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PREPARATION AND ACELLULAR IN-VITRO BIOACTIVITY OF SOLID STATE SINTERED 45S5 BIOACTIVE CERAMICS USING BIO-WASTES AS ALTERNATIVE RESOURCES FOR BIOMEDICAL APPLICATIONS

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Abstract

In this research, rice husk ash (RHA) and eggshell ash (EGA) were used as biogenic materials for total replacement of pure quartz (SiO_2) and calcium oxide (CaO) respectively in the traditional 45S5 bioactive glass composition by powder metallurgy route. Body formulation with nominal composition 45% RHA (SiO_2), 24.5 EGA (CaO), 24.5% Na_2O and 6% P_2O_5 was composed. The batch material was properly mixed with addition of 2% PVA (Polyvinyl alcohol) as binder and compacted at 70 MPa to produce compact samples of 40 x 20 mm. The samples were then allowed to dry in an ambient temperature followed by sintering at 1000°C for 2 h, then allowed to cool to room temperature. Selected samples were immersed inside prepared simulated body fluid (SBF – pH 7.4) at 37 °C for 5, 9, and 18h respectively. Physical, microstructure and phase evaluation were conducted to examine the developed bio-ceramic. The results showed the bio-waste based 45S5 bioceramic has bulk density and porosity of 1.02 g/cm^3 and 62% respectively while deposits of carbonate-hydroxyapatite were found to increase with immersion period showing good bioactivity and affirm that the developed bio-waste based bioceramics are bioactive and can find suitable application bone repair.

Keywords: *Deep beams; RC; strengthening; GFRP; web openings*

Introduction

Over the years, several bioactive ceramics and glasses have been widely studied to repair diseased or damaged hard tissue in the medical field as a result of their compositional resemblance to bone minerals and greater biomineralization characteristics [1, 2]. Bioactive ceramics which are mainly a family of calcium phosphate based such as hydroxyapatite, tricalcium phosphate and biphasic calcium phosphate have been widely used to improve bone formation, integration and osteoconduction [3] while bioactive glasses such as 45S5 bioglass, phosphate-based glasses and borate-based glasses are effective for strong bone integration and osteogenic properties [4].

The recent high cost of health care as well as high demand for biomaterials have placed a great pressure on government funding agencies and researchers to develop cost-effective and suitable biomaterials for tissue regeneration. One of the approaches to meet this task is through waste utilization as alternative materials to some of the expensive traditional materials used in developing these biomaterials [5].

Globally, there has been an increasing concern on the escalation of both organic and inorganic wastes accumulation arising from increase in human population coupled with their lifestyle. Among these wastes, chicken eggshells and rice husks are prominent bio-wastes, which are of

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environmental concern [6, 7]. There is therefore needed to harness these wastes to serve new and useful purposes by incorporating them into the development of biomaterials like bioactive ceramics. It has been reported that silica present in rice husk ash (RHA) exhibited better biocompatibility over the synthetic form of silica [8] while chicken eggshells has been proven to be high in CaO upon thermal calcination with other biologically beneficial ions [9] and has potential efficacy to act as an osteoconductive bone substitute [10].

Therefore, exploring the use of these bio-wastes which are renewable, sustainable, cheaper, easily accessible and biologically compatible as alternate materials to synthetic materials to develop 45S5 bioactive ceramics locally for biomedical applications will not only reduce overall cost of production but also impart tremendous beneficial effects biologically. The use of these bio-wastes will also make this bioactive material readily available and cost-effective for a developing country.

Few investigations have been conducted and reported on the use of rice husk ash (RHA) and eggshell ash (EGA) as bio-waste materials to substitute for commercial silica (SiO_2) and lime (CaO) respectively, for the synthesis of 45S5 bioglasses and glass-ceramics.

