

Preparation and Characterization of Hydroxyapatite from Black Tilapia Fish Scales using Spray-drying Method

Izyan Khairani Mohd Ismail¹, Che Nor Aiza Jaafar^{1,*}, Ismail Zainol² and Mohd Zuhri Mohamed Yusoff¹

¹Department of Mechanical and Manufacturing Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43000 Serdang Selangor, Malaysia.

²Chemistry Department, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia.

Abstract

Black tilapia (*Oreochromis mossambicus*) fish scales were used as a source of natural hydroxyapatite (HAp) since it is cheaper than synthetic hydroxyapatite and safer than animal origin such as bones. This study investigates the preparation of natural hydroxyapatite from tilapia fish scales (FsHAp) by thermal technique followed by ball milling and spray dried to produce FsHAp powder. The effect of milling time on the particles size of FsHAp was investigated. In this study, Mastersizer 2000 was applied to measure the particles size of FsHAp. Field emission scanning electron microscope (FESEM) was used to investigate the morphology of the FsHAp powders whereas x-ray diffraction (XRD) and Fourier transform infrared (FTIR) were used to verify the presence of FsHAp. From the experimental results, it was found that the optimum milling time is 48 hours which produced smallest particles of FsHAp at about 1.86 μm . The spray-drying method produced different sizes of FsHAp ranging from 2.18 micron to 6.36 μm . The FESEM analysis revealed that the FsHAp particles agglomerated in the spray dryer. FTIR and XRD analyses have showed the prominent peaks corresponding to high quality of FsHAp from fish scales.

Keywords: hydroxyapatite, spray dry, fish scales

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*Corresponding author: Che Nor Aiza Jaafar; e-mail: cnaiza@upm.edu.my

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Introduction

Hydroxyapatite (HAp) is common biomaterials for bone implants and dental restoration [1]. This is because HAp has chemical structure similar to bones and teeth and they are bioactive, osteoconductive and biocompatibility to the human tissue. Besides that, HAp is also widely used as fillers in polymer composites such as high density polyethylene/hydroxyapatite (HDPE/HAp) composite. This composite is one of the successful materials being used in biomedical applications [2].

Commercially available HAp is commonly synthesized through chemicals reaction such as chemical precipitation method [3]. However, due to high manufacturing cost of synthetic HAp, many researchers have focused on natural resources to produce economical HAp. Biological sources such as animal's bones such as bovine and pig are among the major natural resources for HAp [4]. However due to animal diseases, natural resources such as fish scales become the best alternatives source of HAp [5]. Most of fish scales were reported to contain around 50 wt% of organic and inorganic components known as collagen and HAp [6]. Extraction of HAp from fish scales is more economical and they are safer than animals's bone resources. There are a few methods can be used to extract HAp from fish scales such as alkaline treatment, enzymatic and direct burning. Direct burning method is among the easiest and cheapest methods to extract natural HAp from fish scales. In this method, fish scales are burnt in furnace at about 1000 °C to remove organic composition whereas HAp is remained as ash [7].

The fish scales HAp (FsHAp) produced from burning process can be reduced its particle size by milling process to suite for specific applications. Wet ball milling technique is usually carried out to reduce the particle size of HAp [8] followed by drying in oven. However, the dried HAp slurry need to be grinded into powders and sieved for final applications. Spray dry is an alternative drying method for large scales production of HAp.

Application of spray dryer in production of synthetic HAp powder was reported by many researchers [9, 10]. However, not much research has been reported to apply spray-drying method in production of natural HAp powder. Kusrini et. al., (2012) studied the effect of ultrasonic on the properties of spray dried bovine bones [11]. They found that by increasing sonication time, the particles size decreased to 25 nm. None of the literature has reported the application of spray-drying method in production of FsHAp powder. Therefore this study was focused on the effect of ball milling on the particle size of FsHAp slurry and the effect of spray-drying method on the particles size FsHAp powder. The particle size is important parameter if the FsHAp are to be used as fillers in polymer composites as it will affect the final properties of composite [12]. The black tilapia (*Oreochromis mossambicus*) fish scales was chosen in this study since this fish is the third largest cultured fish in Malaysia.

