Sea urchin *Tripneustes gratilla* is one of the valuable fishery products in Taiwan. The edible part of sea urchin is about 10% and the rest is regarded as waste. In this study, sea urchin shell was used here as an ingredient to synthesize magnesium substituted beta-tricalcium phosphate (β-TCMP). Shell powder of the echnoid *Tripneustes gratilla* was converted by hydrothermal reaction at 180°C for 24 h (referred as SU-180-24 product), and calcinated into tablet at 800°C for 4 h to form the target ingredient (SU-800-4 product). Then these products were tested for chemical composition analysis and bioassays. These products are confirmed to be rich in magnesium and constitute as β-TCMP. Results from bioassays showed that SU-180-24 and SU-800-4 products increased cell viability in human osteosarcoma cell (MG-63). Alkaline phosphatase (ALP) activity of MG-63 products are confirmed to be rich in magnesium and constitute as β-TCMP. Results from bioassays showed that SU-180-24 and SU-800-4 products increased cell viability in human osteosarcoma cell (MG-63). Alkaline phosphatase (ALP) activity of MG-63 cell cultured with SU-800-4 tablet also showed significant increase when compared to commercial β-TCMP. It indicated that sea urchin’s β-TCMP materials including SU-180-24 and SU-800-4 products exhibited the potential for applying as the bone graft material.

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Key-words: *Tripneustes gratilla*, Magnesium substituted beta-tricalcium phosphate, Hydrothermal reaction, Biomaterial

1. Introduction

Autogenous graft is the first bone graft applied to bone regeneration since it is divided from the patient itself. This material doesn’t occur any immune responses even though autogenous graft is the golden material for bone implant. The disadvantage of autogenous graft is its limited content. Other bone grafts also encounter problems such as immune responses, infectious diseases or tumor.1) For those reasons, bioimercial bone substitutes received wide attention and have been developed for many years for avoiding those disadvantages and reducing the cost of bone implant.2) Bone grafting is a vital component to facilitate the repair of bone defects or fusions. Calcium-based bone graft substitutes provide surgeons with an alternative or an additional material to graft the site and participate in the healing process.3),4) Bioceramics like tricalcium phosphate (TCP) and hydroxyapatite (HA), which are ceramic with chemical composition similar to the mineral components of bone, have received attention for their biocompatibility and osteoconductivity.5) An optimal scaffold for bone tissue replacement should fulfill several criteria, (1) appropriate pore size distribution, (2) interconnectivity of pores and (3) surface porosity.5) At the very least a scaffold should have sufficient stability to maintain its architecture during in vitro culturing and handling throughout implantation.5) HA and other calcium derivatives (TCP, etc.) are the most commonly used calcium phosphates, due to their calcium/phosphorus (Ca/P) ratios being close to that of natural bone and their stability being high when in contact with physiological environment.3),4) Calcium-based bone graft substitutes provide surgeons with an alternative or an additional material to graft the site and participate in the healing process. Beta tricalcium phosphate (β-TCP) ceramic, due to its bio-compactibility, resorbability and chemical stability in vivo, is one of the most used material when applied to bone graft. It can be obtained from different commercial source with same chemistry but with differences in handling characteristics and resorption rate.10) The chemical synthesized pathway is the most common way to obtain ceramic ingredient.11) Using CaCO3 as template to transfer into different ceramics is one of the most used way to obtain biomedical material.12) The main product via transformation by using CaCO3 are mainly HA and TCP. Both of them have good bio competitive, no inflammatory effect and available in multiple forms.13) They had been applied to bone implant industry for many decades.13)–15)

Sea urchin body wall is mainly composed of CaCO3. Since its body wall is rich in Mg, the composition of CaCO3 was substituted by (Ca,Mg)CO3, thus magnesium substituted tricalcium phosphate [(Ca,Mg)3(PO4)2, β-TCMP] is formed after hydrothermal reaction with phosphate solution. In previous study, researchers using pencil sea urchin spine as template with hydrothermal reaction to transfer spine into β-TCMP, and proofed that this material could offer better compression ability when compared to commercial β-TCMP.15)–18) To utilize sea urchin shell, the potentials on biomaterial availability of calcinated products from sea urchin shell were evaluated.
2. Materials and methods

2.1 Sample collection

Sea urchins (Tripneustes gratilla) were collected from the Bi-sha seafood market in Keelung. All samples were dissected, separated into meat (gonad) and shells. Sea urchin shell was brushed and washed by water at 4°C for 1 h. Shells were then immersed in 1% NaOCl for 24 h to remove residue, and then washed with de-ionized water to eliminate NaOCl. Then, the shells were freeze-dried and crushed into powder (abbreviated as SU-Raw product) and stored at room temperature for further use.

