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IN SITU SYNTHESIS OF COMPOSITE HYDROGEL BASED ON GELATIN AND CHITOSAN/ALGINATE/CHONDROITIN SULFATE FOR BONE REGENERATION

Specialization: Organic Chemistry

Code: 62440114

SUMMARY OF DOCTORAL THESIS IN CHEMISTRY

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The Doctoral thesis was defended at the Board of Examiners of Graduate University of Science and Technology-Vietnam Academy of Science and Technology at …..

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INTRODUCTION

1. The necessity of research

The injury caused by trauma is currently at an unprecedented high globally and ranks as the fourth leading cause of death across all age groups (6%). However, there have also been significant advancements in the field of musculoskeletal healthcare during the past few decades [1]. The development of biomaterials, which will eventually replace conventional graft materials like metals and alloys, is currently receiving more attention. The technique of bone tissue engineering (BTE) involves designing scaffold structures that mimic the natural bone structure such as hydrogel scaffolds. Hydrogels are composed of three-dimensional hydrophilic polymer chains, offering a suitable nutrient environment for the growth of endogenous cells because it can mimic the natural extracellular matrix (ECM) composition of bone. However, the inherent soft and elastic nature of hydrogels makes them less suitable for cases requiring high mechanical strength. Therefore, to improve the mechanical capabilities of hydrogels without losing their advantageous properties, the design of synthetic hydrogel materials with the advantages of hydrogels and additional reinforcing components becomes crucial. Researchers have focused on studying graft materials for regeneration based on composite hydrogels containing biphasic calcium phosphate (BCP), as BCP exhibits excellent biocompatibility, high biological activity, and bone healing potential due to its resemblance to the mineral component of bones. Moreover, BCP can gradually dissolve in the body to release Ca^{2+} and PO_4^{3-} ions, promoting bone cell development and growth. However, BCP in powder form with large particle sizes poses challenges in providing minerals to bones [2].

Based on the advantages of the hydrogel framework, combined with the ability to provide Ca^{2+} and PO_4^{3} ions to form bone minerals from BCP nanoparticles, the topic " **In situ synthesis of composite hydrogel based on gelatin and chitosan/alginate/chondroitin sulfate for bone regeneration**" was selected for study.

Research objective

In this study, we aimed to synthesize new materials based on gelatin with polysaccharides (chitosan, alginate, chondroitin sulfate) combined with nano biphasic calcium phosphate particles to create

materials that have biocompatibility, stimulate bone growth, have degradation time suitable for bone development time to be able to apply in the field of bone regeneration.

Research content

The research consists of Introduction, Conclusion, New Contributions of the Research and 3 chapters (Chapter 1: Literature Overview, Chapter 2: Research content, Chapter 3: Results and Discussions). The thesis has 6 tables of data, 53 figures and 3 related works have been published. The appendix includes 13 figures and tables.

CHAPTER 1. LITERATURE OVERVIEW

1.1. Bones and the treatment of bone-related problems

Bones are solid internal components that form a solid framework that is responsible for supporting, protecting, moving, creating blood and and serving as a mineral reservoir (calcium and phosphorus) that the body can mobilize when needed. [3].

Currently, the common methods of treating bone-related problems involve the use of materials and devices for bone fracture fixation (titanium, titanium alloy, stainless steel, polymer-based composite materials, bioceramic,...); as well as bone grafting techniques (autografts, allografts, xenografts, etc.). Some materials such as ceramics, metals, polymers, hydrogels, etc. can also be utilized as replacement materials in bone grafting [9,10].

1.2. Hydrogel

Hydrogel is a three-dimensional network of polymer chains with significant properties such as high water absorption capacity, biodegradability, and biocompatibility. The structure and composition of hydrogel can be modified based on the original materials and synthesis processes, making hydrogel a promising material in the field of biomaterials.

Hydrogel can be synthesized from natural polymers (gelatin, chitosan, chondroitin sulfate, etc.), synthetic or semi-synthetic [20]. Natural polymers often possess good biocompatibility and biodegradability, and most are water-soluble. Most of them are natural components of the extracellular matrix (ECM), and provide a conducive surface for cell adhesion, proliferation, and differentiation. Hydrogels can be synthesized through physical crosslinking (physical gelation) or chemical crosslinking (chemical gelation). In

particular, the Horseradish peroxidase (HRP) method has recently been used for the in-situ synthesis of various hydrogel types.

1.3. Composite hydrogels

Composite materials are synthesized from two or more different materials, resulting in a new material that combines the properties of the original materials. Synthesizing composite hydrogels by combining the favorable characteristics of polymer networks and auxiliary materials can enhance the mechanical properties of hydrogels without compromising their beneficial properties.

Osteogenic composite hydrogel materials possess physical properties that can stimulate cell growth and guide the bone healing. In composite materials commonly used to create composite hydrogels, biphasic calcium phosphate (BCP) has garnered attention due to its favorable influence on bone regeneration compared to single-phase hydroxyapatite (HAp) or β-tricalcium phosphate (β-TCP). BCP's dissolution rate is suitable for bone regeneration time, and it has the ability to stimulate bone formation.

1.4. Previous studies

- Domestic: In 2011, the research group of Tran Ngoc Quyen successfully synthesized in situ gel of chitosan derivative rutin-tyramine-chitosan-PEG under the presence of enzyme horseradish peroxidase (HRP) and hydrogen peroxide (H2O2) and applied it for wound healing in skin [2]. In 2014, Nguyen et al. synthesized composite hydrogel Hyaluronic acid (HyA)-Gelatin (Gel)/biphasic calcium phosphate (BCP) for bone regeneration. In 2015, Nguyen Thi Phuong, Institute of Materials Science and Application conducted a study on synthesizing new materials for bone grafting and regeneration based on biocomposite hydrogel consisting of biphasic calcium phosphate and biopolymer (gelatin, chitosan) for bone regeneration [3].
- Abroad: Some foreign research works:

Barbani et al. (2012): "Hydroxyapatite/gelatin/gellan sponges as nanocomposite scaffolds for bone reconstruction" Hunter et al. (2013) evaluated the development and differentiation of human bone stem cells in culture conditions (in vitro) of hydroxyapatite–chitosan–gelatin composite membrane

Pasqui et al. (2014) studied Carboxymethyl cellulose—hydroxyapatite hybrid hydrogel for bone material

