American Journal of Engineering Research (AJER)
e-ISSN: 2320-0847 p-ISSN : 2320-0936
Volume-9, Issue-4, pp-166-170
www.ajer.org
Research Paper Open Access

Synthesis of Natural Hydroxyapatite from Fish Scales and Its Potential Application as Fillers in Dental Composites

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ABSTRACT :*Natural hydroxyapatite was produced from fish scales using thermal degradation method. The fish scales ash was ball milled and spray dried to produce fish scale hydroxyapatite (FsHAp) powder. The particle size of FsHAp powder was analysed using Mastersizer 2000. The X-ray diffraction (XRD) and Fourier transform infrared (FTIR) were used to verify the presence of natural hydroxyapatite. The effect of FsHA and Silica reinforcing fillers in dental resin-based composite (RBCs) was investigated. The composites were prepared by mixing 80 to 100 wt% FsHA and 0 to 20 wt% silica into dental resin. The resins used were mixing of bisphenol A glycidyl methacrylate (BisGMA)/triethylene glycol dimethacrylate (TEGDMA)/hydroxyethyl methacrylate (HEMA) in the ratio of 50:25:25 wt%. The ratio of fillers and organic resins were fixed at 70:30 wt%. The composites were prepared by mixing process and cured using UV light for 60 second. The flexural and compressive strength, Vickers hardness and cytotoxicity test were evaluated. The surface morphology and fillers distribution in composites were observed using field emission scanning electron microscope (FESEM). FTIR and XRD analyses reveals the prominent peaks corresponding to high crystallization of FsHAp from fish scales. The results indicated that the composite with 85 wt% of FsHA and 15 wt% of silica filler exhibited satisfactory mechanical and physical properties value of flexural strength (42.74 MPa), compressive strength (174.28 MPa) and Vickers hardness (43.7 HV). Cytotoxicity test demonstrated the composites were non-toxic. The overall results were met the standard requirement of dental composite, thus the composites developed become a promising material for dental filling application.*
KEYWORDS*fish scale, hydroxyapatite, dental composite*

Date of Submission: 31-03-2020 Date of acceptance: 18-04-2020

I. INTRODUCTION

Hydroxyapatite (HAp) widely used as bone implants and dental restoration [1] due to their excellent biological properties and similar chemical structure to bones and teeth. HAp is commonly synthesized through chemicals reaction such as chemical precipitation method [2]. However, due to high manufacturing cost of synthetic HAp, thus researchers have focused on natural resources to produce inexpensive HAp such as animal bones and fish scales and bones [3]. However due to animal diseases, natural resources such as fish scales become the best alternatives source of HAp [4]. Fish scale was reported to contain around 50 wt% of organic and inorganic components known as collagen and HAp [5]. Direct burning method is among the easiest and cheapest method to extract natural HAp from fish scales. In this method, fish scales were burnt in furnace at about 1000 ^{o}C to remove organic composition whereas HAp is remained as ash [6].

Dental resin-based composites (RBC) frequently used in dentistry as restorative material to repair decayed or damaged teeth due to their acceptable mechanical properties, superior aesthetics and the ability to bond to tooth tissues [7,8] as compared to amalgams and ceramics [7]. However, the major drawback of dental composites is insufficient mechanical strength especially in load-bearing areas due to polymerisation shrinkage. [7]. Efford have been made to improve the performance of the composite by modified resin and applications of suitable fillers. Due to aesthetic demand of the dental composites, hydroxyapatite is the best choice. The RBCs that meet this demand are generally made up of organic matrix and inorganic filler particles. The polymer matrix is the main organic component consists of monomers Bis-GMA, TEGDMA and HEMA while silica are main inorganic filler to improve the mechanical and physical properties of resin-based composites. Fillers can

be used to reduce polymerisation shrinkage with the purpose of enhancing mechanical properties and its clinical applications.

This study focus on the effect of fish scales hydroxyapatite (FsHAp) and silica fillers on the mechanical and biological properties of dental composite resin. It is expected that the silica particles not only fill in the gaps between HA particles but also occupied the empty spaces between the organic resins, subsequently enhance the packing density and further improve the mechanical properties of the composite [9].