Materials and Methods

Extraction of hydroxyapatite from fish scale

Black tilapia (*Oreochromis mossambicus*) fish scales (Fs) were collected from wet market at Tanjong Malim, Perak. The Fs were washed, cleaned and dried in oven. The Fs were heated in furnace for 2 hours at 800 °C and then 1200 °C for 2 hours. The obtained white fish scales ash were collected and wet grinded using ball milling for 72 hours. The sample of FsHAp

slurry was collected after milled for 0, 24, 48 and 72 hours and characterized for particle size using Mastersizer 2000 (Malvern Analytical Ltd, UK).

Spray dryer of hydroxyapatite slurry

Spray dry machine manufactured by Agridon Technology Sdn. Bhd, Selangor Malaysia. The FsHAp slurry was spray dried into the form of fine mist in main chamber at temperature at around 200 °C. The slurry mist was evaporated into fine FsHAp powders and they were collected in the main chamber (MC), secondary chamber 1 (SC1) and secondary chamber 2 (SC2). The pressure of the spray drying was set at 5 psi. The FsHAp slurry was constantly stirred using mechanical stirrer to control the homogeneity of the slurry during spray drying process. Figure 1 shows spray dryer setup that used in production of FsHAp powder.



Figure 1. Spray dryer setup used in production of FsHAp powder

Characterization of FsHAp fine powder

Fourier transform infrared (FTIR) (model Nicolet 6700) was used to identify the functional groups of FsHAp powder using KBr disc technique. The sample discs were scanned from 400 to 4000 cm^{-1} for 32 times with a spectral resolution of 4 cm^{-1} . X-ray diffraction (XRD) (model D8 Advanced, Bruker, Germany) was used to characterize the phase presence in FsHAp samples. Data was taken from the diffraction angle 2θ between 10° to 70° with a scan speed of 2 $^\circ\text{C}/\text{min}$. The XRD results obtained were compared with the diffraction pattern that was indexed by file standard powder diffraction (ICDD) 00-009-0432 card for HAp hexagonal structure. The surface morphology of the FsHAp particles and the calcium (Ca) and phosphorous (P) elements ratio were analysed using field emission scanning electron microscope (FESEM), model Hitachi SU 8020 UHR attached with EDX Horiba. The sample was coated with platinum using Quorum Q150R S before analysis.

Results and Discussion

Bimodal distribution of FsHAp particle size of slurry produced from Mastersizer 2000 is shown in Figure 2. Bimodal distribution refers to two peaks of particles size which is common result from a process involving breakup of large particles. The particles size results is summarised in Table 1 show that 10% of sample mass ($D_{0.1}$) comprised of particles with diameter below $0.68 \mu\text{m}$ and 90% of sample mass ($D_{0.9}$) with particle size below $7.27 \mu\text{m}$. About 50% ($D_{0.5}$) of FsHAp has particles size around $1.86 \mu\text{m}$ and taken as an average FsHAp particle size.

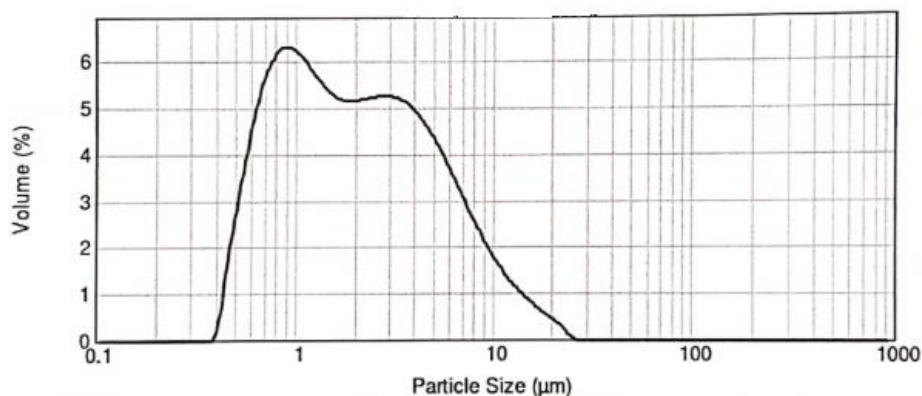


Figure 2. Bimodal particle size distribution of FsHAp ash slurry after 48 hours milling time.