2.2 Material transformation by hydrothermal reaction and modeling

The transformation was processed by hydrothermal reaction, which was referred from Vecchio et al. with slight modification. After SU-Raw product was cleaned with de-ionized water and freeze-dried, 4 g of SU-Raw powder were sealed into a bomb furnace (RMF-20, Taipei, Taiwan) at 600 and 800°C for 4 h and 1050°C for 2 h. After calcination, the calcinated tablets were washed by de-ionized water until the pH reach neutral and abbreviated as SU-180-24, SU-180-48 and SU-180-96 product depending on reaction time.

After hydrothermal reaction, SU-180-24 product was chosen for next evaluation, pressed into tablet and calcinated by muffle furnace (RMF-20, Taipei, Taiwan) at 600 and 800°C for 4 h and 1050°C for 2 h. After calcination, the calcinated tablets were collected and abbreviated as SU-600-4, SU-800-4 and SU-1050-2 product, respectively. These samples were stored at room temperature until use for biomedical availability assay.

2.3 Phase characteristics of products processed with hydrothermal reaction and calcination

2.3.1 Powder X-ray diffraction analysis

The phase characterization was conducted by X-ray diffraction analysis (XRD, MD-10, USA) using monochromatic CuKα radiation at a step size of 0.02°, 2θ = 15–70°, voltage at 25 kV and electric current at 25 mA. Standards for this experiment were calcium carbonate (CaCO₃), hydroxyapatite [(CaHPO₄)₂(OH)₂, HA] and β-tricalcium phosphate [β-Ca₃(PO₄)₂, β-TCP], purchased from Sigma (St. Louis, MO, USA).

2.3.2 Analysis of chemical groups

An analysis of chemical groups was conducted using the Fourier Transformation Infrared Spectrometer (FTIR, FTS 155 Win-nr, Bio-Rad, USA). Infrared spectra of KBr or sample mixtures were obtained over the frequency range of 650 to 4,000 cm⁻¹ at a resolution of 4 cm⁻¹. The sample was thoroughly mixed with KBr (100:1, v:v), and the dried mixture was then ground and pressed to generate a sample disk.

2.4 Metal content analysis

0.2 g of SU-180-24 product was subjected to 10 ml of concentrated nitric acid, put in a glass vial and placed on a hot plate (90°C) for 4 h. Then, all samples were quantified with Milli-Q water into 20 ml and immediately tested for metal content by using the Inductively Coupled Plasma-optical Emission Spectrometry (ICP-OES, Perkin Elmer Optima 2000, USA).

2.5 Element dissolution test

Each 0.1 g sample of SU-Raw and SU-180-24 product was immersed into 10 ml 0.1 M phosphate buffer (pH = 7.4) and then 1 ml of the solution was collected at each time point (on day 0 (30 min), 1, 2, 3, 4, 7 and 14). All samples were diluted to appropriate concentration and tested for the calcium ion by using ICP-OES.

2.6 Scanning electron microscopy

The morphology of the sample surface was studied by scanning electron microscopy (SEM) using a Hitachi SEM S-8800 (Hitachi, Tokyo, Japan). The elemental compositions of the products were qualitatively identified by energy dispersive X-ray spectroscopy (EDS) in SEM.

2.7 Cell viability evaluation

2.7.1 Cell culture

Human osteogenic sarcoma (MG63) cell lines were obtained from Bioresource Collection and Research Center (Shinchu, Taiwan). Cells were incubated in modified eagle medium (MEM) with 10% fetal bovine serum in an incubator (37°C, 5% CO₂), and subcultured every 3 to 5 days. After incubation, cells were treated with trypsin-EDTA and planted in a cell culture dish at a density of 5 × 10³ cells per well for 96-well plates and 5 × 10⁴ cells per well for 24-well plates for further study.