Derakhshan et al. (2015) synthesized hydrogel based on chondroitin sulfate combined with hydroappatite for bone tissue engineering

Bhisham et al. (2019) developed chitosan and chondroitin sulfate hydrogel system combined with bio-glass with nano size to overcome the disadvantages of mechanical properties and lack of stability in the structure of chitosan

CHAPTER 2. RESEARCH CONTENT

2.1. Research Content

- Synthesis of materials: preparation and investigation of the properties of BCP nanomineral particles.
- Synthesize and evaluate the structure and morphology of gelatin-tyramine (GTA) hydrogels and nanocomposite hydrogels.
- Synthesis and evaluation of structure, morphology of chitosan 4-hydroxyphenylacetic acid (CHPA) hydrogels and nanocomposite hydrogels.
- Synthesize and evaluate the structure, morphology of alginate-tyramine (ATA) hydrogels and nanocomposite hydrogels.
- Synthesize and evaluate the structure and morphology of chondroitin sulfate-tyramine (CDTA) composite hydrogels and hydrogels
- Synthesis and determination of properties of hydrogel and nanocomposite hydrogel based on GTA with CHPA, ATA, CDTA materials with different ratios.
- Evaluation of the mineralization ability of hydrogel and nanocomposite hydrogel based on GTA with each material CHPA, ATA, CDTA with different ratios.
- Comparison of degradation, biocompatibility and mineralization of 3 nanocomposite hydrogel matrices.

2.2. Research Methodology

- Synthesis of BCP by ultrasonic method with Ca/P ratio: 1.57 and pH = 7. XRD analysis for component and structure determination. SEM method for particle size and morphology of BCP.
- Synthesis of hydrogels GTA, CHPA, ATA, CDTA by mixing method using HRP and H_2O_2 enzymes
- Determine the content of TA in hydrogels GTA, ATA, CDTA by UV-Vis method.
- Synthesis of nanocomposite hydrogels GTA/BCP, CHPA-GTA/BCP, GTA-ATA/BCP, GTA-CDTA/BCP by mixing method using HRP and H_2O_2 enzymes.
- Determine the product structure by ^{1}H-NMR nuclear magnetic resonance spectroscopy and FT-IR Fourier transform infrared spectroscopy.
- Morphological investigation of hydrogel and nanocomposite hydrogel by FESEM
- Investigate the gelation time of hydrogel and nanocomposite hydrogel by time forming a solid gel and when upside down the material does not flow down for 1 minute
- Surveying the weight loss of hydrogel and nanocomposite hydrogel in a simulated biological environment (PBS solution containing collagenase enzyme)
- Evaluation of the mineralization ability of hydrogels and nanocomposite hydrogels by EDS measurement
- Cell toxicity assessment on nanocomposite hydrogel materials

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1. BCP Synthesis

Phase structure analysis of BCP by XRD diffraction method showed that with mol Ca/P ratio = 1.57 at pH = 7, BCP (β-TCP and HAp) was successfully synthesized. The morphological results of the synthesized BCP were observed using scanning electron microscopy (SEM), indicating that the product had nano size, the particle size was relatively uniform and ranged from 60-100 nm.

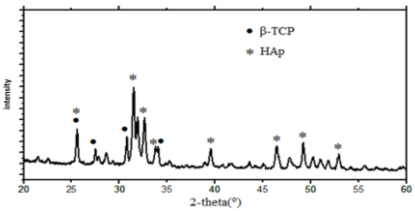


Figure 3.1. XRD pattern of BCP with mol ratio Ca/P = 1.57 at pH = 7

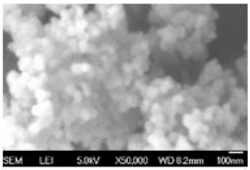


Figure 3.2. SEM image of BCP

3.2. GTA Synthesis Results

- The 1H-NMR spectrum of GTA in D2O and the FTIR spectra of Gelatin, Tyramine and GTA confirmed the successful synthesis of GTA.

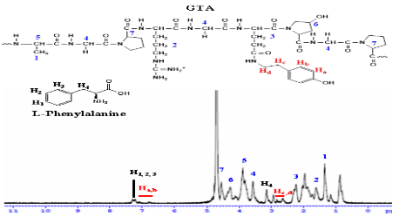


Figure 3.3. GTA ^{1}H-NMR spectrum in D_2O

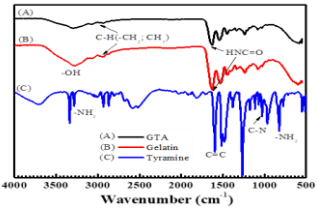


Figure 3.4. FTIR spectrum of GTA

The results of measuring the absorbance A of TA and GTA obtained by UV-Vis (λ = 275 nm) show that the minimum amount of H2O2 needed to react with TA in 10 mg GTA to form gel is 0.014% in 10% GTA solution (not higher than 0.25% because it will be toxic to cells).

3.3. CHPA Synthesis

3.3.1. Product Structural Determination

- The ^{1}H-NMR spectrum of CHPA in D_2O and the FTIR spectrum of CHPA show that CHPA has been synthesized successfully.

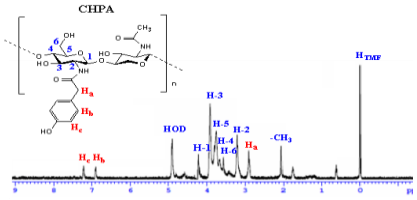


Figure 3.5. CHPA ^{1}H-NMR spectrum in D_2O

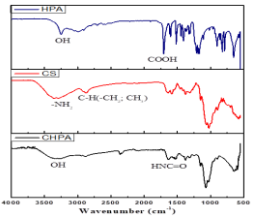


Figure 3.4. FTIR spectrum of CHPA

- The results of measuring absorbance (A) of HPA and CHPA obtained by UV-Vis (λ = 275 nm) show that the minimum amount of H2O2 required to react with HPA in 10 mg of CHPA to form a gel is 0.046% in CHPA 5% solution (not exceeding 0.25% to avoid cytotoxic effects on cells).

3.3.2. Properties Investigation of CHPA-GTA/BCP Hydrogel and Hydrogel Nanocomposite

Gelation time

The results show that the gelation time of GTA hydrogel is quite variable. When increasing the concentration of H2O2 up to 0.05%, the gel formation increased in proportion to the decrease in gelation time. With the concentration of H2O2 from 0.05 to 0.07%, the gel formation process slows down corresponding to the increasing gelation time. The gelation time of the nanocomposite hydrogel did not change much compared with that of the hydrogel.