Table 1: Hydroxyapatite particle size after milling for 48 hours

Sample	Particle size (μm)			Surface area (m^2/g)
	$D_{0.5}$	$D_{0.1}$	$D_{0.90}$	
HAp	1.86	0.68	7.27	4.06

$D_{0.5}$, median particle size; $D_{0.1}$ and $D_{0.9}$ the size below which 10% and 90% of the particle diameter lie, respectively.

The effect of milling time on the FsHAp slurry particle size is summarized in Figure 3. The particle size of fish scale ash before milling was $446.98 \mu\text{m}$, however after milling for 24 hours the FsHAp particle size was decreased to $2.55 \mu\text{m}$ while after milling for 48 hours and 72 hours the particles size were found to be $1.86 \mu\text{m}$ and $2.06 \mu\text{m}$, respectively. The smallest particle size was observed for FsHAp milled for 48 hours. It is interesting to note that milling for 72 hours produced FsHAp with median particle size bigger than sample milled for 48 hours.

During milling process, particle breakage occurred at initial stage of milling then equilibrium achieved between re-agglomeration (due to excessive surface energy that accumulate on small particle) and de-agglomeration [13]. 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. However, after milling time extending to 72 hours there was increasing in particle size. This observation was also reported by other researchers [14] and this phenomena occurring probably due to particles start to experience agglomeration after prolong milling as the energy generated during milling process have reached their maximum particles breaking. Therefore, in this study, the milling time of 48 hours was used to process high volume production of FsHAp powder.

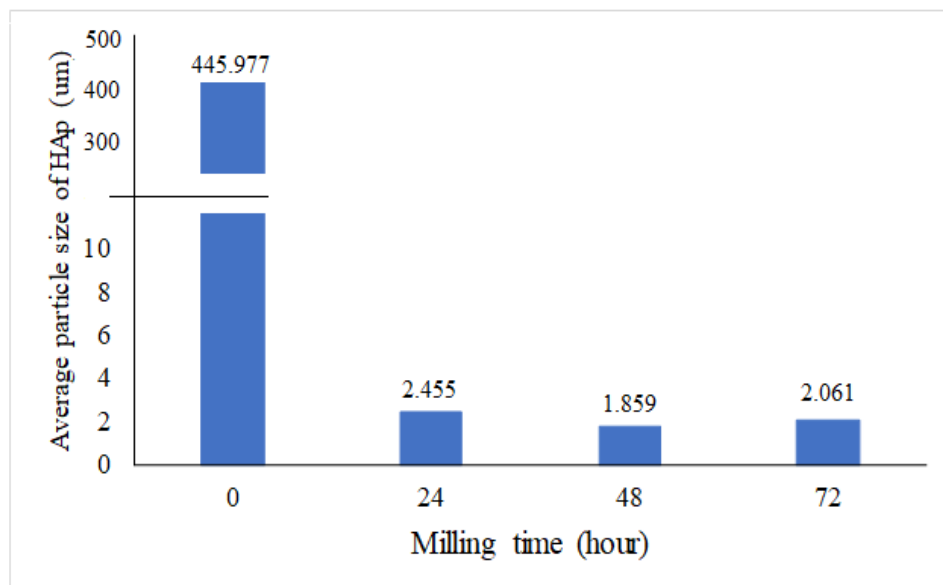


Figure 3. Average size ($D_{0.5}$) of fish scales hydroxyapatite (FsHAp) by milling process at different milling time.

Spray dry is the most common technique to process food product in high volume production. In this study, FsHAp slurry that milled for 48 hours was used in the spray dry process. The slurry mist was evaporated into fine dry FsHAp particles and collected in the main chamber (MC) collector, secondary chamber 1 (SC1) and secondary chamber (SC2) collectors.

