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Research Article

Preparation and characterization of bioactive cockle shell aragonite/ gypsum composite as bone cement for bone repair

Nguyen Xuan Thanh Tram[1,2,*]

ABSTRACT

Introduction: As the demand for bone defect treatment grows, a natural bone graft cannot be fulfilled the order. Therefore, the development of artificial bone graft is essential for biomedical applications. In this study, the feasibility of bioactive cockle shell Aragonite/ Gypsum composite as bone cement for bone repair has been demonstrated. It is meaningful to use the by-products from the food industry, such as cockle shells for the preparation of valuable biomedical materials. **Method:** Bioactive cockle shell Aragonite/Gypsum composite as bone cement was prepared using cockle shell and Calcium Sulfate Hemihydrate ($CaSO_4$. $0.5H_2O$) powder mixtures with different weight ratios. X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and Scanning electron microscope (SEM) were used to characterize phase composition and structure. Mechanical strength was evaluated in terms of Diametral Tensile Strength (DTS), and *in vitro* bioactivity was investigated using Simulated Body Fluid (SBF) solution. **Results:** The results indicated that cockleshell Aragonite/Gypsum composite as bone cement is bioactive, and its properties depend on weight mixing ratios. In conclusion, cockle shell Aragonite/Gypsum composite is ideal for reconstructing bone defects due to its bioactivity and abundant supply.

Key words: bone cement, cockle shell aragonite, gypsum cement and Simulated Body Fluid

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INTRODUCTION

Bone-related diseases and bone fractures are so problematic for older people since delayed bone healing is always associated with increasing age. Therefore, a bone graft is needed to enhance bone reconstruction and prevent further bone fractures that can lead to serious health consequences. Autograft, allograft, and xenograft are used to cover the bone defects. However, there are many disadvantages of these natural bone grafts. Both autograft and allograft have limited supplies due to the severe shortage of the donor sites and the donors. In addition, allograft and xenograft have genetic barriers and disease transmission that significantly affect the bone healing process[1,2]. And hence, the alternative method is artificial bone graft since it can overcome the complicated issues of natural bone grafts and satisfy the demand for bone defect treatment.

For implantation, bone substitute materials must be fabricated in a particular shape to prevent an inflammatory response. Different biomaterials have been developed as bone substitutes such as block, granule, and cement[3–5]. Cement is advantageous in filling the unspecified form of the bone defect since it is flexible to be shaped. The cement's setting and hardening reaction result from the continuing reaction between the powder and the liquid phase that can develop the proper mechanical strength of the material. The main challenge of bone substitutes is to achieve bioactivity, biosorption, the appropriate mechanical strength, and the final success of bone reconstruction. The composition of cement material, is the key factor deciding its consequence properties. The study of various compositions of cement materials is necessary for clinical applications. Gypsum (Calcium sulfate) cement has the longest story to be applied as an artificial bone graft due to its osteoconductivity. However, the low bioactivity and the rapid resorption of gypsum is needed to be considered. Most of the resorption of gypsum is due to physical-chemical dissolution[6,7]. If the resorption was not caused by osteoclast, the formation of new bone by osteoblast would be slow. Once implant material had been disappeared quickly before new bone formation was produced, it could lead to a significant loss in microstructural stability and the failure of implantation.

Calcium carbonate ($CaCO_3$) plays an important role in human life. It has three polymorphous as Calcite, Aragonite, and Vaterite. Calcite granules demonstrated good bone formation when implanted in the bone defect of 12 weeks old rat femur[4]. Aragonite has been shown to be an ideal bone substitute due to its bioactivity and biocompatibility[8–10]. Cockleshell

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Aragonite is readily available in countries with long coastline, and it is also the by-product of the food industry. Research trends in the recycling of materials have been popular recently, in which many studies reported on the biomaterials being created from waste products [11–15].

In order to introduce various compositions of bioactive cement for bone substitute materials, this study proposes cockle shell Aragonite/Gypsum composite. Adding Cockleshell Aragonite is for regulating the biological performance and is expected to achieve fast new bone formation. On economic aspects, the preparation of valuable biomaterials from aquaculture and food wastes is a meaningful study since it can solve many environmental issues.