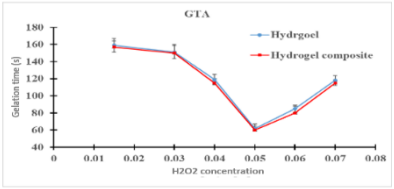


Figure 3.5. Gelation time of GTA hydrogel and nanocomposite hydrogel with HRP concentration 0.05 mg/mL

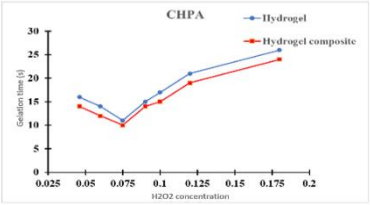


Figure 3.6.. Gelation time of CHPA hydrogel and nanocomposite hydrogel with HRP concentration 0.07 mg/mL

- The results of surveying the gelation time of hydrogel and CHPA-GTA/BCP nanocomposite hydrogel at the ratio 1:1 with the concentration of HRP 0.07 mg/ml show that the gelation time of the hydrogel is quite fast in a few minutes, and the concentration of H2O2, the amount of HRP had an influence on the gelation time

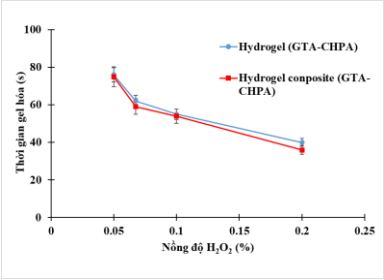
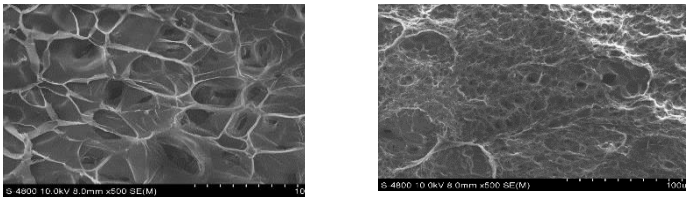


Figure 3.7. Gelation time of CHPA hydrogel and composite hydrogel at 1:1 ratio with HRP concentration 0.07 mg/mL

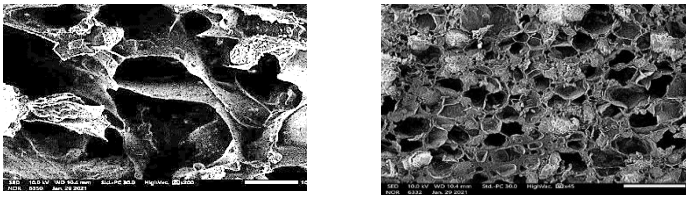
Morphology of CHPA-GTA hydrogel and CHPA-GTA/BCP composite hydrogel with 1:1 and 1:2 ratio

- SEM results at 100 µm magnification: the structure of the material consists of numerous pores with a three-dimensional structure, the size of the pores is about 30-50 µm. The size of these pores ranged from approximately 30 to 50 µm. In the case of the composite hydrogel, BCP particles were observed to cover the material's surface.



(a) **(b)**

Figure 3.8. SEM image of CHPA-GTA hydrogel at 1:1 (a) and 1:2 (b) ratio



(a) **(b)**

Figure 3.9. SEM image (a) of CHPA-GTA/BCP composite hydrogel 1:1 (a) and 1:2 (b)

The weight loss of CHPA-GTA hydrogel and CHPA-GTA/BCP composite hydrogel

- The results, as depicted in Figure 4.1, indicate that the degradation of GTA hydrogel occurred quite rapidly within 42 hours. As for the CHPA-GTA Hydrogel, an increase in the CHPA ratio resulted in a slower degradation rate (with a 2:1 ratio showing an 89.5% reduction over 762 hours). When BCP particles were added (Figure 4.2), the degradation proceeded was slower than that of the hydrogel.

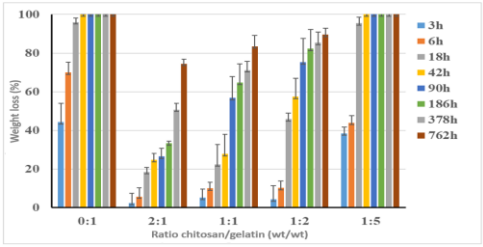


Chart 3.1. Chart of % weight loss of CHPA-GTA hydrogel by ratios (C/G) in PBS solution

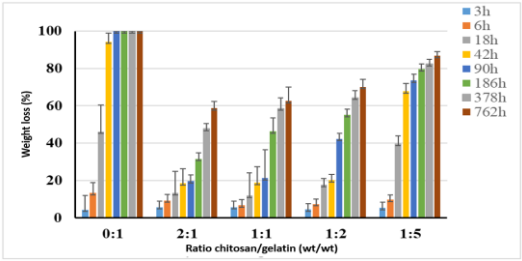


Chart 3.2. Chart of % reduction in weight of composite hydrogel by ratios (C/G) in PBS solution

Evaluation of mineralization ability of CHPA-GTA hydrogel and CHPA-GTA/BCP composite hydrogel

• XRD Diffraction Pattern:

- Structure analysis of CHPA-GTA hydrogel (1:1), GTA-CHPA (1:2) and CHPA-GTA/BCP composite hydrogel (1:1), CHPA-BCP/BCP (1:2) before and after soaking in SBF solution for 28 days showed that GTA-CHPA/BCP composite hydrogel was effective in the formation and development of carbonate apatite mineral.

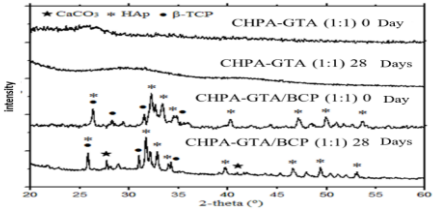


Figure 3.10. XRD pattern of CHPA- GTA (1:1) hydrogel and CHPA- GTA/BCP composite hydrogel (1:1) before and after soaking in SBF solution for 28 days

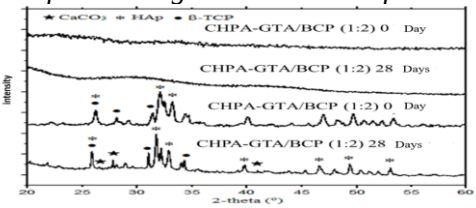


Figure 3.11. XRD pattern of CHPA- GTA (1:2) hydrogel and CHPA- GTA/BCP composite hydrogel (1:2) before and after soaking in SBF solution for 28 days

• Investigation of Carbonate Apatite Formation:

- From the EDS analysis results (Figure 3.16, 3.17, 3.18, 3.19), it can be observed that the CHPA-GTA/BCP composite hydrogel (1:1) and CHPA-GTA/BCP composite hydrogel (1:2) exhibit significantly higher mineral formation compared to the CHPA-GTA hydrogel (1:1) and CHPA-GTA hydrogel (1:2).