2.7.2 Cell viability assay

Cell viability was performed with a WST-1 [4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzene disulfonate] assay. After treatment of the different samples (0.01 g sample/ml) for 24 and 48 h, each well was mixed with 10 µl WST-1 solution and incubated at 37°C for 2 h. After incubation, 90 µl supernatants were transferred into new plate, inspected with ELISA and tested for 450 nm absorbance. Cell viability was presented as a percentage and calculated using the following formula:

\[
\text{Cell viability (\%) = } (\text{Ac} - \text{Ab}) / (\text{Ac} - \text{Ab}) \times 100\%
\]

As : sample group absorbance.
Ac : control group absorbance.
Ab : sample blank absorbance.

2.7.3 Alkaline phosphatase releasing evaluation

Alkaline phosphatase releasing evaluation was referred from Ishang-Riley et al. with slight modification. Briefly, the MG63 cell was used here for alkaline phosphate (ALP) activity test. MG63 cell was co-incubated with transferred biomaterial SU-800-4 product and tested for the ALP content on the 1st, 4th, 7th and 14th day. The absorbance was tested for 405 nm. Average ALP activity (U/ml) = A/V/T

A (µmol) = pNP produced by sample
V (ml) = Sample total volume of each well
T (minutes) = Reaction time

2.8 Statistical analysis

The data were presented as mean ± SD. The differences between the mean values were analyzed by a one-way analysis of variance (ANOVA) followed by the Duncan test at p = 0.05. The statistical analysis was performed by SPSS 10.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

The different shell powders of sea urchin T. gratilla were obtained from different hydrothermal reaction as SU-Raw, SU-180-24, SU-180-48 and SU-180-96 products. In phase characterization of those products, FTIR and XRD were used for identifying the chemical composition. The results are shown as Figs. 1 and 2. Figure 1 showed the FTIR spectra of before and
after hydrothermal reactions of sea urchin shell powder. SU-Raw product showed the same spectra when compared to CaCO₃. The pure calcium carbonate showed the presence of strong bands centered around 710, 870 and 1395 cm⁻¹ for C-O bonding. After hydrothermal reaction at 180°C for 24, 48 and 96 h, three spectra of SU-180-24, SU-180-48 and SU-180-96 products showed the same peaks in 568, 607, 1044 and 1089 cm⁻¹, which were similar to those of commercial β-TCP. As shown in Fig. 2, the XRD spectra of SU-Raw product exhibited the similar pattern when compared to CaCO₃, which was recognized in 2 theta = 29.86, 36.43, 40.23, 43.69, 47.67 and 48.65 [Fig. 2(a)]. After transformation, SU-180-24 product showed the same spectra when compared to SU-180-48 and SU-180-96 products, which displayed signals in 2 theta = 17.74, 26.37, 28.28, 31.03, 34.25 and 52.79. While comparison with commercial β-TCP, the XRD spectrum of SU-180-24 product was similar pattern with a little shift to right side [Fig. 2(b)]. Thus it indicated that SU-180-24 product had transferred into tricalcium phosphate and the reaction was completed after 24 h reaction at 180°C. Based on those results, SU-180-24 product was chosen for calcination. After calcination, the XRD spectra of SU-600-4, SU-800-4 and SU-1050-2 products were shown in Fig. 2(c). The spectra were the same with those of SU-180-24 product, indicating no structure transformation occurred after calcination at higher temperatures than 600°C for different times.

The metal contents were further tested by ICP-OES. SU-180-24 products was found to be rich in Ba, Mn, Mg and Sr, specifically for Mg (Table 1), while Se, Pb, Cd, Ni, Cr, Zr, Ti and Li were not detected (LOD < 10 ppb). Those ingredients indicated that the sea urchin powders were rich in Mg, Si and Sr.
ion (465,300 ± 1,300, 229,800 ± 2,700 and 40,500 ± 1,000 ppb, respectively). Since Zn, Ba, Mn, Mg, Sr were detected in the product here and Ca was also important, those elements were chosen to test the dissolution variation of SU-Raw and SU-180-24 products. The results are shown in Table 2. Within 14-day experiment period, the contents of Ca, Mg and Si ion increased in PBS solvents gradually in SU-180-24 product, while Zn, Ba and Sr showed slight release trend after 14 days. Noted that Mg, Sr for SU-Raw and Ca for both group showed highly ion released phenomenon in the day 0 (30 min) and decreased in day 1, then raised after day 3. The Mg, Ca and Si ion content on day 14 reached to 18,704, 1,145 ± 120 and 1,673 ± 55 ppb for SU-180-24 product, respectively. The Zn, Ba and Sr ion content on day 14 reached to 18 ± 1, 28 ± 1 and 30 ± 3 ppb for SU-180-24, respectively. SU-Raw product showed release activity on day 0 (30 min) with high Ca concentration (17,660 ± 1,000 ppb), but significantly decreased to 2,804 ± 70 ppb on day 3, and then slightly increased to 5,299 ± 704 ppb on day 14. While Mg ion content showed the same trend when compared to Ca ion in SU-Raw product, the Mg ion was recorded for 5,380 ± 228 ppb on day 0 (30 min), and gradually decreased to 4,778 ± 1,453 ppb on day 14. Zn, Ba and Mn were not detected after 14 days in SU-Raw product (Fig. 6).