Table 2 shows the results of FsHAp particle size analysis at different chambers of spray dryer and FsHAp slurry. The efficiency of the spray dry machine was determined to be 88.3%. Different chamber produced different size of FsHAp powders due to each chamber has ability to trap different size of particles (cyclone collection efficiency) [15]. The FsHAp particles from main chamber (MC) shows the mean particle size of 6.36 μm , which was larger than that of slurry particle size i.e. 1.86 μm . This was expected since the powder agglomeration occurred during spray dry process in the chamber. The $D_{0.5}$ particle size from secondary chamber 1 (SC1) however slightly smaller than from MC i.e 5.67 μm . The small portion of HAp powders were collected in SC2 with smallest mean particle size i.e 2.18 μm .

Table 2. FsHAp Particle Size of Different Chambers from Spray Dryer

No	Sources	Particle size ($D_{0.5}$, μm)
1	HA slurry (after 48 hours milled)	1.86
2	Main chamber (MC)	6.36
3	Secondary Chamber 1 (SC 1)	5.67
4	Secondary chamber 2 (SC 2)	2.18
5	Mixture ((MC +SC1+SC2)	5.18

These results indicated that largest particles size was found in sample from MC, followed by SC1 and SC2. Since all FsHAp particles from different chambers has particle size below than 10 μm , thus they were mixed together to be used as fillers in polymer composite with average particle size of 5.18 μm .

The results of particle size and morphology of the FsHAp powders can be clearly seen in SEM micrograph shown in Figure 4. The micrographs show irregular shape of HAp particles with some degree of agglomeration due to static force between particles especially for sample from MC. However, for synthetic HAp, the particles were more spherical, or doughnut shapes as reported by other researchers [16]. As can be seen in Figure 4(a) the morphology of HAp powders were most agglomerated as compared to more separated particles in secondary chambers of SC1 and SC2 (Figure 4(b) and (c)). The biggest agglomeration of FsHAp particles in MC was probably due to the effect of high moisture content in MC which led to particles attached together to form agglomeration.