Most recently, Owoeye et al. [11] investigated fabrication and characterization of 45S5 bioglass-ceramics using RHA and EGA as resources by microwave assisted melting and sintering. Their results showed that the use of these bio-wastes tremendously improved the bio-mechanical properties of the developed bioactive product. Although melting and subsequent sintering was achieved using microwave, but the two-way process used still add to the cost and energy requirement of producing the bioglass-ceramics

The effects of sintering temperature on the properties of 45S5 bioglass-ceramics using rice husk ash as bio-alternative to commercial silica was also studied by Leenakul et al. [12]. In their report, it was affirmed that the use of the rice husk ash improved mechanical property and the formation of hydroxyapatite when the samples were placed in simulated body fluid. However, all the thermal parameters of the samples containing rice husk ash were lower than that found in sample containing commercial silica. This had significant influence on the phase transformation and morphology of the sintered bioglass-ceramics.

Yucel et al. [13] in their investigation on preparation of melt-derived 45S5 bioglass using rice hulls as alternative resource, reported that bioglass containing rice hull ash showed good bioactivity in vitro and affirmed that it can be used as an alternative to commercial silica.

Mattu and Singh [14] studied structurally modified bioglasses produced from agro-food wastes (rice husk ash and eggshells) and conventional sources in a hybrid formulation for bone regeneration applications. In their study, both biowastes and conventional chemicals were used to prepare bioglasses. It was affirmed based on the promising results obtained that biowastes can be used as resources for producing bioglasses without impeding their bioactivity. However, they could not achieve 1.67 standard stoichiometry ratio of Ca/P for bioglasses containing biowastes due to other trace elements contained in biowastes resulting in heterogeneity in the hydroxyapatite (HAp) layer formation.

Sharma et al. [15] in their study also investigated the optical and thermal properties of glasses and glass-ceramics derived from agricultural wastes. Ashes of rice husk and sugar cane leaves were used, respectively, as resources, while the melt-quenching approach was used to obtain glasses, then heated-treated to obtain glass-ceramics. It was reported that the use of agro-waste ashes increased the tendency for glass formation. However, it was reported that samples containing higher content of rice husk ash (RHA) could not form glasses and will require higher temperature for proper melting to form glass. This is attributed to higher silica content inherent in the RHA.

Adams et al. [16] also explored bioactive glass 45S5 from diatom biosilica using the sol-gel technique followed by sintering at 950^{o}C for 3 hours. In their report, diatom biosilica from cultured cells of diatom Aulacoseira granulate was used as a substitute for expensive TEOS (tetraethyl orthosilicate). Their results showed the developed 45S5 bioactive glass from diatom biosilica showed considerable compression strength and good bioactivity, with the formation of carbonated hydroxyapatite (HCA) after incubation in SBF attributed to good surface chemistry. They further affirmed from the results obtained that diatom biosilica could be a potential economically friendly starting material for large-scale fabrication of bioactive glasses.

Araujo et al. [17] also investigated the in vitro bioactivity and antibacterial capacity of 45S5 bioglass-based compositions containing alumina and strontium. In their study, a modified 45S5 bioglass containing 2 mol% alumina and 2 mol% strontium was prepared. Their samples were then immersed in simulated body fluid for hydroxyapatite formation, while cytotoxicity was also performed using the NCTC clone 929 cell line. The results showed that the bioactive glass demonstrated safe disinfection for E. Coli and good apatite deposition. For these reasons, the samples were considered a promising alternative for the reconstruction of bone defects and the treatment of bone infections.

Palakurthy et al. [18] investigated a cost effective SiO_2 – CaO – Na_2O bioglass derived from bio-waste resources for biomedical application. In their study, SiO_2 – CaO – Na_2O bioglass was successfully synthesized through melt-quenching, utilizing RHA and eggshells as bio-wastes. It was reported that the bio-waste based bioglass showed great bioactivity with the formation of hydroxyapatite (HA) and the cytocompatibility results showed no toxicity. Their study however established demonstrated RHA and eggshells are beneficial low-cost substitute resources for CaO and SiO_2 respectively to produce bioglasses.

While all these previous studies are focused on the use of melt-quenching followed by crystallization sintering to produce the bioceramics from the initial bioglass, there is need to employ a less cumbersome and cheaper route of solid-state sintering to produce 45S5 bio-ceramics without altering the necessary properties needed for biomedical applications. Hence, the need for this present work.

In this regard, the present work aimed to develop bio-wastes based 45S5 bioactive ceramics using both eggshell ash and rice husk ash as materials by solid state sintering approach.