MATERIALS AND METHOD

Preparation

Cockleshell was firstly removed residues, then rinsed with water. It was then immersed in distilled water for 10 minutes at 100^oC to remove the other debris. For completely cleaning, the cockleshell was soaked in 2 mol/L sodium hydroxide (NaOH, 7571-4400, Daejung chemical & metals Co. Ltd, Gyeonggi-do, Korea) for 24h at room temperature. Then, it was washed and filtered several times with distilled water using a vacuum pump until the filtrate was neutral. As - prepared cockle shell was dried in the oven for 24h and then ground until cockle shell powder (denoted as CSP) can pass through the 90-μm sieve. The cockleshell/Gypsum powder mixture was prepared by mixing Calcium sulfate hemihydrate ($CaSO_4.0.5H_2O$, 2522 - 4000, Daejung Chemicals & Metals Co., Ltd, Gyeonggi - do, Korea) powder with CSP in a $CaSO_4.0.5H_2O$: CSP weight ratio of 100:0, 75:25, 50:50 and 25:75. The cement paste was prepared by hand, mixing the powder mixtures and distilled water with the liquid to powder (L/P) ratio of 0.5. The paste was packed into stainless steel mold (6mm x 4mm). Mold was then placed inside the oven at 37^oC for 24 h with humidity. After setting, mold was unpacked to obtain composite block for characterization.

Phase characterization

The phase composition was done by means of powder X-ray diffraction (XRD) analysis. The sample was ground to fine powder, and the XRD patterns were recorded using a diffractometer system (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany) using Vario1 Johansson focusing monochromator and high flux CuKalpha radiation generated at 40 kV and 40 mA. The samples were scanned from $2\theta = 10^o$ to $2\theta = 60^o$ (where θ is the Bragg angle) in a continuous mode. Fourier Transform Infrared Spectrometer (FTIR) analysis was used to confirm the phase composition via verification of functional groups.

Setting time evaluation

Setting time measurement is based on the ISO 1566 method using the Vicat apparatus with a movable rod weight 400g and 1 mm^2 needle [5]. First, mixing of the cement and distilled water was done for 1 minute. Then the cement paste was transferred into the mold and kept at 37^oC with humidity. Setting time is the time from this stage to when the needle no longer penetrates a distinctive circular indentation on the surface of the cement sample. In this standard, testing samples (n = 3) were maintained at 37°C and 100% relative humidity, mimicking the clinical condition.

Microstructure properties

Morphology microstructure was observed by a Scanning Electron Microscope (SEM: S-3400N, Hitachi High-Technologies Co., Tokyo, Japan) at 10 kV of accelerating voltage after gold sputter coating. Total porosity was estimated based on the measurement of volume and weight. The average value was calculated from the porosity value of 5 specimens.

Mechanical properties

Mechanical strength was evaluated in Diametral Tensile Strength (DTS) using Universal Testing Machine (AGS-J, Shimadzu Corporation, Kyoto, Japan). The test was done with 5 specimens. The average DTS value was calculated from the DTS value of 5 specimens.

In vitro test

Bioactivity was evaluated using Simulated Body Fluid (SBF) solution. SBF solution was prepared according to the method of Kokubo *et al.*, 2006 [16]. The samples were immersed in SBF solution and kept in a water bath with a fixed temperature of 37°C. SEM was used to prove the apatite formation on the surface of the cement sample after immersion in SBF for 7 and 28 days.

RESULTS

Figure 1 showed the XRD patterns of cement samples after setting at 37^oC for 24 h with humidity, reference $CaSO_4.2H_2O$, Aragonite, and $CaSO_4.0.5H_2O$, respectively. It can be seen that cockleshell powder (denoted as CSP) is aragonite corresponding to the standard JCPDS PDF Card No. 05-0453. The XRD patterns also presented the peaks of Calcium Sulfate ($CaSO_4.2H_2O$, denoted as CS). Calcium sulfate hemihydrate ($CaSO_4.0.5H_2O$, denoted as CSH) transformed almost completely to CS in the cement. Sample with no CSP content (marked as 100% $CaSO_4$. $0.5H_2O$ in Figure 1) has peaks of CS at 11.6, 20.7, and 29.09 degrees with strong intensity, suggesting that setting reaction occurred promptly. the weight percentage of CSP increased, the setting reaction might be delayed since it indicated a decrease in the intensity of main CS peaks.