The surfaces of samples were further observed by SEM which were shown in Fig. 3. The surface of SU-180-24 product was rough. The surface of SU-600-4, SU-800-4 and SU-1050-2 products were smoother when compared to SU-180-24 product which was observed by SEM. The surface of product was getting more compactness with temperature elevated up. The porousities of SU-600-4, SU-800-4 and SU-1050-2 products were less than 5 µm, and SU-1050-2 product showed the most compactness in pore distribution. The result of element weight percentage of

Table 1. Analysis of metal elements in SU-180-24 product by using inductively coupled plasma-optical emission spectrometer

| Element          | SU-180-24 (ppb) |
|------------------|-----------------|
| Selenium (Se)    | N.D.            |
| Zinc (Zn)        | 300 ± 100       |
| Lead (Pb)        | N.D.            |
| Cadmium (Cd)     | N.D.            |
| Nickel (Ni)      | N.D.            |
| Barium (Ba)      | 6,100 ± 500     |
| Manganese (Mn)   | 7,200 ± 700     |
| Chromium (Cr)    | N.D.            |
| Magnesium (Mg)   | 465,300 ± 1,300 |
| Zirconium (Zr)   | N.D.            |
| Titanium (Ti)    | N.D.            |
| Strontium (Sr)   | 40,500 ± 1,000  |
| Lithium (Li)     | N.D.            |
| Silicon (Si)     | 229,800 ± 2,700 |

*N.D.: not detected (<10 ppb).

Table 2. Dissolution test of elements (Zn, Ba, Mn, Mg, Ca and Sr) in SU-Raw and SU-180-24 products after 0, 1, 2, 3, 4, 7 and 14 days (ppb)

| Sample | Day | Zinc (Zn) | Barium (Ba) | Manganese (Mn) | Magnesium (Mg) | Calcium (Ca) | Strontium (Sr) | Silicon (Si) |
|--------|-----|-----------|-------------|----------------|----------------|--------------|---------------|--------------|
| SU-Raw | 0   | N.D.      | N.D.        | N.D.           | 5,380 ± 228a   | 17,660 ± 1,000b | 192 ± 7c     | 376 ± 23d    |
|        | 1   | N.D.      | N.D.        | N.D.           | 3,643 ± 30a    | 4,317 ± 432bc | 56 ± 5bc     | 294 ± 47a    |
|        | 2   | N.D.      | N.D.        | N.D.           | 3,372 ± 185a   | 3,520 ± 460ab  | 42 ± 1b      | 417 ± 62a    |
|        | 3   | N.D.      | N.D.        | N.D.           | 3,145 ± 22a    | 2,804 ± 70a   | 36 ± 1a      | 713 ± 68c    |
|        | 4   | N.D.      | N.D.        | N.D.           | 3,346 ± 102a   | 2,960 ± 304a  | 37 ± 4a      | 753 ± 23c    |
|        | 7   | N.D.      | N.D.        | N.D.           | 3,613 ± 455a   | 3,599 ± 564ab | 46 ± 10b     | 1022 ± 31d   |
|        | 14  | N.D.      | N.D.        | N.D.           | 4,778 ± 1,453b | 5,299 ± 704c  | 69 ± 22c     | 1859 ± 33e   |
| SU-180-24 | 0   | N.D.      | 18 ± 1a    | N.D.           | 11,811 ± 318a  | 1,617 ± 99c   | 32 ± 1b      | 236 ± 62a    |
|        | 1   | 11 ± 1a   | 24 ± 8a    | N.D.           | 22,807 ± 1,202b | 538 ± 76a   | 28 ± 2a      | 272 ± 10b    |
|        | 2   | 11 ± 1a   | 28 ± 2a    | N.D.           | 30,536 ± 1,024b | 618 ± 60b   | 29 ± 2b      | 505 ± 17b    |
|        | 3   | 13 ± 1b   | 21 ± 6a    | N.D.           | 32,384 ± 1,093d | 495 ± 116c  | 27 ± 1a      | 675 ± 26c    |
|        | 4   | 15 ± 1c   | 23 ± 3a    | N.D.           | 34,722 ± 119g   | 578 ± 53b   | 29 ± 1b      | 668 ± 30b    |
|        | 7   | 17 ± 1d   | 27 ± 10d   | N.D.           | 36,438 ± 1,056d | 469 ± 88a   | 28 ± 2a      | 857 ± 89d    |
|        | 14  | 18 ± 1d   | 28 ± 1a    | N.D.           | 42,813 ± 2,074c | 1,145 ± 120b | 30 ± 3b      | 1673 ± 55c   |