II. MATERIAL AND METHODS

Tilapia's fish scale (Fs) was collected from wet market at Tanjong Malim, Perak. The Fs was washed, cleaned and dried in oven. The Fs were heated in furnace for 2 hours at 800 oC and then 1200 °C for 2 hours. The white fish scales ash obtained was collected and wet grinded using ball milling for 72 hours. The chemicals for organic matrix are bisphenol A glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), camphorquinone (CQ), Ethyl 4-(dimethylamino) benzoate (EDMAB) purchased from Sigma Aldrich. Silica (SiO2) powder (particle size 4.966 µm) manufactured from Scharlab S.L. All the reagents were used without further purification.

The dental composites (DC) were prepared by mixing the organic matrix and fillers in a total mass ratio of 30:70 wt%. The monomers of Bis-GMA, TEGDMA, HEMA were mixed in different ratio; 50:25:25 wt% as shown in Table 1. This mass ratio was selected because it gives the most suitable mechanical properties for composites [10]. After an hour, the mixture was kept in a dark room. Then, 0.5 wt% CQ as an initiator and 0.5 wt% EDMAB as an accelerator were added and stirred for another 12 hours in a dark ambience.

The ratio of FsHAp and silica as stated in Tables 1. The mixture were added as inorganic fillers to the prepared resin mixture and stirred thoroughly for six hours to obtain homogeneous composite. The composites were placed in the mould and pressed between a pair of glass slides. The sample was photo-cured using UV-light (Bluephase N® MC) on both side for 60 s. The specimen was removed from the mould and polished using sand paper. In this study four different ratios of hydroxyapatite/silica particles were prepared to study the fillers effect on mechanical properties. The sample code and composition of the composites was listed in Table 1.

Table 1. Ratios of resin-based composites

Composite	Organic matrix (30 wt%)			Inorganic filler (70 wt%)	
	Bis-GMA (wt%)	TEGDMA (wt%)	HEMA (wt%)	FsHAp (wt%)	SiO_2 (wt%)
DC-80	50	25	25	80	20
DC-85	50	25	25	85	15
DC-95	50	25	25	95	5
DC-100	50	25	25	100	0

The sample of FsHAp slurry was collected after milled for 48 hrs and characterized for particle size analysis using Mastersizer 2000 (Malvern Analytical Ltd, UK). The FsHAp was characterised using X-ray diffraction (XRD) and Fourier transform infrared (FTIR). The mechanical properties such as flexural and compressive strength were carried out using universal testing machine (Shimadzu Universal Testing Machine, Japan) according to ISO 4049. The hardness of the sample was tested using Vickers hardness tester. Cytotoxicity test was done on the DC-85 sample due to their best mechanical properties.This test was assessed based on the ISO 10993 specifications in order to determine the biological response of L929 mouse subcutaneous connective tissue fibroblast cells (Mus musculus, NCTC clone 929, CCL-1TM) (American Type Culture Collection - ATCC).

III. RESULTS AND DISCUSSION

The results of particle size analysis of FsHAp slurry is shown in Fig.1. The results show bimodal distribution of particle size with average was determined around 1.859 µm. During milling process, particle breakage happened at initial stage of milling then equilibrium achieved between re-agglomeration (due to excessive surface energy that accumulate on small particle) and de-agglomeration [10]. From this research, the particle breakage occurred up to 28 hours milling time and achieved equilibrium at 48 hours of milling time as proved by the smallest mean particle size.

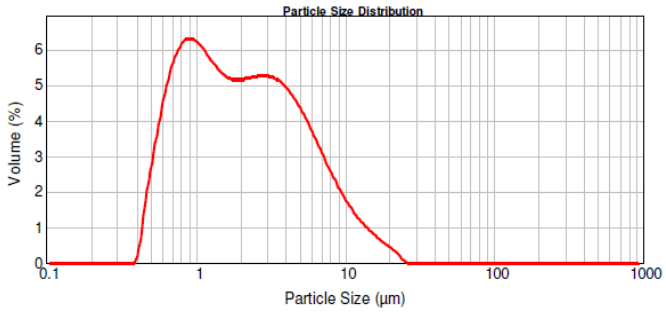

Fig. 1. Bimodal particle size distribution of FsHAp slurry after 48 hours milling time.