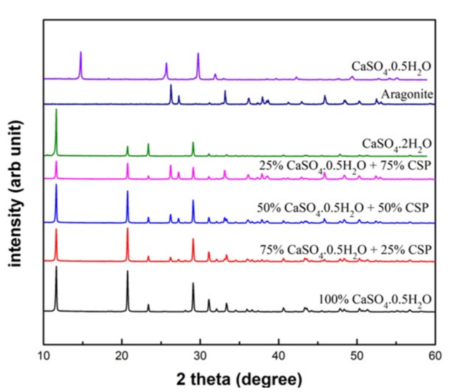

Figure 1: XRD patterns of cement samples (marked as 100%$CaSO_4$. $0.5H_2O$, 75% $CaSO_4$. $0.5H_2O$+25% CSP, 50% $CaSO_4$. $0.5H_2O$+50% CSP and 25% $CaSO_4$. $0.5H_2O$+75% CSP) after setting at 37^{o}C for 24 h with humidity, reference $CaSO_4.2H_2O$, Aragonite, and $CaSO_4.0.5H_2O$ respectively. XRD pattern indicated that peaks of CSP belonging to the aragonite phase (main peaks at 2theta = 26, 33, 45, and 52 degrees). Sharp peaks of CS at 11.6, 20.7, and 29.09 degrees proved that setting reaction occurred.

Table 1 indicated setting time and CS crystal size of cement samples. The calculation of crystalline size is based on the Scherrer equation[11,17]. It is noted that setting time is extended as increasing the amount of CSP. On the other hand, CS crystal size is inversely proportional to CSP content.

For further characterization of phase composition, Figure 2 illustrated the FTIR spectra of cement samples after setting at 37^{o}C for 24 h with humidity, reference Aragonite, $CaSO_4.0.5H_2O$, and $CaSO_4.2H_2O$, respectively. Bands of $SO_4{}^{2-}$ group at 602 - 670 cm^{-1}, 1150 cm$^{-1,}$ and water (H-O-H bending) at 1620 -1680 cm^{-1} belong to CS. There is no band fitting with CSH in FTIR spectra of cement samples as compared to the FTIR spectrum of reference $CaSO_4.0.5H_2O$.

Typical bands of $CO_3{}^{2-}$ group of reference aragonite at 860 cm^{-1} and 1480 cm^{-1} appeared in the cement samples with CSP content. The intensity of these bands is amplified as increasing of the CSP content. This is also in agreement with XRD results suggesting that adding CSP would lead to slow phase transformation from CSH to CS.

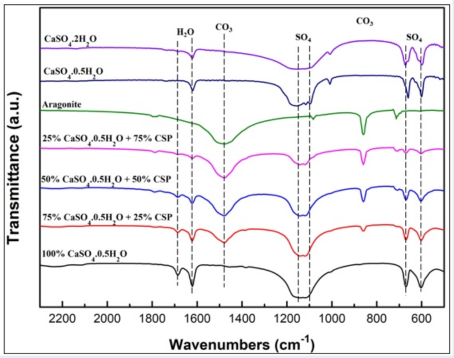

Figure 2: FTIR spectra of cement samples (marked as 100%$CaSO_4$. $0.5H_2O$, 75% $CaSO_4$. $0.5H_2O$+25% CSP, 50% $CaSO_4$. $0.5H_2O$+50% CSP and 25% $CaSO_4$. $0.5H_2O$+75% CSP) after setting at 37^{o}C for 24 h with humidity, reference Aragonite, $CaSO_4.0.5H_2O$, and $CaSO_4.2H_2O$ respectively. Bands of $CO_3{}^{2-}$ group at 860 cm^{-1} and 1480 cm^{-1}is typical bands of aragonite. CS has vibration bands of $SO_4{}^{2-}$ group at 602 - 670 cm^{-1}, 1150 cm^{-1} and water (H-O-H bending) at 1620 -1680 cm^{-1}.

Figure 3 showed Diametral Tensile Strength (DTS) of cement samples before and after soaking in SBF for 7 days and 28 days. Generally, mechanical strength is inversely proportional to the content of CSP. This can be explained by the setting reaction level. The higher the amount of CSP was added, the slower the setting reaction happened. Besides, the mechanical strength of cement samples was also varied before and after soaking in SBF for different periods of time. In particular, cement sample with 50 wt% CSH – 50 wt% CSP after setting at 37^{o}C for 24 h with humidity had a mean DTS value of 1.23 ± 0.09 MPa and this value was changed slightly after this cement sample was soaked in SBF for 7 days (1.07 ± 0.05 MPa) and for 28 days (0.79 ± 0.02 MPa).

