Niğde Ömer Halisdemir Üniversitesi Mühendislik Bilimleri Dergisi
Nigde Omer Halisdemir University Journal of Engineering Sciences

ISSN: 2564-6605
Araştırma / Research

THE EFFECT OF HYDROXYAPATITE ADDITIVE PRODUCED FROM DIFFERENT CALCIUM SOURCES TO STRENGTH OF BONE CEMENT

Nida GELİBOLU [1], Özge Can ÖZTÜRK[2], Nevin KARAMAHMUT MERMER[3], Fatma Tuğçe ŞENBERBER[4], Nurcan TUĞRUL[5,*], Mehmet Burçin PİŞKİN[6]

[1,2,3,5] Yildiz Technical University, Department of Chemical Engineering, 34142, Istanbul, Turkey
[4] Nisantasi University, Department of Civil Engineering, 34398, Istanbul, Türkiye
[6] Yildiz Technical University, Department of Bioengineering, 34142, Istanbul, Turkey

ABSTRACT

Acrylic bone cement at high strength is produced by adding hydroxyapatite additive which was produced using two different calcium sources ($Ca(OH)_2$ and $CaCO_3$) was aimed. Seashell was used as a $CaCO_3$ source. Characterizations were performed using XRD, and FT-IR. Thermal behaviours and surface morphologies were determined using DTA/TG and SEM techniques. The addition of hydroxyapatite synthesized from $Ca(OH)_2$ and $CaCO_3$ increased the compressive strength (from 74 MPa to 106 MPa and 103 MPa) of the resultant cements respectively. These new bone cements have mechanical strengths comparable with commercially available cements and are believed to be more biocompatible since hydroxyapatite is a natural mineral present in the bone structure.

Keywords: Acrylic bone cement, Hydroxyapatite, Seashell, Characterization, Compression strength

FARKLI KALSİYUM KAYNAKLARINDAN ÜRETİLMİŞ HİDROKSİAPATİTİN KEMİK ÇİMETOSU DAYANIMINA ETKİSİ

ÖZET

Bu çalışmada, iki farklı kalsiyum kaynağı ($Ca(OH)_2$ and $CaCO_3$) kullanılarak üretilen hidroksiapatit katkısı eklenerek yüksek mukavemetli akrilik kemik yapıştırıcısı üretilmiştir. $CaCO_3$ kaynağı olarak deniz kabuğu kullanılmıştır. Üretilen örnekler, XRD ve FT-IR kullanılarak karakterize edilmiştir. Termal davranış ve yüzey morfolojileri DTA/TG ve SEM teknikleri kullanılarak belirlenmiştir. $Ca(OH)_2$ ve $CaCO_3$ kullanılarak üretilen hidroksiapatit ilavesi, ortaya çıkan yapıştırıcıların basınç dayanımını (74 MPa'dan 106 MPa ve 103 MPa'ya) arttırmıştır. Bu yeni kemik yapıştırıcıları, ticari olarak temin edilebilen örneklerle karşılaştırılabilecek mekanik dayanımlara sahiptir ve hidroksiapatitin kemik yapısında mevcut doğal bir mineral olduğu için daha biyolojik olarak uyumlu olduğuna inanılmaktadır.

Anahtar kelimeler: Akrilik kemik yapıştırıcısı, Hidroksiapatit, Deniz kabuğu, Karakterizasyon, Basınç mukavemeti

1. INTRODUCTION

In orthopedics recovery and regeneration of the human bones are common challenge. Titanium, titanium alloy and 316L stainless steel materials have been utilized in bone tissue substitution. Bone cement is used to fix to prosthesis into bone [1].

Polymethylmethacrylate (PMMA) and related polymers have been used more than 40 years as bone cement in orthopedic surgery and dentistry, is the most effective synthetic biomaterial to fix prosthesis into bone. Bone cement is generally used as grouting agent between bone and the prosthesis and it provides transfer of the load from the prosthesis to bone [2-4].

Acrylic bone cements include two phases, a powder and a liquid. Generally, the ratio of powder/liquid is 2:1. The powder consists of PMMA as a polymer, benzoyl peroxide (BPO) as a polymerization initiator, barium sulphate ($BaSO_4$) as a radiopacifier. The liquid phase consists of methylmethacrylate (MMA) as a monomer, N,Ndimethylparatoluidine (DMPT) as an activator [5].

---

* Sorumlu yazar / Corresponding author, e-posta / e-mail: ntugrul@yildiz.edu.tr, ntugrul@hotmail.com
Geliş / Recieved: 18.06.2019 Kabul / Accepted: 15.02.2020 doi: 10.28948/ngumuh.579474

Hydroxyapatite (HAp - $Ca_{10}(PO_4)_6(OH)_2$) has similar properties with the mineral composition of bones and teeth that used in orthopedic, dental and maxillofacial applications. Hydroxyapatite is a material that is proved its biocompatibility, osteoconductive properties and commonly used in orthopedics. HAp can be obtained from biogenic materials such as eggshell [6-8], seashell [9], coral [10], and by some synthetic chemical methods. Production of HAp can be successed by using hydrothermal reactions [11], sol-gel methods [12], solid state reactions [13, 14] and mechano-chemical methods by using of different starting materials [18]. Due to the structure properties of HAp, many studies are carried out about its effect on bone cement.

