การพัฒนาไฮดรอกซีอะพาไทต์ขนาดนาโนจากของเหลือทิ้งชีวภาพเพื่อประยุกต์ใช้งานในหลายรูปแบบ

Development of Nanosized Hydroxyapatite from Biowaste for Variety Applications

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บทคัดย่อ

ไฮดรอกซีอะพาไทต์ HA ($Ca_{10}(PO_4)_6(OH)_2$) เป็นวัสดุอนินทรีย์ที่มีความเป็นผลึกและเสถียรภาพทางเคมีสูง ไฮดรอกซีอะพาไทต์สังเคราะห์จากวัสดุเหลือทิ้ง เช่น เปลือกไข่หรือเถ้ากระดูกวัวควายเพื่อลดต้นทุนการผลิต HA ที่มีขนาดนาโนเกิดขึ้นจากเทคนิคการตกตะกอน การวิเคราะห์ลักษณะเฉพาะ เช่น TEM, XRD, TGA และ FTIR ถูกนำมาใช้เพื่อยืนยันโครงสร้างของ HA ผงของ HA นำมาทำการขึ้นรูปหลายอย่าง เช่น เส้นใยสั้น โครงสร้างที่มีรูพรุน และแผ่นรองรับที่ประกอบด้วยเส้นใยนาโน ผลิตภัณฑ์เหล่านี้สามารถประยุกต์ใช้งานทางการแพทย์และสิ่งแวดล้อม งานวิจัยครั้งนี้ ทุกกระบวนการดำเนินงานโดยปราศจากสารเคมีที่เป็นพิษและเป็นวัสดุที่เป็นมิตรต่อสิ่งแวดล้อม

คำสำคัญ: ของเหลือทิ้งชีวภาพ ไฮดรอกซีอะพาไทต์ อนุภาคขนาดนาโน การตกตะกอน

ABSTRACT

Hydroxyapatite, HA (Ca10(PO4)6(OH)2), is an effective inorganic material with high degree of crystallinity and chemical stability. HA was synthesized from disposal waste such as egg shells or cattle bones to reduce production cost. The nanosized HA was obtained from precipitation technique. Many characterization techniques such as TEM, XRD, TGA, FTIR, were used to ascertain HA structure. HA was fabricated in a variety of forms, i.e.; whisker, porous structure and HA substrate with nanofiber. These products can be applied in biomedical and environmental purposes. In this research work, all processes were carried out with nontoxic chemicals and environmentally friendly substances.

Keywords: biowaste, hydroxyapatite, nanosized , precipitation

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Introduction

Hydroxyapatite (HA) is a calcium phosphate compound with a chemical formula of Ca10(PO4)6(OH)2. Typically, HA has excellent biocompatibility, bioactivity, non-inflammatory and non-toxic properties (Marugan, et al., 2004). HA has been used in regenerative medicine, drug delivery systems, toothpaste formulations, wound healing purposes and heavy metal removal in waste water treatment (Uskkovic, et al., 2010, Krajewski, et al., 2000, and Elliott, 1994). HA can be prepared from low cost animal biowaste, i.e., pig bones, fish bones, cattle bones and egg shells (Huang, et al., 2011, Lorprayoon et al., 1986, Sobczak, et al., 2009). These biowastes were cleaned with hot water to remove organic substances, dried and calcined at 1000 □C. Many researchers (Choochaisangrat, et al., 2015, Huang, et al., 2011, Sobczak, et al., 2009,) used the biowaste as starting materials for HA production. These obtained products were characterized phase and crystallinity using XRD. The results showed that the low crystallinity of HA displayed a high reactivity with surrounding tissue (KuŚnieruk, et al., 2016). The calcium to phosphorous ratio showed a ratio greater or lower than 1.67. At the Ca/P ratio of 1.67, the high stability of the material (inert) inside the human body occurred. The bioactivity of material was observed with one at a Ca/P ratio lower than 1.67 (deficient HA). HA is a good material for bone and teeth substitution. HA can be prepared using different methods such as precipitation, sovo-thermal, solid state, sol-gel, sonochemical microwave and hydrothermal methods. (Arends, et al.,1987, Lorprayoon, et al., 1991, CüneytTas, 2000, Jevtic, et al., 2008, Ma, et al., 2009, and Zhu et al., 2014). The summary of methods, advantages and disadvantages are shown in Laonapakul, 2015 and KuŚnieruk, et al., 2016. In this research work, HA was synthesized from cattle bone ash and egg shells using the precipitation method. The nanosizes of the obtained products were characterized using SEM, XRD and FTIR. Many applications of HA products were also concerned.

