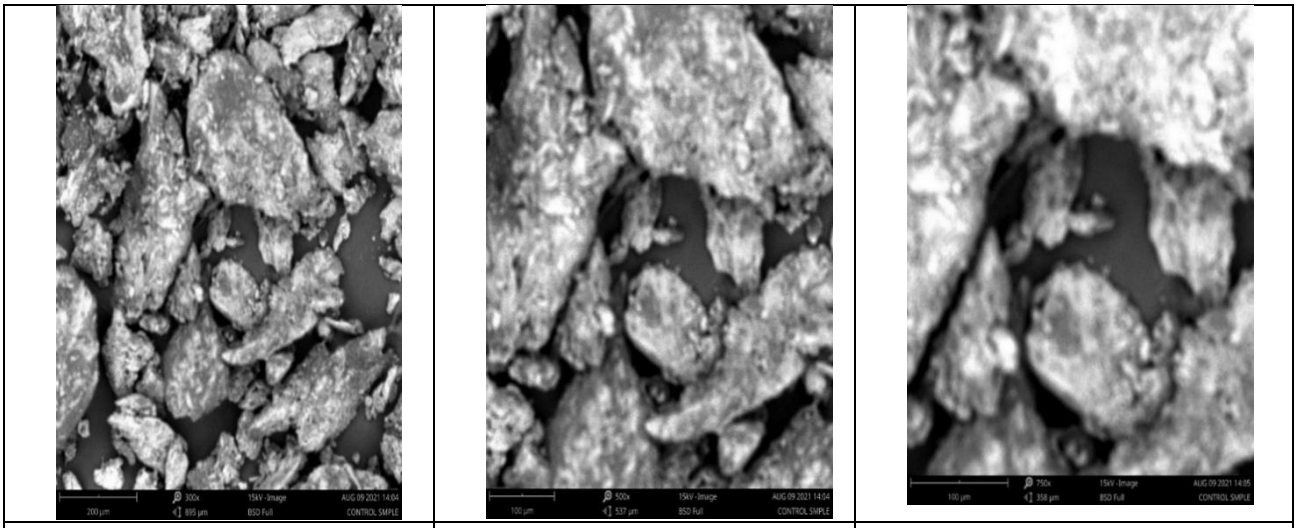

(a) (b) (c)

Figure 9: Microstructure of Bovine bone control sample: (a) 300X (b) 500X (c) 750X

4. CONCLUSION

The result of the AAS showed that, the samples increased in elemental composition with the optimum being Sample 4, fired at 700°C for two hours. After soaking the sample in Simulated Body Fluid (SBF) for two weeks and four weeks respectively, there was no proof of decay in the sample. This is to show that the sample can survive in the mouth environment without any major alteration.

There was a slight decrease in the pH value to indicate an increase in solubility of hydroxyapatite. The average percentage reduction for two weeks is 2% while the one for four weeks is 1.68%.

The disparity in clarity displayed in the SEM results show that the higher the temperature and holding time, the clearer the sample becomes to indicate the removal of oxides and increase in calcium. The SEM results also support the claim that calcium increased after calcination. Also, there was an increase in particle size and decrease in pore spaces.

Therefore, from the above properties revealed through the results, it has been established that an alternative dental filler that can compete favourably with the conventional ones can be produced from Bovine bone calcined at 700ºC for two hours.

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