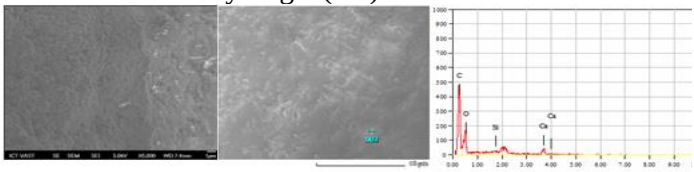


Figure 3.12. SEM and EDS results of CHPA-GTA hydrogel (1:1) after soaking in SBF solution for 28 days

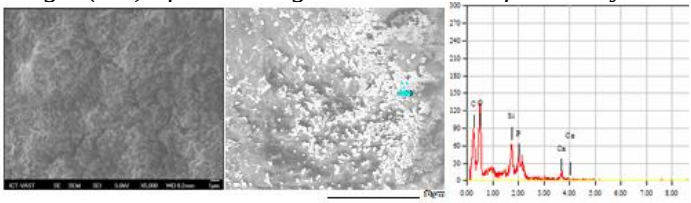


Figure 3.13.. SEM and EDS results of CHPA-GTA hydrogel (1:2) after soaking in SBF solution for 28 days

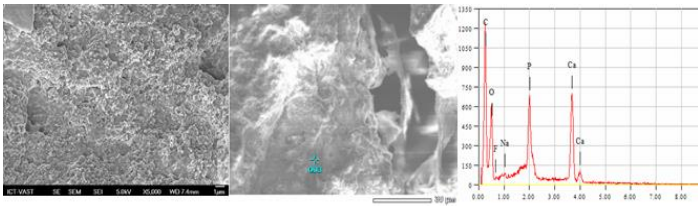


Figure 3.14. SEM and EDS of GTA-CHPA/BCP-1G:1C composite hydrogel after soaking in SBF solution for 28 days

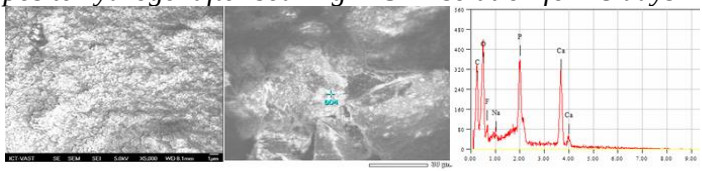
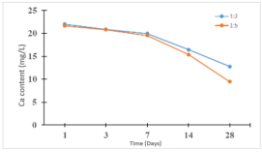


Figure 3.15. SEM and EDS of GTA-CHPA/BCP-2G:1C composite hydrogel after soaking in SBF solution for 28 days

• Examination of Ca and P Content in SBF Solution:

- Surveying the content of Ca, P in SBF solution after soaking hydrogel composite showed that after 1 day the amount of Ca, P ions increased, then the amount of Ca, P decreased steadily. The hydrogel composite samples showed no significant statistical difference between the 2 ratios.



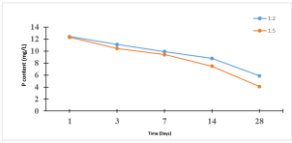


Figure 3.16. Ca content in SBF solution soaked composite hydrogel for 28 days with ratio 1:1 and 1:2

Figure 3.17. P content in SBF solution soaked composite hydrogel for 28 days with the ratio 1:1 and 1:2

Conclusion: Based on the results from EDS, XRD, and ICP analysis, the composite hydrogel material CHPA-GTA/BCP demonstrates a better ability to promote mineralization and apatite formation, which is more favorable for bone interaction compared to the hydrogel CHPA-GTA

. • Cell toxicity evaluation:

- Based on the MTT results and fluorescence images, it shows that the composite hydrogel material is safe, does not cause cell toxicity after degradation

3.4. Results of ATA Synthesis

3.4.1. Product Structural Determination

- The ^{1}H-NMR spectrum of CHPA in D_2O and the FTIR spectrum results of ATA prove that ATA has been successfully synthesized.

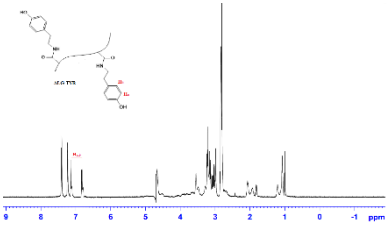


Figure 3.18. ^{1}H-NMR spectrum of ATA in D_2O

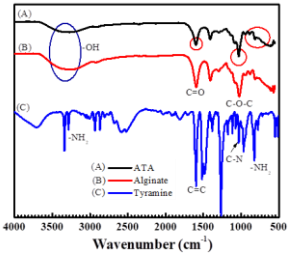


Figure 3.19. FTIR spectrum of ATA

- The results of measuring the absorbance (A) of TA in ATA by UV-Vis (λ = 275 nm) show that the minimum amount of H2O2 needed to react with TA in 10 mg ATA to form gel is 0.012% in 10% ATA solution (not higher than 0.25% because it will be toxic to cells).

3.4.2. The results of surveying the properties of hydrogel and nanocomposite hydrogel ATA-GTA/BCP

Gelation Time

- The results show that the gelation time of hydrogel is quite fast in a few minutes and changes according to the concentration of H2O2, ***nanocomposite*** hydrogel exhibits faster gel formation time than hydrogel.