Material and Methods

Materials

The starting materials used for this work are rice husks and eggshell wastes from rice mill and poultry respectively, anhydrous sodium carbonate (Na_2CO_3, 99.8% purity), ammonium di-hydrogen orthophosphate ($NH_4H_2PO_4$, 99.9 % purity) as precursor for P_2O_5, and Polyvinyl Alcohol (PVA) granule as binder.

Preparation of rice husk ash (RHA) and eggshell ash (EGA)

Combustion method inside furnace was used for the preparation of silica from rice husks in form of rice husk ash. The as-received rice husks was rinsed thoroughly with water to remove adhered sand and dirt that can interfere with the purity of the ash followed by oven dried at 110^{o}C, then subjected to heating inside muffle furnace at 1000^{o}C for 2 hours to obtain white RHA known to be highly rich in silica. In the same vein, as-received chicken eggshells to be used as bio-source of calcium oxide (CaO) was collected from poultry and boiled inside in water at 100^{o}C for about 20 minutes in order to properly remove attached membrane and collagen. It is later rinsed thoroughly with water followed by drying in an oven at 110^{o}C. The dried eggshells was then calcined in an electric muffle furnace at 1000^{o}C for about 2 hours to transform the eggshells to eggshell ash (EGA) which is rich in calcium oxide.

Sample preparation

A batch of 200g was composed comprising the starting materials formulated in Table 1. The materials were weighed and poured into the porcelain-lined mortar and pestle and thoroughly mixed for several minutes to ensure homogeneity of the material. After mixing, it was poured into a porcelain lined mixer to mix the materials properly with gradual addition of prepared 2% PVA solution and blended thoroughly to ensure a uniform batch. The batch material was poured into a cylindrical polymeric mould of 40 mm x 20 mm and was pressed at a pressure of 70MPa using hydraulic powder pressing machine. The samples were then allowed to dry in an ambient temperature followed by sintering at 1000^0C for 2 h at the rate of 5^0C/min. The samples were then allowed to cool within the furnace.

Table 1. Batch Composition (wt. %)

Samples	SiO_2	CaO	Na_2O	P_2O_5	EGA	RHA
BGC_W	-	-	24.5	6	24.5	45

Preparation of simulated body fluid (SBF)/in vitro bioactivity test

Corrected SBF solution was prepared by dissolving chemical-grade reagent in accordance to Kokubo and Takadama [19]. Selected samples were then immersed in the SBF solution placed inside a plastic beaker. The plastic beaker was then placed inside the water bath whilst the temperature is maintained at 37°C for varying soaking time of 5, 9 and 18 h respectively. After each period, the respective sample was evacuated and dropped inside acetone for 5 seconds to stop the surface mineralization followed by drying in a desiccator. The samples were afterward analysed by scanning electron microscopy and X-ray diffraction to assess its bioactivity by assessment of CHA/HA (carbonate-hydroxyapatite) phase deposited.

Characterization

The microstructure of the sintered bioceramic samples were characterized using scanning electron microscope (JSM-6100 JEOL, Japan) while X-ray diffractometer (Rigaku miniflex 600, Japan) was used to investigate the crystalline phases after sintering and evolution of the hydroxyapatite layer respectively after in-vitro test Other tests carried out include bulk density and porosity.

Results and Discussion

Morphology

Fig. 1 (a – d) show the representative morphologies of the as-sintered BGC_W and the SBF immersed counterparts for 5 (BC_WSBF5hrs), 9 (BC_WSBF9hrs) and 18 hrs (BC_WSBF18hrs) respectively. It can be observed that the as-sintered BGC_W samples before and after immersion in SBF showed a somewhat dense bone-like microstructure containing varying macro and several micropores on the surface. The dark portions of the morphology indicate the BGCs matrix as also reported by Aswad et al. [20].

However, samples BC_WSBF5hrs, BC_WSBF9hrs and BC_WSBF18hrs respectively showed a contrast morphology as observed for as-sintered sample prior to immersion as their surfaces are well covered with somewhat whitish layers deposition. These deposited whitish layers are attributed to the formation of HA (hydroxyapatite) layers deposited due to good bioactivity reaction during immersion in SBF solution [21]. It is also observed that the HA layer became more compact and higher with an increase in immersion period; thus, indicating good bioactivity reaction in SBF.