Materials and method

Materials

Egg shells were obtained from household waste. Cattle bones were collected from Pratum Thani market. Nitric acid (65%), calcium nitrate $Ca(NO_3)_2.4H_2O$, and ammonium hydroxide (30%) were purchased from Carlo Erba. Phosphoric acid (85%) received from Lab Scan. Tris [hydroxymethyl] aminomethane (Tris-base) was Ultrapure MB grade.

Equipment

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Method

Hydroxyapatite prepared from cattle bone ash

(modified from Lorprayoon, 1991)

HA was prepared from selected parts of cattle bones. Organic materials had been removed by calcination at 1000^{o}C for 3 h. The calcined bones were crushed, milled and screened through a sieve (140 mesh; 106 μm) to obtain fine powder. The powder of the cattle bone

ash was composed of big particles of HA with some impurities. So, the HA powder was further purified by dissolving the cattle bone ash with 1 N of HNO_3, adding 10% w/v of $Ca(NO_3)_2.4H_2O$ and precipitating with 30% of NH_4OH with a pH of about 10. The precipitate was filtrated, washed with hot water and dried in a hot air oven. The obtained products were characterized by XRD, TGA, TEM, FTIR. and the Ca/P molar ratio was also examined.

Hydroxyapatite prepared from egg shells

(modified from K-hasuwan *et al.*, 2012)

Normally, egg shells are composed of 95% calcium carbonate, an organic component, and mineral salts (Rivera, *et al.*, 1999). Egg shells can be used as a calcium source for HA preparation. Egg shells were cleaned in hot water and dried in a hot air oven. They were then calcined at 1000°C to totally turn them into CaO. The obtained powder was dissolved with 1 M of HNO_3. H_3PO_4 (85%) was added into the solution to bring the Ca/P molar ratio to 1.67. The tris-base solution (12% w/v) had been poured into the solution to precipitate the HA. The precipitates were filtered, washed with dionized water and dried in a hot air oven. The obtained products were characterized using XRD, TGA, TEM, and FTIR. Ca/P and the molar ratio of the purified products were also studied.

After preparation, the purified HA powder was fabricated into products such as whiskers, the porous structure of the HA and substrate for the PVA-chitosan nanofiber membrane attachments.

Results and discussion

Morphology and phase of purified HA

HA was prepared from cattle bones or egg shells. An x-ray diffractometer was equipped with Cu Kα radiation, with the step time of 0.5, the step 0.02 and scanning range of 5-50° in the study phase of the HA products. The big particles of cattle bone are shown in fig 1a) with phase of hydroxyapatite (JCPDS card 090432) as shown in fig 1b). The HA from cattle bone ash contained some drawbacks such as poor mechanical properties, low surface area and contaminated with some impurities. So, cattle bone ash was purified by the method after Lorprayoon *et al*., 1991. After chemical treatment, nanosized HA occurred as shown in figure 2a). The nanosized HA from egg shells is also displayed in fig 2b). The pure phase of both types of HA was recorded in fig 2c). The tubular particles of HA were 20x40 nm and 10x50 nm for HA synthesized from cattle bones and egg shells respectively.

Ca/P molar ratio

The Ca/P molar ratio of the HA was detected using SEM-EDS. The electron microscope was carried out with an energy dispersive x-ray attachment for elemental analysis. The Ca/P ratio of the HA synthesized from cattle bones and egg shells were 1.64 and 1.67 respectively. The Ca/P molar ratio affects the reactivity, mechanical properties and stability of HA products (Rivera-Muñoz *et al.,* 2012, and Laonapakul, 2015).