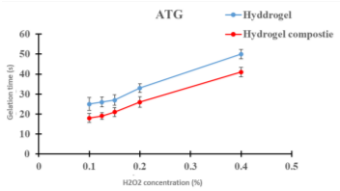


Figure 3.20. Gelation time of hydrogel and nanocomposite hydrogel ATA with HRP concentration 0.0125mg/ml mg/mL

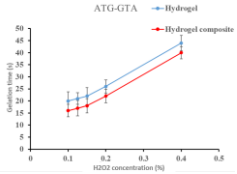
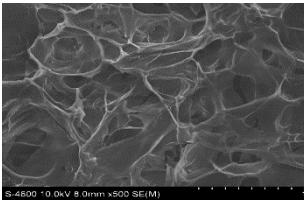


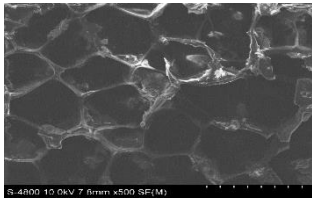
Figure 3.21. Gelation time of hydrogel and hydrogel nanocomposite ATA on GTA background with HRP concentration 0.0125mg/ml

The morphology of hydrogel ATA-GTA and hydrogel nanocomposite ATA-GTA/BCP

SEM results at a magnification of 100 µm reveal the structure of the material, featuring numerous porous voids with a three-dimensional spatial arrangement, with the pore size ranging around 20-40 µm. In the case of the nanocomposite hydrogel, SEM images show the uniform presence of nano-sized BCP particles covering the entire porous structure's surface

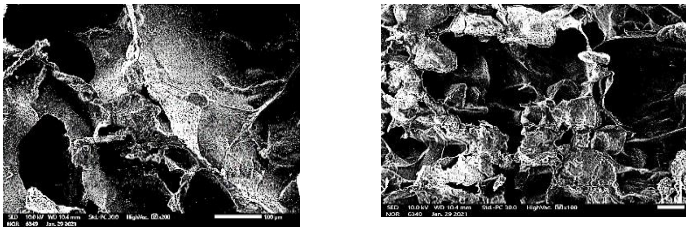


(a)



(b)

Figure 3.22. SEM image of ATA-GTA hydrogel 1:1 (a) and 1:2 (b)



(a) **(b)**

Figure 3.23. SEM image (a) of ATA-GTA/BCP nanocomposite hydrogel at 1:1 (a) and 1:2 (b) ratio

The weight loss of hydrogel CHPA-GTA and hydrogel nanocomposite CHPA-GTA/BCP

- *The results of surveying the weight loss comparing GTA with hydrogel of ATA on GTA base show that hydrogel GTA biodegrades very quickly. With nanocomposite hydrogel, when adding BCP particles, the weight loss occurs slower than hydrogel (chart 3.4).*

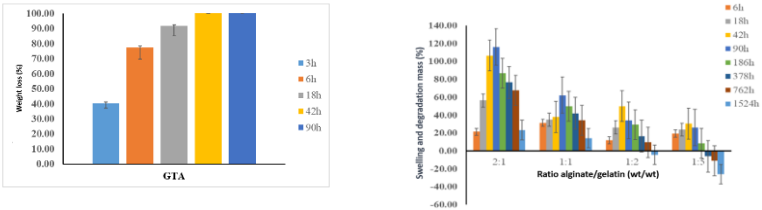


Chart 3.3. The graph of % weight loss of GTA and ATA hydrogels on GTA background by ratios (A/G) in PBS solution

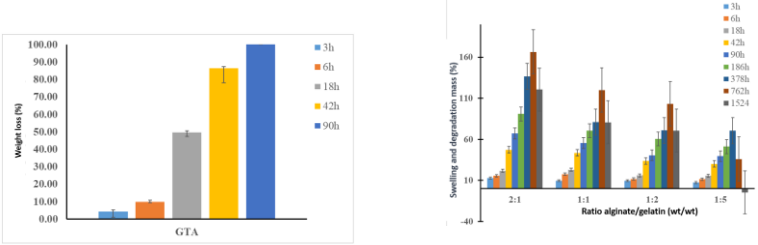


Chart 3.4. The graph of the percentage of biodegradation of GTA and ATA nanocomposite hydrogels on GTA background by ratios (A/G) in PBS solution

Đánh giá khả năng tạo khoáng của hydrogel ATA-GTA và hydrogel nanocomposite ATA-GTA/BCP

• XRD diffraction pattern

- Analysis of the structure of hydrogel CHPA-GTA (1:1), GTA-CHPA (1:2) and nanocomposite hydrogel CHPA-GTA/BCP (1:1), CHPA-BCP/BCP (1:2) before and after soaking in SBF solution for 28 days shows that hydrogel nanocomposite ATA-GTA/BCP is effective in the formation and development of carbonate apatite mineral.

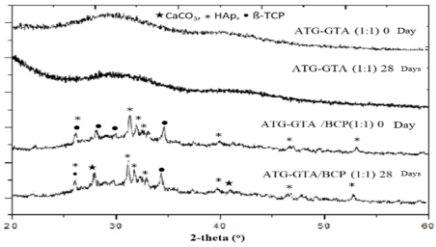


Figure 3.24. XRD pattern of hydrogel ATA- GTA (1:1) and nanocomposite hydrogel ATA- GTA/BCP (1:1) before and after soaking in SBF solution for 28 days

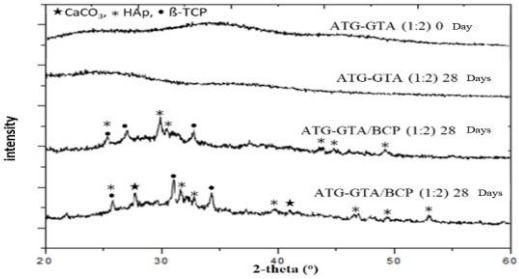


Figure 3.25. XRD patterns of hydrogel ATA- GTA (1:2) and nanocomposite hydrogel ATA- GTA/BCP (1:2) before and after soaking in SBF solution for 28 days

- **Surveying the formation of carbonate apatite mineral**

- From the EDS analysis results, it shows that nanocomposite hydrogel ATA-GTA/ BCP (1:1) and ATA-GTA/ BCP (1:2) have higher mineral formation than hydrogel ATA-GTA (1:1) and ATA-GTA (1:2).