(a) (b)

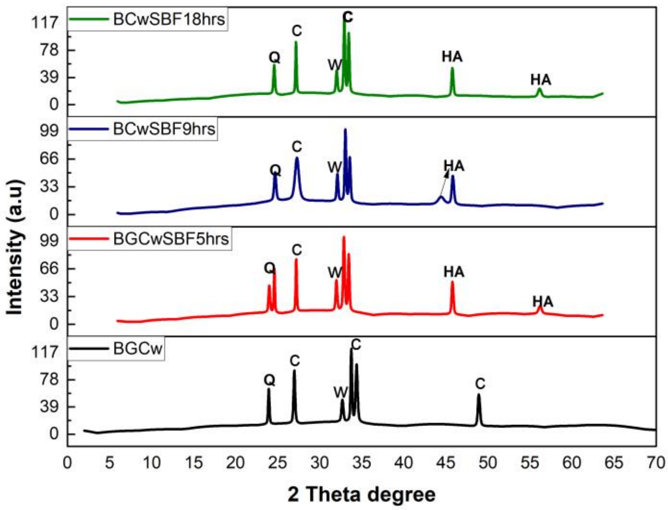
(c) (d)

Fig. 1. SEM images of: a) BGC_W; b) BC_WSBF5hrs; c) BC_WSBF9hrs; d) BC_WSBF18hrs

Phase analysis

Fig. 2 shows the representative phase evaluation of the BGC_W before and after immersion in SBF for 5, 9 and 18 h respectively. For the BGC_W, it can be observed that the XRD pattern of the bioactive ceramics indicates the presence of crystalline phases. These crystalline phases has been attributed to the presence of silicate and phosphate network as well as the possible phase separation of the two phases during sintering [22]. It is observed that the main and prevalent crystalline phase present is Combeite (sodium-calcium-silicates, $Na_2Ca_2Si_3O_9$), while other phases such as wollastonite and unreacted quartz (SiO_2) were also observed. These crystalline phases are in agreement with previous studies [23, 24] and have been affirmed to be the major determinant of any bioactive glass and glass ceramic material to be considered a bioactive material either for dental or bone regeneration.

For the samples immersed in SBF, in contrast to the phases observed before immersion in SBF solution, there is presence or formation of hydroxyapatite (HA). The presence of HA on the samples after immersion in SBF showed good bioactivity in-vitro and indicated bone bonding ability when used in-vivo. Similar results have been reported by previous studies [24, 25].



C – Combeite, W – Wollastonite, Q – Quartz, HA – Hydroxyapatite

Fig. 2. XRD spectra of (a) BGC_W (b) BC_WSBF5hrs (c) BC_WSBF9hrs (d) BC_WSBF18hrs

Conclusions

This present work has successfully dealt with development and characterization of bio-wastes based 45S5 bioceramics for intended application as biomedical material either for dental or bone regeneration. The following conclusions can be drawn within the limit of this work:

The RHA and EGA showed good potential to be utilized as bio-alternative source to commercial quartz (SiO_2) and calcium oxide (CaO) respectively in the bio-wastes based bio-ceramics.

The presence of HA after immersion in SBF showed good bone bonding potential. This gets stronger as the immersion period increases as depicted by the SEM micrograph.

The results of the phase evolution showed combeite as the major and prevalent phase while other phases such as wollastonite and quartz were also present in the control sample (BGC_W). However, after immersion in SBF, the emergence of HA peaks is obtained.

Future directions and limitations

After confirming promising in vitro bioactivity, further directions include conducting in vivo studies using animal models to evaluate bone formation, biocompatibility and long-term functionality. With positive in vivo results, progress towards clinical trials to assess safety and efficacy in humans shall be explored.

While promising, this research also faces some limitations such as unpredictable nature of biowastes as regard their composition variation and cost-effectiveness for widespread adoption. Also, long-term biocompatibility and potential degradation of bioceramics derived from biowastes need further investigations.

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