FTIR analysis of HA powder

FTIR was used to detect in the range of 4000-650 cm^{-1} with a resolution 64 times in the ATR mode. The spectra of the obtained products are shown in fig 3. The characteristic bands were assigned as shown in table 1. The HA synthesized from cattle bones and egg shells was composed of OH^- and PO_4^{3-} groups of calcium phosphate. The carbonate in the HA occurred from the absorption of carbon dioxide from the atmosphere within a synthetic mixture (Eslami *et al*., 2018). The HA from cattle bone purified

showed the absorption band at 1421 and 875 cm^{-1} due to the carbonate ion substituted at the PO_4^{3-} site or type B. The HA synthesized from egg shells displayed the absorption band at 1541 cm^{-1} due to the carbonate ions substituted at OH^- sites (type A). Nanosized particles of carbonated apatite were expected to be high bioactivity materials (Othman, *et al.*, 2016).

Thermal stability of HA powder

A themogravimetric analyzer was used in air atmosphere at a heating rate of 10°C/min up to 1000°C to examine the heat stability of the HA powder. The results are shown in fig 4a) and 4b). The reactions that occurred on the HA powder during heat treatment were divided into 3 steps i.e., the evaporation of water, dehydration and decomposition (Modal, *et al*., 2016). The results can be concluded in table 2.

HA can be prepared from biowastes such as cattle bones or egg shells. The properties of as-synthesized products are shown in table 3.

Applications of hydroxyapatite

The HA powder was fabricated in various forms, i.e., porous structures, whiskers, and substrate with nanofiber membrane. The products were applied for biomedical or water treatment purposes.

Hydroxyapatite whisker

The HA powder from purified cattle bone was mixed with 1 M acetic acid, stirred for 3 h and put into an autoclave at 150°C for 6, 12 and 24 h. The HA whiskers are shown in fig 5a), 5b) and 5c). The aspect ratio of a HA whisker was greater than 20. The obtained products were expected to be used for reinforcing in polymer matrix in order to improve the mechanical properties and applied for biomedical purposes (Kuanchertchoo, *et al*., 2013).

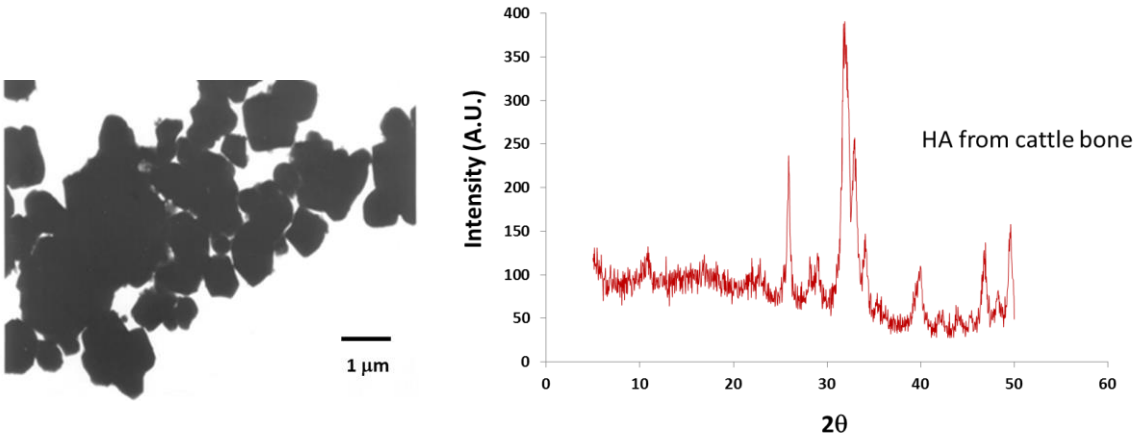


Figure 1 HA from cattle bone ash a) TEM micrograph show the big particle of HA b) XRD pattern revealed the phase of hydroxyapatite.