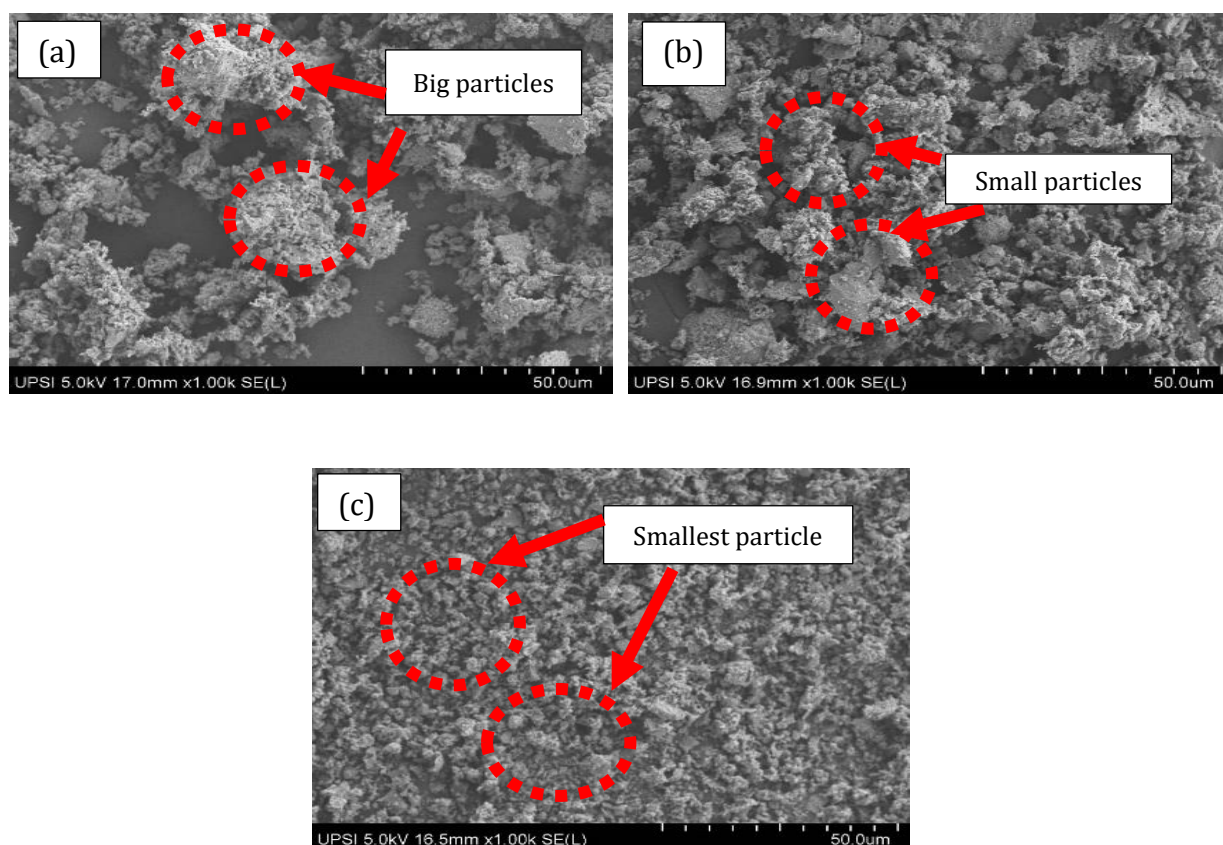


Figure 4: SEM micrographs of HAp powder from (a) main chamber (MC) (b) secondary chamber (SC1) and (c) secondary chamber (SC2)

The EDX analysis results of FsHAp is shown in Figure 5. It is found that the Ca and P peaks with their ratio (Ca/P) is 1.75 are closed to the theoretical values.

Figure 6 shows FTIR spectrum of FsHAp powder. The sharp peak appeared at 3569 cm^{-1} which corresponded 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 corresponded to phosphate groups [6]. The FTIR result observed a typical FTIR spectrum for highly crystalline HAp as reported by several researches [5,17].