On the other hand, there was a considerable difference in DTS value in the cement samples without CSP content before and after soaking in SBF. Cement sample with 100 wt% CSH – 0 wt% CSP after setting at 37^{o}C for 24 h with humidity had a mean DTS value of 3.07 ± 0.14 MPa. After soaking in SBF for 28 days, cement samples with no CSP content had a DTS value being reduced to half (1.58 ± 0.06 MPa).

Table 1: **Setting time and CS crystal size of cement samples**

Sample	Setting time (mins)	CS crystal size (Å)
100 %wt CSH - 0 %wt CSP	5 ± 0.3	1400.6
75 %wt CSH - 25 %wt CSP	9 ± 0.2	1185.1
50 %wt CSH - 50 %wt CSP	15 ± 0.5	962.9
25 %wt CSH – 75 %wt CSP	23 ± 0.4	855.9

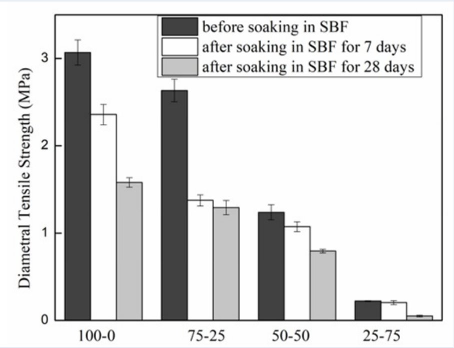

Figure 3: Diametral Tensile Strength (DTS) of cement samples before and after soaking in SBF for 7 days and 28 days.

Figure 4 shows the total porosity of cement samples before and after soaking in SBF for 7 days and 28 days. It is noted that mechanical strength and total porosity have a trade-off relationship[18,19]. Therefore, the results of total porosity (as shown in Figure 4) agree with the hypothesis. The interlocked molecular architectures were cracked as the dissolution of cement samples occurred, which caused the increase in porosity after soaking in SBF.

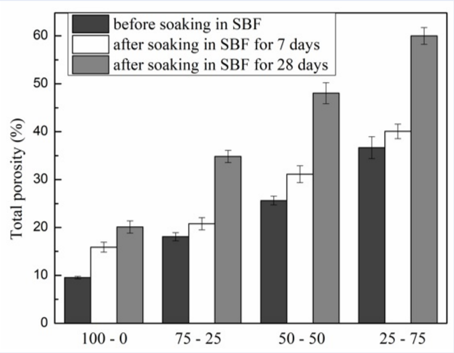

Figure 4: The total porosity of cement samples before and after soaking in SBF for 7 days and 28 days.

The microstructure of cement samples before and after soaking in SBF for 7 days and 28 days was presented in SEM images (Figure 5). The Gypsum crystals display a needle-like structure. After soaking in SBF for 7 days, the cement with 50 wt% CSH – 50 wt% CSP (marked as 50-50-7) exhibited partially the modification of morphology that is from needle-like layered structures to sheet-like aggregation. Prolonging the soaking period to 28 days extended the formation of sheet-like layers. On the contrary, the cement with 100 wt% CSH – 0 wt% CSP (marked as 100-0-7) showed no morphological change after soaking in SBF for 7 days. After soaking for 28 days, the surface of cement with 100 wt% CSH – 0 wt% CSP (marked as 100-0-28) appeared a few agglomerates in the matrix of needle-like shapes. It was determined that the variation of the morphology was due to the growth of the new precipitation phase.

DISCUSSION

The solubility of CSH is 0.9 g per 100 g of water at 20 °C. CSH was dissolved in water, releasing ion Ca^{2+} and $SO_4{}^{2-}$. The setting reaction occurred because this solution is supersaturated concerning CS since its solubility is 0.2 g per 100 g of water[6,7]. The presence of CSP prevented CS formation; therefore, it took a longer time for the cement paste with CSP to set. And so on, the larger the amount of CSP was added, the smaller the crystal size of CS was achieved. Besides, increasing amounts of setting gypsum led to the higher DTS value of cement sample.