Fig. 1 shows FTIR spectrum and XRD pattern of FsHAp powder. From Fig.1(a), the sharp peak appeared at 3569 cm^{-1} which correspond to OH group from FsHAp. Peaks in the region of 472 cm^{-1}, 569 cm^{-1}, 601 cm^{-1} ,632 cm^{-1}, 1046 cm^{-1} and 1091 cm^{-1} were correspond to phosphate groups [5]. The FTIR result observed a typical FTIR spectrum for highly crystalline HAp as reported by several researches [11]. XRD pattern for FSHAp powder is shown in Fig. 1(b). The sharp peaks indicated high crystallinity of FsHAp was produced [12]. The prominent peaks related to HAp were observed, very similar to standard hydroxyapatite peaks correspond to the planar (211), (300) and (112). Similar results have been reported by the previous study [11]. It is interesting to note that the presence of β-tri-calcium phosphate (β-$Ca_3(PO_4)_2$, β-TCP) as secondary phases was observed at peaks (210), (214) and (220) located at 2θ angle 31.76 °, 27.97 ° and 34.59 °. The β-TCP phase present as a result of decomposition of the FsHAp at high temperature (1200 °C) [12]. However, this phase was not observed from other natural HAp [11]. Both analyses i.e FTIR and XRD were confirmed that natural hydroxyapatite was successfully produced from thermal degradation of fish scales in this study.

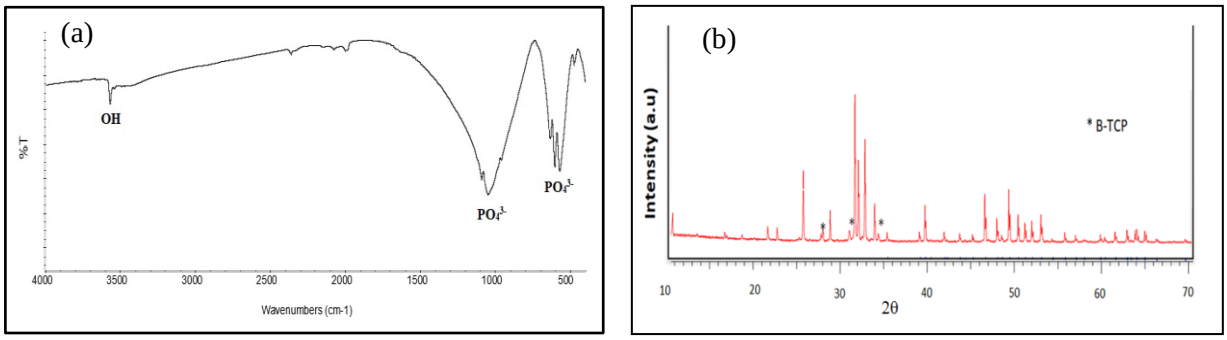

Fig. 2. (a) FTIR spectrum and (b) XRD pattern of FsHAp powder

Figs. 3a and 3b showed the flexural and compression strength results respectively for different composition of FsHAp and Si of DC composites. Both results show a similar trend where the flexural and compression strength were improved with the addition of FsHAp up to 85wt% but decreased as the FsHAp increased to above 85 wt%. The maximum flexural and compression strength of the DC composite was 36.2 MPa and 174.3 MPa respectively. It can be observed that increasing of Si content increased the flexural and compression strength. This finding is in agreement with the results of Liu et al. [7]. In addition, Samuel et al. [13] stated that the incorporation of microsilica tend to improve the abrasion resistance, hydrolytic stability, and shrinkage of restorative composite resins. However, the incorporation of 95wt% FsHA and 5 wt% Si resulted in the decrease of flexural strength and compression strength due to the presence of small voids indicating that less filler occupied in the organic matrix which responsible for the reduction of flexural strength value.