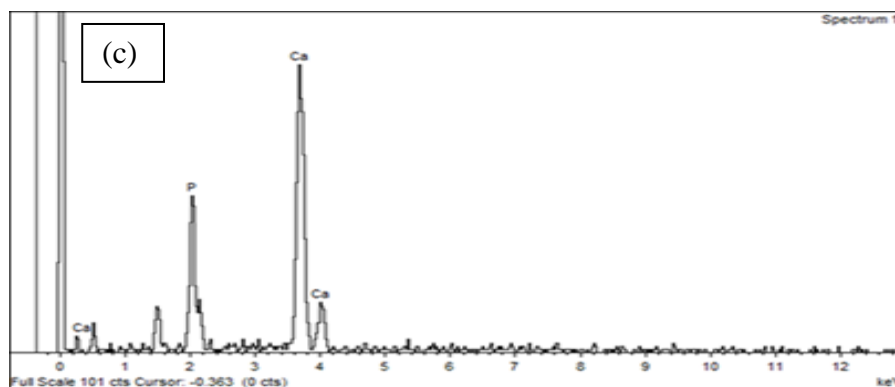


Figure 5. EDX spectrum of FsHAp sample

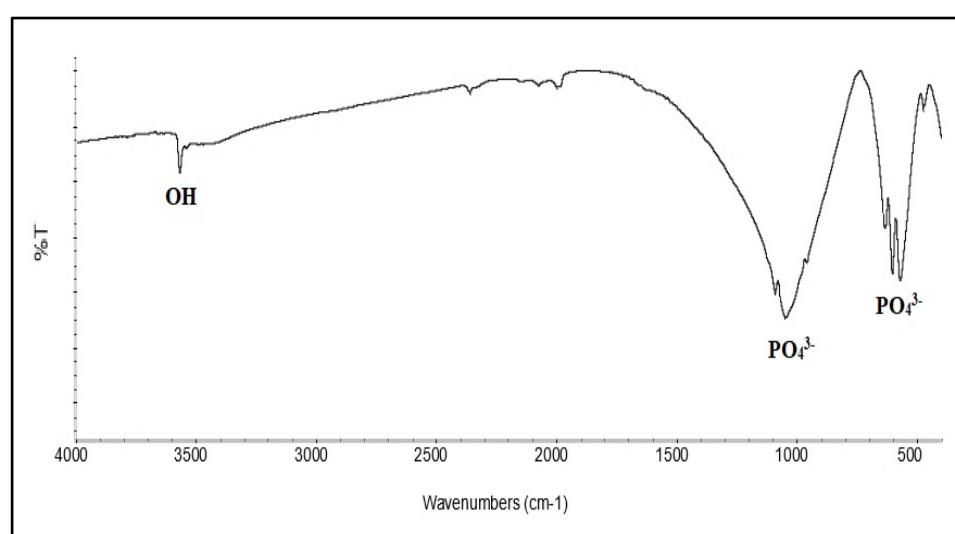


Figure 6. FTIR results for FsHAp samples.

The crystalline phase of the FsHAp powder was characterized using X-ray diffraction analysis as shown in Figure 7. The XRD pattern from FsHAp sample was compared with standard XRD pattern of HAp according to ICDD cards 00-009-0432 for hexagonal structure. These results have proved the high crystalline HAp was extracted from the fish scales. The prominent peaks related to HAp were observed at 2θ angle (31.86° and 31.78°), (33.04° and 32.90°) and (32.53° and 32.20°) associated with the planar hkl (211), (300) and (112) respectively. Similar results have been reported by the previous study [20]. Some other peaks were also observed at hkl (002), (210), (210), (202), (310), (222), (312), (212), (321), (410), (402) and (004) as shown in Figure 6. The sharp peak indicated that the FsHAp sample is highly crystalline materials [9].

It is interesting to note that the presence of β -tri-calcium phosphate (β -Ca₃(PO₄)₂, β -TCP) as secondary phases was observed at hkl peaks (0210), (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) [13]. However, this phase was not observed for bovine bones [1].

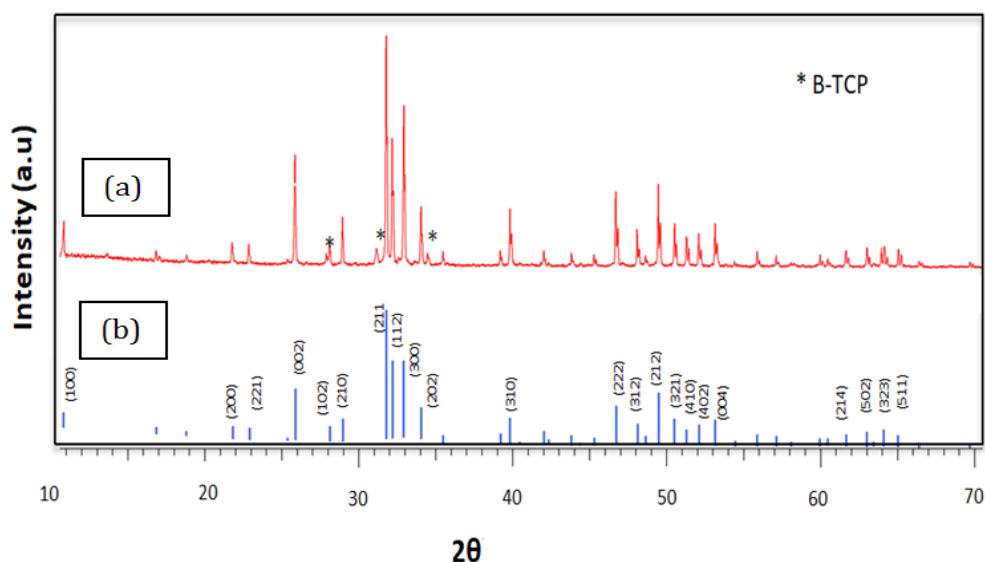


Figure 7. XRD patterns of (a) FsHAp sample and (b) HAp standard

Conclusion

High crystalline natural FsHAp powder successfully extracted from Tilapia fish scale and dried using spray-drying method. The optimum particle size of FsHAp powder was obtained using ball milling for 48 hours. The average particle size of FsHAp powder is 5.18 μm using spray-drying method and they were found to be suitable for filler in polymer composites. Analysis shows that sintered FsHAp have high phase purity and crystalline grade.

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Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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