On the contrary, as the setting reaction of cement paste was delayed, CS crystals were not developed and not fastened firmly together interlocking shapes. This is why the mechanical strength of the cement sample with 25 wt% CSH – 75 wt% CSP was very small. Changing the mechanical strength after soaking in SBF could be attributed to the dissolution and precipitation of the new apatite phase. It is known that SBF is a solution highly supersaturated concerning apatite[16]. As soaking in SBF, the cement sample is dissolved in the solution and supplies ions that are required for the precipitation of the apatite phase. If

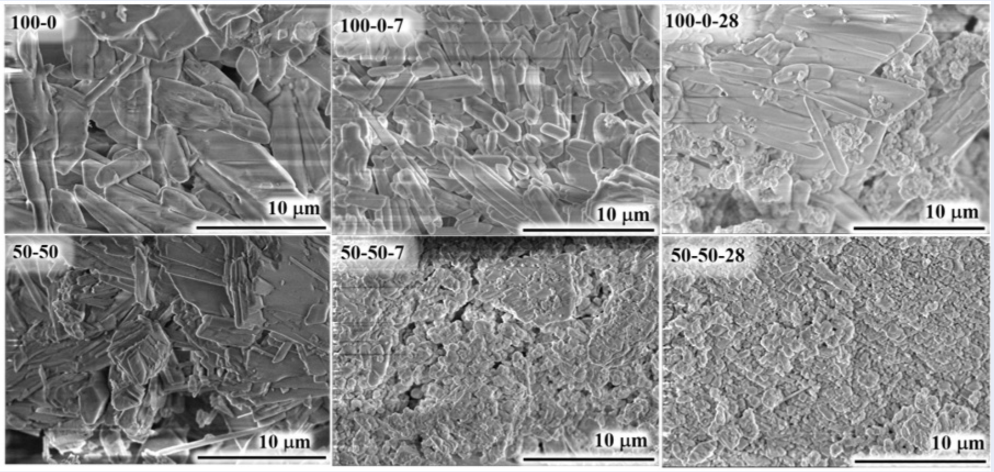

Figure 5: SEM images showing the morphology of cement samples before (marked as 100-0 and 50-50) and after soaking in SBF for 7 days (marked as 100-0-7 and 50-50-7) and 28 days (marked as 100-0-28 and 50-50-28).

the materials were extremely bioactive, a new apatite phase could be formed quickly to maintain the rigidity of cement samples. The apatite forming ability of cement indicated the level of bioactivity. Hence, cement sample without CSP content is less bioactive than cockle shell Aragonite/ Gypsum composite. That means the biological performance of cockle shell Aragonite/ Gypsum composite might be improved if it was applied as bone substitutes.

CONCLUSIONS

Adding Cockleshell Aragonite to Gypsum bone cement was found to be effective for the promotion of bioactivity. Apatite forming ability of the cement in SBF solution is preliminary *in vitro* test to indicate if materials were proper for bone repair application. However, it is better to conduct cell culture and *in vivo* to confirm exactly the biological performance of this bioactive cockle shell Aragonite/Gypsum composite. Moreover, its mechanical strength also needs to improve to overcome the initial stress in the beginning stage of implantation.

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ABBREVIATIONS

CSP: Cockle shell powder
CS: Calcium Sulfate
CSH: Calcium sulfate hemihydrate
XRD: X-ray diffraction
FTIR: Fourier-transform infrared spectroscopy
SEM: Scanning electron microscope
DTS: Diametral Tensile Strength
SBF: Simulated Body Fluid
L/P: liquid to powder

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Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Xã hội và Nhân văn
ISSN: 2588-1043
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 62%
Phí xuất bản: Miễn thu phí đối với tác giả là CBVC của ĐHKHXHNV, ĐHQG-HCM; Tác giả khác: 500.000 VNĐ/bài
Thời gian phản biện: 75 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit
SCAN ME


ISSN 2588-1078
Sci. Tech. Dev. J.-Sci. Earth Environ.
Issue 1, Vol 3, 2019
Science and Technology Development Journal
SCIENCE OF THE EARTH & ENVIRONMENT
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Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Trái đất và Môi trường
ISSN: **2588-1078**
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt và tiếng Anh
Tỉ lệ chấp nhận đăng 2021: 87%
Phí xuất bản: liên hệ tòa soạn
Thời gian phản biện: 45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit
SCAN ME


ISSN 2734-9446
Science and Technology Development Journal
HEALTH SCIENCES
A sub-journal of Science and Technology Development Journal
Indexed & Abstracted by:
Google Scholar, OCLC
Worldcat, Dimension, Scilit
http://stdjhs.scienceandtechnology.com.vn

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Sức khỏe
ISSN: 2734-9446
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 70%
Phí xuất bản: Miễn phí
Thời gian phản biện: 30 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit
SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ, Đại học Quốc gia Tp.HCM
25 năm xuất bản học thuật (1997-2022)

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