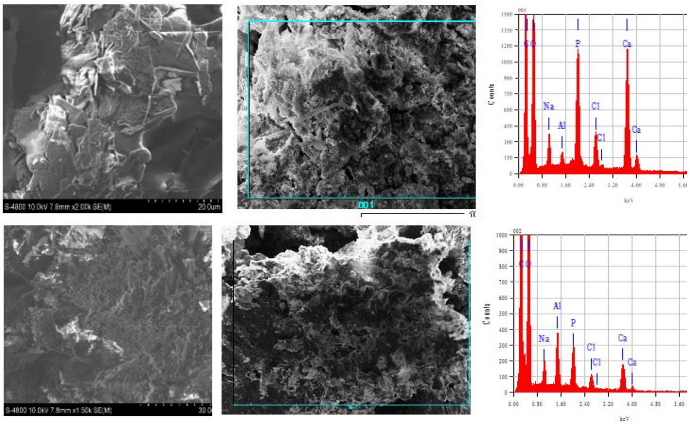


Figure 3.26. SEM and EDS results of ATA-GTA (1:1) and ATA-GTA (1:2) hydrogels after soaking in SBF solution for 28 days

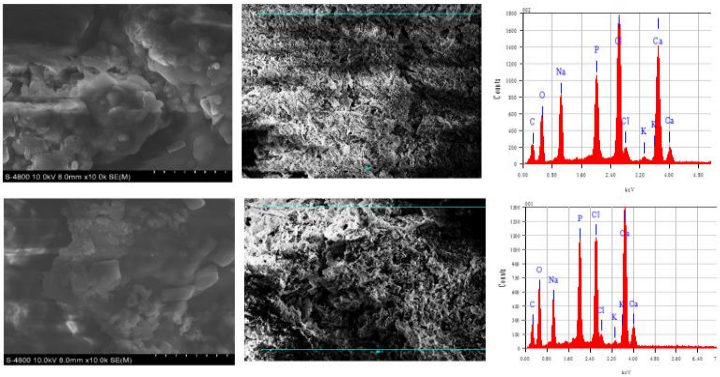


Figure 3.27. SEM and EDS results of ATA-GTA (1:1) and ATA-GTA (1:2) hydrogels after soaking in SBF solution for 28 days

- Surveying the content of Ca, P in SBF solution

- Surveying the content of Ca, P in SBF solution after soaking nanocomposite hydrogel shows that after 1 day, the amount of Ca, P ions increases, then the amount of Ca, P decreases steadily. The hydrogel nanocomposite samples show no significant statistical difference between the 2 ratios

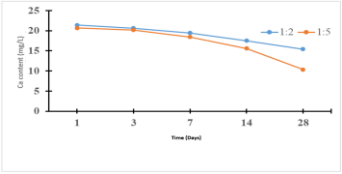


Figure 3.28. Ca content in SBF solution soaked in nanocomposite hydrogel for 28 days with ratio 1:1 and 1:2

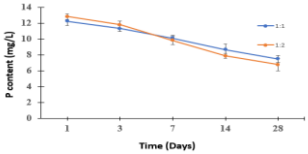


Figure 3.29. P content in SBF solution soaked in nanocomposite hydrogel for 28 days at the ratio 1:1 and 1:2

Conclusion: From the results of EDS, XRD and ICP analysis, it shows that nanocomposite hydrogel ATA-GTA/BCP has the ability to create mineral and form apatite that has a good effect on bone compared to hydrogel ATA-GTA.

- Cell toxicity evaluation:

Based on the MTT results and fluorescence images, it shows that hydrogel nanocomposite material is safe, does not cause cell toxicity after degradation.

3.5. Synthesis results of CDTA

3.5.1. Determining the structure of the products

- The ^{1}H-NMR spectrum of CDTA in D_2O and the FTIR spectrum results of CDTA prove that CDTA has been successfully synthesized.

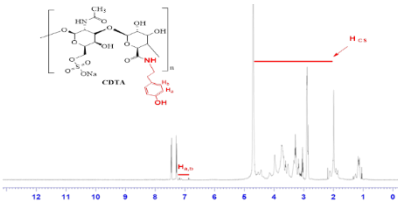


Figure 3.30. GTA ^{1}H-NMR spectrum in D2O

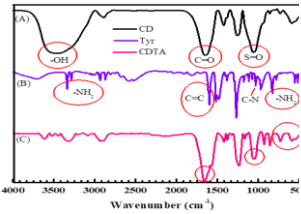


Figure 3.31. FT-IR spectrum of CD_Tyr. (A) CD; (B) Tyr; (C) CDTA

- The results of measuring the absorbance A of TA and CDTA obtained by UV-Vis (λ = 275 nm) show that the minimum amount of H2O2 needed to react with TA in 10 mg CDTA to form gel is

0.014% in 10% CDTA solution (not higher than 0.25% because it will be toxic to cells)

3.5.2. The results of surveying the properties of hydrogel and nanocomposite hydrogel CDTA-GTA/BCP

Gelation time

The graph shows that the gelation time of hydrogel is quite fast in a few minutes, and the amount of H2O2, the amount of HRP affect the gel formation time. For hydrogel nanocomposite CDTA-GTA /BCP (1:1), the gel formation time is faster from 29 to 94 seconds, but in general there is no significant change compared to hydrogel.

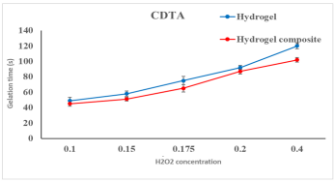


Figure 3.32. Gelation time of CDTA hydrogel and nanocomposite hydrogel with HRP concentration 0.125 mg/mL

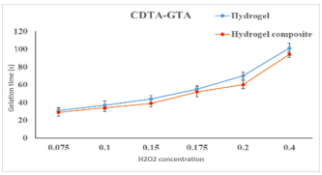
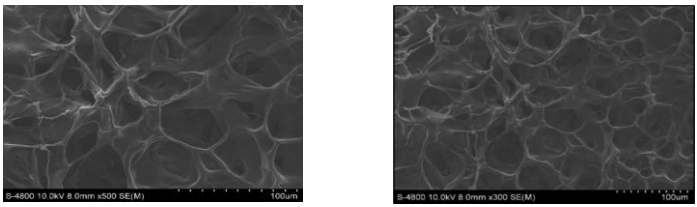


Figure 3.33. Gelation time of CDTA hydrogel and nanocomposite hydrogel on GTA background with HRP concentration 0.0125mg/ml