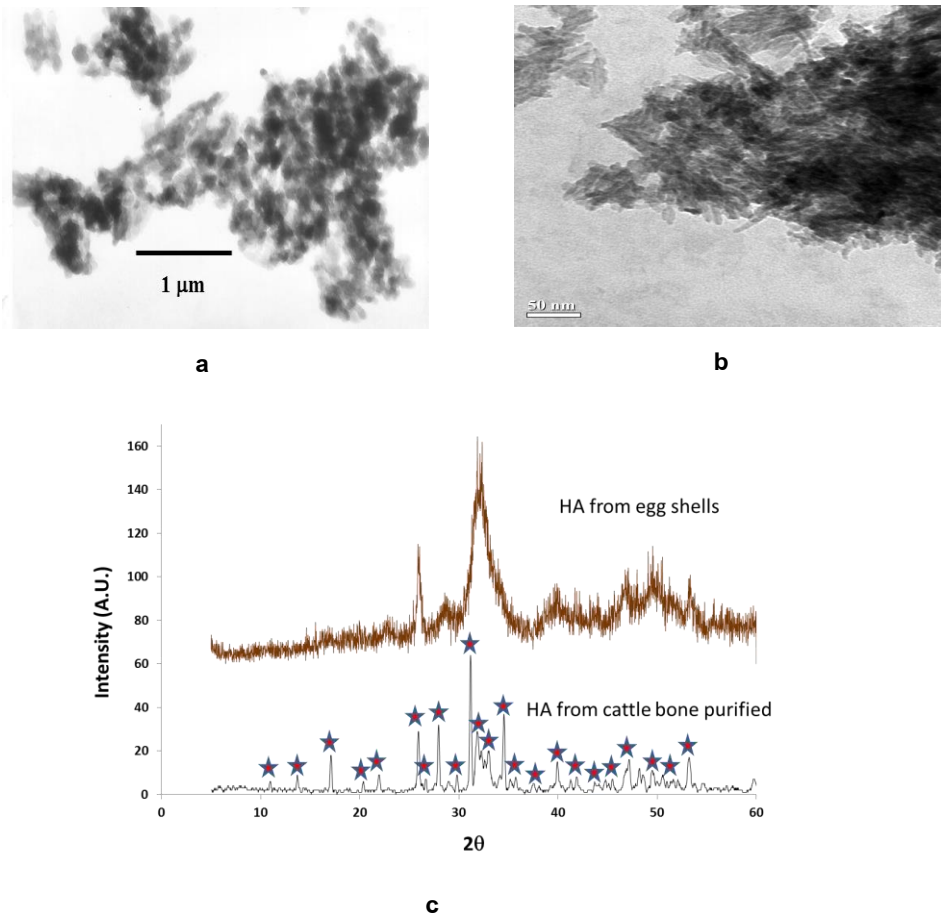


Figure 2 Nanosized HA was synthesized from a) cattle bones and b) egg shells and c) XRD patterns displayed the pure phase in both types of HA