*N.D.: not detected (<10 ppb).
The qualitative results indicated the element distributed in SU-Raw surface were mainly calcium and oxygen (37.31 ± 3.27% and 60.02 ± 3.45%, respectively), with small amount of magnesium, strontium and silicon (1.75 ± 0.18%, 0.95 ± 0.20% and 0.34 ± 0.07%, respectively).

In SU-180-24 surface, the elements were mainly calcium, oxygen and phosphorous (32.11 ± 1.21%, 46.51 ± 1.76% and 18.62 ± 0.16%, respectively), with small amount of magnesium, strontium and silicon (1.46 ± 0.26%, 0.98 ± 0.25% and 0.33 ± 0.04%, respectively).

Before evaluating biomedical availability of SU-800-4 product, the viability of SU-180-24 product to MG-63 cell was evaluated. After 24 and 48 h co-incubated, SU-180-24 product showed no toxicity toward MG-63 cells when compared to the standard commercial β-TCP (Fig. 4(a)). When co-incubated with SU-180-24 product, the cell viability was 91.52 ± 2.40% after 24 h and 101.19 ± 6.14% after 48 h. While co-incubated with in SU-Raw product, the cell viability were 79.88 ± 2.91% and 87.96 ± 11.63% for 24 and 48 h respectively, indicating the products of sea urchin shell after hydrothermal reaction are not toxic.

After hydrothermal reaction and calcination of sea urchin shell, SU-800-4 product who used to evaluate the material availability by MG-63 cell. The result is shown in Fig. 4(b). The viability increased with increasing of experiment periods and reached to 120.21 ± 5.21% on day 14. Meanwhile, the ALP activity were also evaluated in this period (Fig. 5). The ALP activity were significantly higher than control on day 7 (0.2442 ± 0.006 U/ml), and increased to 0.2463 ± 0.0002 U/ml after 14 days, indicating SU-800-4 product could enhance the viability of MG-63 cell growth and help bone regeneration.

4. Discussion

Using natural product as base to transfer into bio-medical material had been studied for many decades and had already applied into surgery regeneration for many years.20) By hydrothermal reaction, the base ingredient CaCO$_3$ could be transferred into ceramics like hydroxyapatites (HA) and tricalcium phosphates (TCP). Among the existing calcium orthophosphates, only certain compounds are useful for biomedical applications, because those having a Ca/P ionic ratio less than 1 are not suitable for implantation into the body due to their high solubility and acidity. In present, the most common used ceramic were hydroxyapatite (HA) and tricalcium phosphate (TCP).21) In previous study, there had been applied ox bone or coral exoskeleton to transfer to HA and implanted into animal body.22) Vecchio et al.16) have used sea urchin Heterocentrotus species...
spine to transfer into β-TCP and proved that it could be applied to animal bone implant, which was less absorbed in animal body and offered stronger compression force when compared to commercial β-TCP. In this study, T. gratilla’s body walls was used for the propose of increasing the availability of the shell. Since echinoid contained high amount of Mg, and the ossicle were composed of 54.9–95.8% CaCO₃ with 4.1–45.7% MgCO₃. In this experiment, sea urchin body wall was crushed, ground into powder and processed with phosphate buffer by hydrothermal reaction. After hydrothermal reaction, samples had been transferred into β-TCP. When compared to commercial β-TCP, the spectra of β-TCP product by XRD analysis showed the similar results with slightly shift to right side in SU-180-24 product, it may be resulted from the magnesium substituted to the calcium position to form (Ca, Mg)₅(PO₄)₃ structure. The lattice parameters of β-TCP were reported to be a=b (1.03596 to 1.03599 nm) and c (3.7175 to 3.7208 nm) depending on the Mg substitute rate. Besides, the lattice were decreased with the increase of the content of Mg⁺⁺ due to the smaller ionic radius of Mg⁺⁺ (0.65Å) compared to Ca⁺⁺ (0.99Å). This result is the same with that reported in previous studies. The FTIR and XRD spectra also indicated the product had been transferred into desired target within 24 h. Ceramic TCP material could exist in animal body for 4–8 months, which had the same period for bone remodeling cycle in human body. Meanwhile, β-TCP have been reported to exist in animal body for longer period when compared to commercial β-TCP. Hence, β-TCP is a proper bone implant for bone regeneration. Thus, sea urchin shell may be a good source of biomedical material for bone regeneration.