Morphology of hydrogel CDTA-GTA and hydrogel nanocomposite CDTA-GTA/BCP

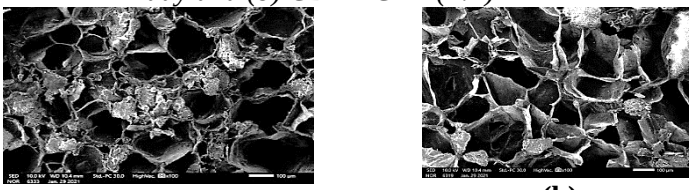
- Through the SEM results with a magnification of 100 µm, it shows that the structure of the material consists of many porous holes with a three-dimensional structure, the size of the porous holes is about 20-40 µm. In the case of hydrogel nanocomposite. SEM image shows the appearance of nano BCP particles evenly coated on the entire surface of the porous structure of the material



(a) **(b)**

Figure 3.34. SEM images (a) CDTA-GTA hydrogel (1:2)

day and (b) CDTA-GTA (2:1)



(a) **(b)**

Figure 3.35. SEM images (a) CDTA-GTA nanocomposite hydrogel (1:2) day and (b) CDTA-GTA (2:1)

Weight loss of hydrogel CDTA-GTA and hydrogel nanocomposite CDTA-GTA/BCP

- The comparison results between the 2 graphs of hydrogel and hydrogel nanocomposite show that, when adding BCP particles, the weight loss occurs slower than hydrogel

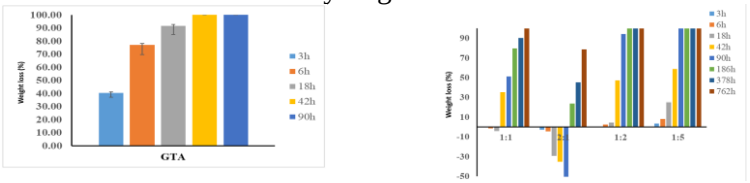


Chart 4.5. Histogram of % weight loss of GTA-based CDTA hydrogel by ratios (CD/G) in PBS solution with collagenase enzyme

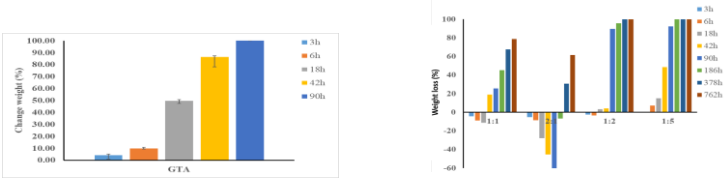


Chart 4.6. The graph of % weight loss of GTA and CDTA nanocomposite hydrogels on GTA platform by ratios (A/G) in PBS solution

Evaluation of mineralization ability of hydrogel CDTA-GTA and hydrogel nanocomposite CDTA-GTA/BCP

• XRD diffraction diagram

- Analysis of the structure of hydrogel CHPA-GTA (1:1), GTA-CHPA (1:2) and nanocomposite hydrogel CHPA-GTA/BCP (1:1), CHPA-BCP/BCP (1:2) before and after soaking in SBF solution for 28 days shows that nanocomposite hydrogel CDTA-GTA/BCP is

effective in the formation and development of carbonate apatite mineral

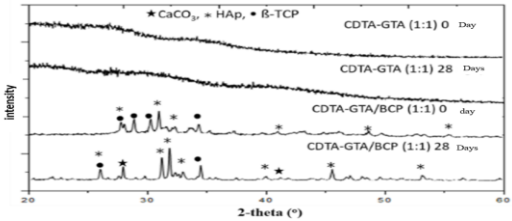


Figure 3.36. XRD pattern of CDTA- GTA hydrogel (1:1) and CDTA- GTA/BCP nanocomposite hydrogel (1:2) before and after soaking in SBF solution for 28 days

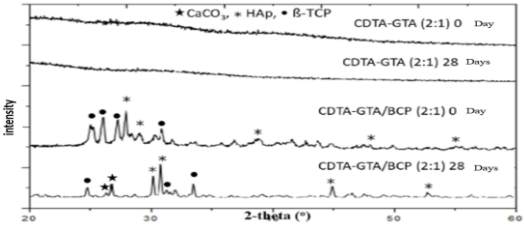
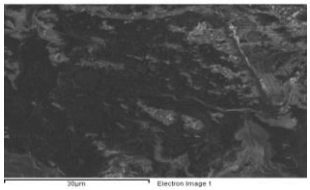


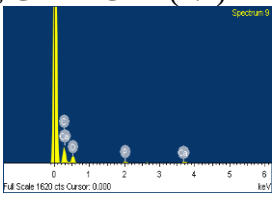
Figure 3.37. XRD patterns of CDTA- GTA (2:1) hydrogel and CDTA- GTA/BCP nanocomposite hydrogel (2:1) before and after soaking in SBF solution for 28 days

- **Survey of the formation of carbonate appatite mineral**

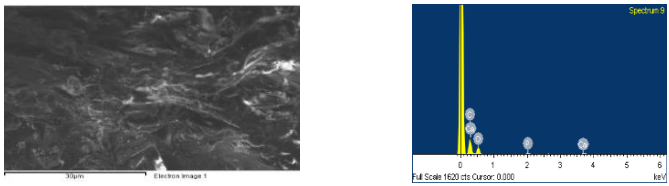
- The EDS analysis results show that nanocomposite hydrogel CDTA-GTA/BCP (1:1), CDTA-BCP/BCP (2:1) have higher mineral formation than hydrogel CDTA-GTA (1:1), CDTA-GTA (2:1)



(a)

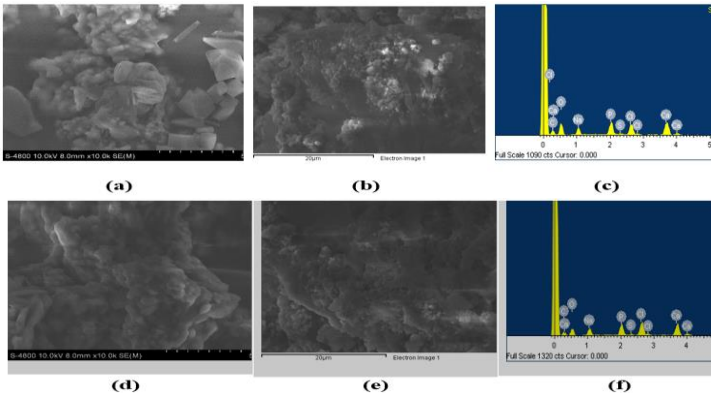


(b)



(c) **(d)**

Figure 3.38. SEM and EDS results of CDTA-GTA (1:1) and CDTA-GTA (2:1) hydrogels after soaking in SBF solution for 28 days



(a) **(b)** **(c)**

(d) **(e)** **(f)**

Figure 3.39. SEM and EDS results of CDTA-GTA nanocomposite hydrogels (1:1) and CDTA-GTA (2:1) after soaking in SBF solution for 28 days

• Survey of Ca, P content in SBF solution

- Survey of Ca, P content in SBF solution after soaking nanocomposite hydrogel shows that after 1 day the amount of Ca, P ions increases, then the amount of Ca, P decreases evenly. The nanocomposite hydrogel samples show no significant statistical difference between the 2 ratios.