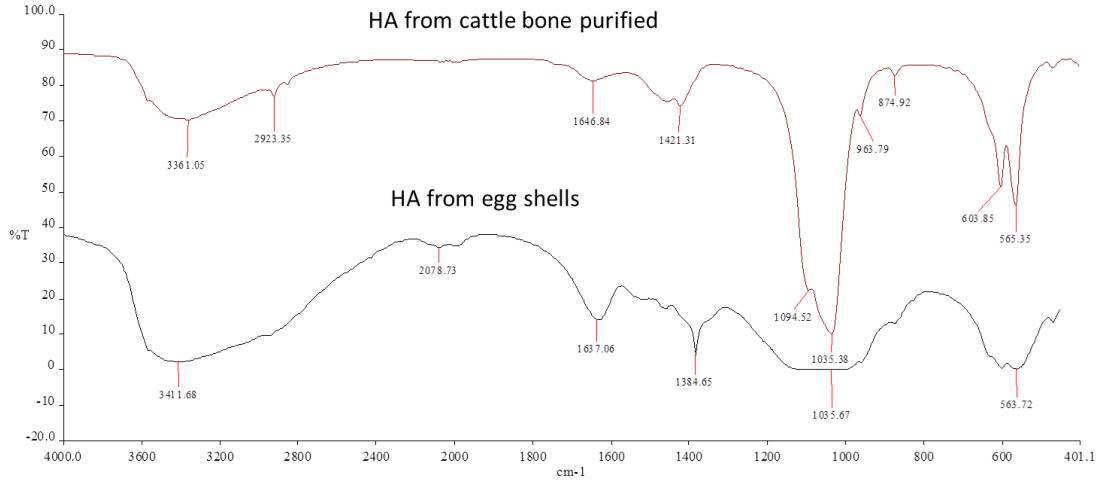


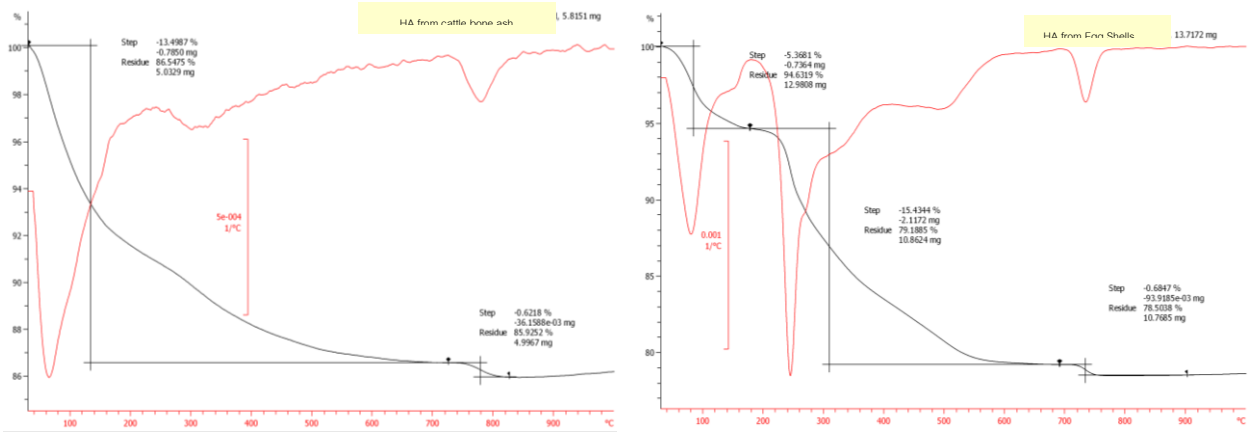
Figure 3 FTIR spectra of HA synthesized from cattle bone purified and egg shells

Table 1 Assignment for FTIR spectra of synthesized HA

Assignment	*HA from cattle bone*	*HA from egg shell*	*References*
OH^- structural	3451 cm^{-1}	3532 cm^{-1}	Eslami *et al*., 2018
PO_4^{3-} bend $\nu 1$	1035,565 cm^{-1}	1036, 564 cm^{-1}	
and $\nu 3$	1094, 1035, 964 cm^{-1}	1035, 955 cm^{-1}	Ito, *et al*., 2014
PO_4^{3-} bend $\nu 4$	604, 565 cm^{-1}	585, 564 cm^{-1}	
CO_3^{2-}	1421, 875 cm^{-1} (type B)	1541 cm^{-1} (type A)	Kamitakahara *et al*., 2015

Table 2 Reaction of HA powder during heat treatment

Temperature (^oC)	*Reactions*
25-150	Evaporation of surface water
150-600	Evaporation of water in lattice
600-800	Decarbonation
800-900	Dehydroxylation of HA

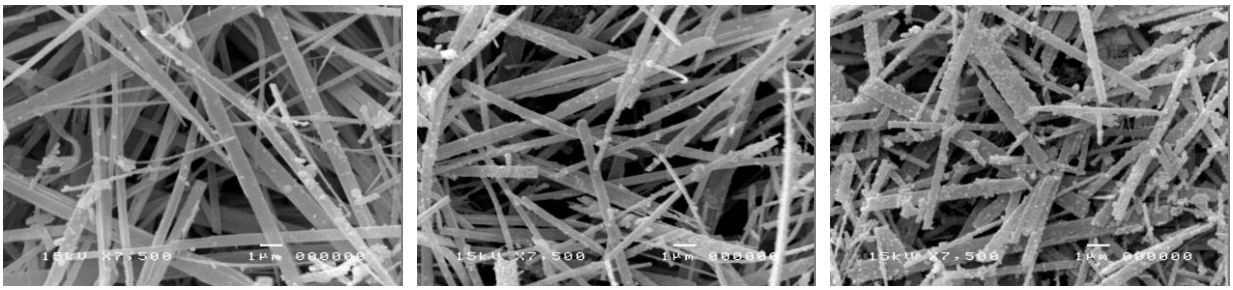


a **b**

Figure 4 Thermogram of HA synthesized from a) cattle bone b) egg shells

Table 3 Properties of HA prepared from cattle bones and egg shells

Properties	*HA from cattle bone*	*HA from egg shells*
Preparation process	Precipitation	Precipitation
Particle morphology and size	Tubular, 20x40 nm	Tubular, 10x50nm
Functional groups	OH^-, PO_4^{3-} and CO_3^{2-} (type B)	OH^-, PO_4^{3-} and CO_3^{2-} (type A)
Phases	Hydroxyapatite with low crystallinity	Hydroxyapatite with low crystallinity
Ca/P molar ratio	1.64-1.65	1.67
% Yield	100%	70-80%