In order to avoid the heavy metal contamination, metal ion contents such as Pb, Cu and Cr were checked, and no harmful metal content was observed in this study. The protein content of SU-180-24 product was also tested with no protein residue detected (data not shown), indicating there’s no immune reaction suspect. On the other hand, the beneficial element for bone grafting, like Mg, Sr and Si, had been observed. Because the Mg content in sea urchin shell is rich and thus is easy to form Mg-riched calcite in the exo-skeleton. In this study, SU-180-24 product could release high amount of Mg⁺⁺ and Sr⁺⁺ ion during 14 days element dissolution test. Magnesium plays an important role in the mineralization of bone and in the promotion of bone formation. With high amount Mg⁺⁺ released from bone implant, it could stimulate bone to release Ca²⁺ into blood, hence the bone regeneration was accelerated. Furthermore, recent studies of Mg ion incorporation by ion-beam implantation in a range of biomaterials have established its stimulatory effect on bone formation, although it is unclear whether this is through its influence on surface chemistry or by direct interaction of Mg ions with cells. Sr has unique function on increasing many biofunctions in animal body, including pre-osteoblast proliferation, osteoblast differentiation and bone matrix mineralization. Si has been found to be essential for normal bone and cartilage growth and development. The high concentration of Si observed in extracellular matrix components implies a role for Si as a biological cross-linking agent that contributes to the architecture and resilience of connective tissue. On the other hand, since ossification, which was important in bone regeneration, required a lot amount of Ca ion for mineralization of osteogenic tissue. It’s important that implant could offer high amount of Ca ion during regeneration period. In this research, SU-180-24 product was found to release Ca ion gradually during experiment period, indicating this ingredient could offer sufficient metal ions during osteogenic duration.

After calcination, material surfaces were observed by SEM. SU-180-24 product showed the rough surface when compared to SU-600-4, SU-800-4 and SU-1050-2 products. The surface of SU-180-24, SU-600-4, SU-800-4 and SU-1050-2 products showed smooth surface and formed pores which the pore sizes were around 1–5 μm. Based on the pore size, these product materials could be applied in neovascularization or attracting cell adhesion and helping cell in growth.

The cell viability of sea urchin shell was tested by using MG-63 cells. It was found that the raw powder of sea urchin shell and its hydrothermal reaction product had no negative effect on MG-63 cells. On the other hand, the cell proliferation of sea urchin shell (SU-800-4) showed a significant increase in cell proliferation. Furthermore, ALP activity assay was further used to evaluate the enzyme releasing activity of SU-800-4 product for 14 day testing for ALP activity. ALP is an enzymes which will be produced by osteoblast which is differentiated from osteoprogenitor cells while bones regenerate. Those results indicating SU-800-4 product could enhance cell proliferation and increase ALP releasing activity during experiment period. Sader et al. reported to use chemical synthesized β-TCP and β-TCP as base to evaluate the proliferation effect on human osteoblasts and then indicated that β-TCP could significantly enhance SaOs2 cells adhesion and proliferation compared to β-
TCP. On the other hand, these ingredients can also combine with different bases such as stem cell or collagen to improve the cell adhesion and growth. Therefore, the SU-800-4 product from sea urchin body wall can be also evaluated with combination of different ingredients in the future.

5. Conclusion

Sea urchin T. gratilla's body wall was transferred into a biomedical material β-TCMP. This biomaterial showed potentials to be bone grafting by evaluated with human osteosarcoma cell, indicating this material could be applied to bio-medical usage.

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