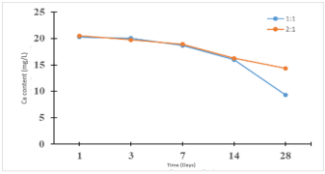


Figure 3.40. Ca content in

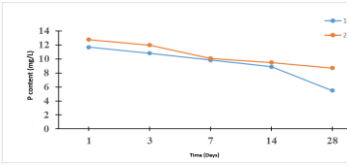


Figure 3.41. P content in

SBF solution soaked in nanocomposite hydrogel for 28 days with ratio 1:1 and 2:1

SBF solution soaked in nanocomposite hydrogel for 28 days at the ratio 1:1 and 2:1

Conclusion: From the results of EDS, XRD and ICP analysis, it shows that the CDTA-GTA/BCP nanocomposite hydrogel material has the ability to mineralize and form apatite that is better for bone than the CDTA-GTA hydrogel.

• Cell toxicity assessment:

Based on the MTT results and fluorescence images, the nanocomposite hydrogel material is safe, does not cause cell toxicity after degradation

CONCLUSION

- Material synthesis: synthesis and investigation of the characteristics of nano BCP mineral particles.
- Synthesis and evaluation of structure, morphology of hydrogels and gelatin-tyramine (GTA) nanocomposite hydrogels.
- Synthesis and evaluation of structure, morphology of hydrogels and 4-hydroxyphenylacetic acid chitosan (CHPA) nanocomposite hydrogels.
- Synthesis and evaluation of structure, morphology of hydrogels and alginate-tyramine (ATA) nanocomposite hydrogels.
- Synthesis and evaluation of structure, morphology of hydrogels and chondroitin sulfate-tyramine (CDTA) nanocomposite hydrogels.
- Study on synthesis and determination of properties of hydrogels and nanocomposite hydrogels based on GTA with each material CHPA, ATA, CDTA with different ratios.
- Evaluation of mineralization ability of hydrogels and nanocomposite hydrogels based on GTA with each material CHPA, ATA, CDTA with different ratios.
- Comparison of degradation, biocompatibility and mineralization of three nanonanocomposite hydrogel bases.

NEW CONTRIBUTIONS OF THE RESEARCH

- Successfully synthesized composite hydrogel materials based on gelatin with polysaccharides such as chitosan, alginate, chondroitin sulfate… combined with nano Biphasic calcium phosphate particles. The synthesized hydrogel products were evaluated for structure by methods such as H1NMR, FT-IR, SEM.
- Investigated the gel formation time of the materials as follows:
  • CHPA - GTA material: For hydrogel, nanocomposite hydrogel has the fastest gel formation time of 11s; 40s (investigated in H2O2 concentration from 0.05-0.2%)
  • ATA - GTA material: For hydrogel, nanocomposite hydrogel has the fastest gel formation time of 25s; 16s (investigated in H2O2 concentration from 0.05-0.2%)
  • CDTA - GTA material: For hydrogel, nanocomposite hydrogel has the fastest gel formation time of 43s; 46s (investigated in H2O2 concentration from 0.1-0.4%).

  The results show that when increasing the H2O2 concentration, the gel formation time will be prolonged due to the enzyme HRP being inhibited from forming cross-links in the polymer chain.

- In the simulated biological environment, the biodegradation time of 3 systems of hydrogel and nanocomposite hydrogel materials was investigated to be proportional to the amount of GTA used, because gelatin is a polymer that is easily hydrolyzed even in physiological and simulated biological environments, so the biodegradation process of the material takes place in a relatively short time. Therefore, combining GTA with polysaccharides (CHPA, ATA, CDTA) will help prolong the biodegradation time of the material. Therefore, depending on the needs and purposes of the material, the GTA ratio can be adjusted to have the desired biodegradation time in the simulated biological environment.
- Comparing between 2 materials with the same BCP ratio, CHPA-GTA and CDTA-GTA showed that CHPA has better mineralization ability. ATA showed the lowest mineralization ability among the three materials. This is explained by the structure of alginate having very high viscosity, making it difficult for nano BCP particles to disperse evenly in the material, leading to poor mineralization ability. For chitosan structure, electrostatic interaction

in chitosan and gelatin helps the material have better mineralization ability.

- Based on MTT results and fluorescence images, nanocomposite hydrogel materials on CHPA-GTA/BCP, ATA-GTA/BCP, CDTA-GTA/BCP systems (with different ratios) are safe, non-toxic to cells after degradation.

LIST OF PUBLICATIONS

1. Nguyen Vu Viet Linh, Nguyen Tien Thinh,Pham Trung Kien, Tran Ngoc Quyen, Huynh Dai Phu, Injectable Nanocomposite Hydrogels and Electrosprayed Nano(Micro) Particles for Biomedical Applications, Novel Biomaterials for Regenerative Medicine, 2018, 225-249, IF: 3.65, Q2
2. Nguyen Tien Thinh, Dang Le Hang, Tran Thi Yen Nhi, Sija Feng, Jun Chen, Nguyen Phuon, Tran Ngoc Quyen, Biphasic calcium phosphate embedded biomimetic hydrogel based chondroitin sulfate and gelatin as an injectable scaffold for bone regeneration, European Polymer Journal, 2023, 189: 111975, IF: 5.5, Q1
3. Tien Thinh Nguyen, Chan Khon Huynh, Ngoc Quyen Tran, Van Thu Le, Minh Dung Truong, Bach Long Giang, Minh Thanh Vu, In situ fabrication of biological chitosan and gelatin-based hydrogels loading biphasic calcium phosphate nanoparticles for bone tissue regeneration, 2019, 31(5), 1062-1070, IF: 0.6, Q4