Fractured surface morphology of the composites is shown in Figure 4. When the composite with 85 wt% of FsHA and 15 wt% of silica, the filler were observed to be well-dispersed within the organic matrix and to have good interfacial adhesion to the matrix. However, the appearance of voids were clearly observed when the silica content was 20 wt.% (DC-80). The presence of voids might be due to the air entrapment during mixing the inorganic fillers and organic resins of the sample. Hyun et al. [14] stated that the homogeneity of the sample during mixing affects the morphology.

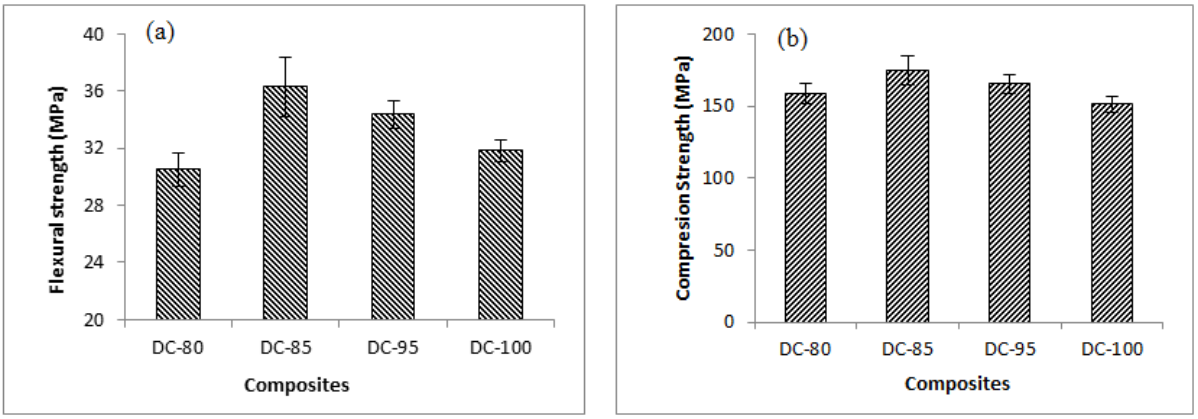

Fig. 3. Mechanical properties of DC dental composites. (a) flexural strength and (b) compressive strength.

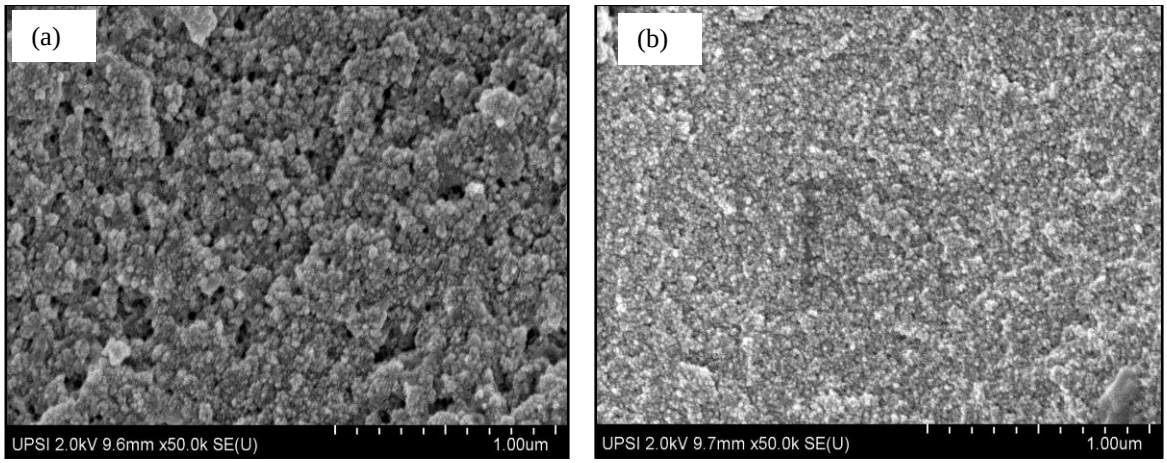

Fig. 4. SEM images of DC dental composites for (a) DC-80 and (b) DC-85 composites.