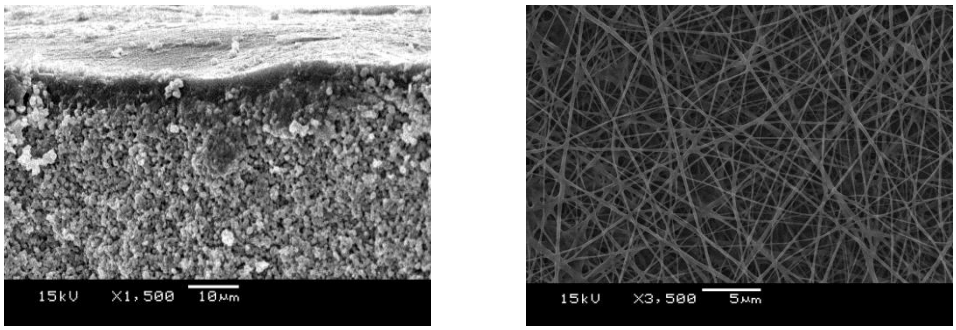


a b c

Figure 5 Hydroxyapatite whisker from purified cattle bone were prepared at 150°C for a) 6 h b) 12 h and c) 24 h in autoclave reactor

a **b** **c**

Figure 6 Porous structure of a) polymeric sponge b) porous HA and c) microstructure of porous ceramic



a **b**

Figure 7 membrane from PVA-chitosan nanofiber on HA substrate a) microstructure of membrane b) PVA and chitosan nanofiber in membrane

Table 4 Efficiency of membrane from PVA-chitosan nanofiber on cattle bone HA substrate in waste water treatment at Pinthong industrial estate, Chonburi, Thailand

Ion in waste water	*Before treatment (ppm)*	*After treatment (ppm)*	*% Removal*
Cr^{3+}	0.5 (±0.133)	0.15 (±0.064)	70
Fe^{2+}	6.20 (±3.025)	2.72 (±2.098)	56
Zn^{2+}	39.52 (±2.991)	0	100

Data from Department of Chemistry, Faculty of Science, Ramkhamhaeng University

Porous structure of hydroxyapatite

HA from cattle bone was turned into a suspension form with some $Ca_3(PO_4)_2$ glass powder. The polymeric sponge (fig 6a)) was used as a template for the replication of its structure. The suspension was pushed into a sponge, squeezed out and these processes were repeated several times. The sample was dried, burnt out sponge and sintering at 1000^{O}C for 3 h. The porous structure of the HA is displayed in fig 6b) and microstructure in fig 6c). The porous HA tends to be used as carrier for drug delivery or bone filler for orthopedic surgery. (Kuanchertchoo, 1996).

Polyvinyl alcohol and chitosan nanofiber membrane deposited on HA substrate

Polyvinyl alcohol (10%w/v) was mixed with 2%w/v of chitosan in an acetic acid solution at the ratio 1:3. The mixture was formed into nanofiber using the electrospinning technique onto substrate of HA from cattle bone. The obtained products are shown in fig 7 and used as membrane for the removal of Fe^{2+}, Zn^{2+} and Cr^{3+} in waste water treatment. The efficiency of the membrane is shown in table 4. This process is environmentally friendly and energy saving. (Butrieng and Wangtrakul, 2014).

Conclusions

Nanosized hydroxyapatite was successfully prepared from biowaste, i.e., cattle bones or egg shells through a precipitation method. Synthesized HA was examined by different techniques, FTIR showed the functional groups of carbonated apatite and TEM displayed the nanorod of HA. TGA ascertained the heat stability of the obtained products. XRD also confirmed the low crystalline of the HA phase. Synthesized HA powder was fabricated into whiskers, porous structure of HA and HA substrate for the nanofiber membrane attachment. The obtained products were applied for biomedical or environmental purposes. Moreover, this research work was also concerned with green and waste utilization in the process.

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