The Vickers hardness of composites is shown in Fig.5. The result show gradual decreased as the FsHAp increased. This results indicated that Si content play a major roles in hardened the dental composites as compared to FsHAp. The highest Vickers harness is shown in DC-80 composites (51.9 HV) followed by DC-85, DC-95 and DC-100 (34.8 HV). The Vickers hardness of human dentin is reported between 53 and 63HV [15]. Previous studies have reported that hardness of the composite increases when silica content increases to above 10% v/v [16]. In this study, the values obtained were very close to the reported value, especially for DC-85 composites.

One-way ANOVA exposed there was no statistically significant differences between mean OD values in concentrations of the composite ($p>0.05$). Multiple comparisons exposed that the mean concentration of 25 (0.729 ± 0.05 nm, $p=0.998$), 50 (0.667 ± 0.07 nm, $p=0.697$), 100 (0.697 ± 0.04 nm, $p=0.983$) and 200 mg/ml (0.706 ± 0.04 nm, $p=0.999$) had no significant differences in mean OD values with negative control group (0.717 nm). However, only 25 mg/ml concentration exhibit slightly higher than the negative control group. It can be seen that the OD values of DC-85 specimen at different concentration was very close to that of the negative control group. Based on Fig. 5, the percentages of cell viability in DC-85 concentration of 25, 50, 100, 200 mg/ml (101.7, 93, 97.2 and 98.5%) was also closed to the percentages of cell viability in negative control group (100%). Therefore, it can be conclude that L929 cells demonstrated good cell viability (98.5%) at the highest concentration showing no toxic effects released from DC-85 composite. Thus the DC-85 composite is identified as non-toxic or biocompatible.

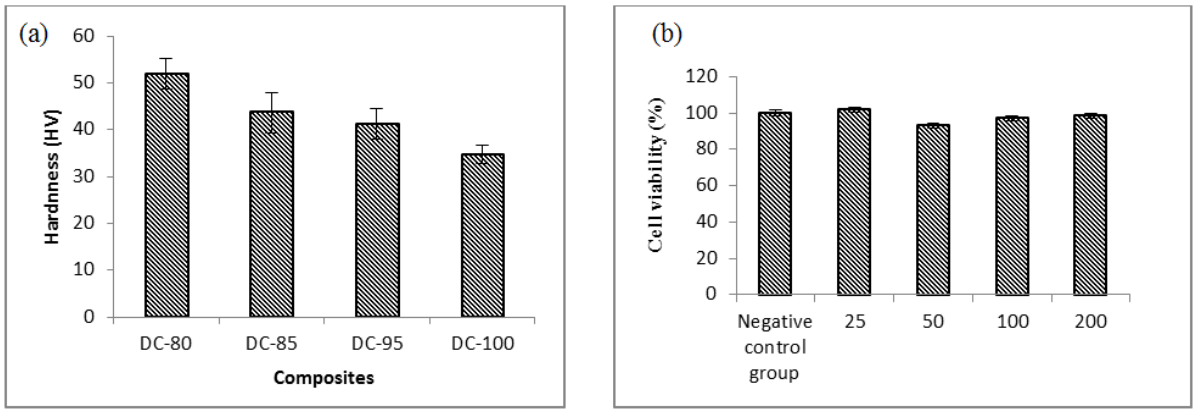

Fig. 4. (a) Vickers hardness of the composites (b) cytotoxicity test of the DC-85 composite

IV. CONCLUSION

The combination of natural hydroxyapatite and silica as fillers were successfully incorporated into the Bis-GMA/TEGDMA/HEMA dental resins. The result showed that the composition of 85 wt% natural HA and 15 wt% silica as a filler enhances the mechanical properties compared to other composites. Silica play a major roles in hardened the composite rather than hydroxyapatite. This composite have a potential material to be used as a dental composite because of the good mechanical properties and biocompatibility properties. The results are meets the requirement standard of ISO 4049 and even better than other dental composite.

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Rafiq Akram Che Razaliet.al."Synthesis of Natural Hydroxyapatite from Fish Scales and Its Potential Application as Fillers in Dental Composites."*American Journal of Engineering Research (AJER)*, vol. 9(04), 2020, pp. 166-170.