In recent years, many studies are carried out to improve mechanical, rheological, biological and radiopacity properties of bone cement. For this purpose, some additives such as hydroxyapatite, chitosan, antibiotics, titanium dioxide, carbon nanotubes etc. are added into bone cement.

Serbetci et al. (2002), improved bone cement including hydroxyapatite and was aimed to investigate the biocompatibility properties of this cement. The bone cement with 7% wt HAp additive was prepared and was compared with tissue reaction of produced bone cement and tissue reaction of commerical bone cement. For in vivo experiments, white rabbits were used. The pathology pattern taken in 1st, 3rd, 7th days showed that the bone cement with 7% wt hydroxyapatite was more biocompatible than commerical bone cement [15]. In another study performed by Korkusuz et al. (2000), production of new bone cements with high mechanical strengths, low setting temperature and composition comparable with commercially available cements were aimed. After addition of 8% wt HAp, polymerization temperature decreased from 111°C to 87°C and compressive strength increased from 110 MPa to 122 MPa [16]. Zebarjad et al. (2011), performed wear, compression and three-point bending tests to measure performance of bone cement with hydroxyapatite. The wear rate decreased by increasing in hydroxyapatite content and the compression strength increased with 2.5% wt hydroxyapatite additive. The results of three-point bending tests showed that the bone cement with 2.5 wt % hydroxyapatite had maximum bending strength value and modulus. Increasing amount of hydroxyapatite additive from 2.5 wt % to 10 wt % did not affect the bending properties significantly [17]. Phakatkar et al. (2020), examined the effects of magnesium phosphate and nanofibers of hydroxyapatite addition to nanocomposites of PMMA bone cement on the materials' mechanical and biological properties [18]. Che et al. (2019), prepared the modified bone cement with the addition of hydroxyapatite and PMMA at different morphologies [19]. Ayatollahi et al. (2018), modified the bone cement with nano particles of hydroxyapatite and examined tension-shear behaviour [20].

The aim of this study was to produce bone cements with high mechanical strength. For this purpose, hydroxyapatite additives produced from different raw materials were used and various compositions with different hydroxyapatite ratios were prepared. The effect of hydroxyapatite additive on the compressive strength of the bone cement was examined. Prepared samples were characterized and compared with each other using the techniques of XRD, FT-IR, SEM and thermal analyses.

2. MATERIALS AND METHOD

2.1. Materials

Calcium hydroxide ($Ca(OH)_2$), phosphoric acid (H_3PO_4) (purity≥85%) and ethanol (C_2H_5OH) (purity≥96%) were obtained from Merck Chemicals. Polymethylmethacrylate ($(C_5H_8O_2)_n$) and dimethyl para toluidine ($C_9H_{13}N$) having a minimum purity of 99% were suplied from Alfa Aesar. Barium sulphate ($BaSO_4$) was obtained from Carlo Erba. Benzoyl peroxide ($[C_6H_5C(O)]_2O_2$) and methyl methacrylate ($C_5H_8O_2$) (purity≥98,5%) received from Sigma Aldrich. Sea shells were retrieved from Tekinler Sea Word. All of the sea shells were crushed and ground using a Retch Agate mortar and sieved using 150 µm sieve. Sea shells were calcined at 900°C for 3 hours. As a reference for the comparison of bone cements characteristic features, a commercial bone cement (ORCEM Standard viscosity bone cement produced by ORTHOPRO Company) was used.

2.2. Preparation of hydroxyapatite

Hydroxyapatite powder was synthesized using two different kinds of calcium sources. The solutions of Ca^{2+} agent and H_3PO_4 were mixed in hydrothermal conditions using magnetic stirrer. Experimental conditions in the synthesis of hydroxyapatite were selected as Ca/P ratio of 1.67, reaction temperature of 75°C, reaction time of 4 hours. Calcium agent was determined as calcium hydroxide [$Ca(OH)_2$] for the first synthesis process, and sea shell for the second synthesis process. After agitation process, the solutions were aged for 4, 24, 48 and 72 hours at room temperature in a dark place, washed with ethyl alcohol and dried in an oven at 105°C for 24 hours. Dried samples were calcined at 1100°C for 2 hours.

Each product was coded by initial letters of the raw materials (C: Calcium hydroxide and S: Sea shell), and agitation time. For instance, "C-24" indicated the product synthesized hydroxyapatite at agitation time of 24 hours using the calcium source of $Ca(OH)_2$.

2.3. Preparation of bone cement reinforced hydroxyapatite

Acrylic bone cements were produced by hand mixing of the powder and liquid phases. The powder phase consisted of 87.5% *wt* PMMA, 2.5% *wt* benzoyl peroxide (BPO), 10% *wt* barium sulphate ($BaSO_4$). The liquid phase consisted of 99.17% methylmethacrylate (MMA), 0.83% N,Ndimethylparatoluidine (DMPT).

Hydroxyapatite samples synthesized using the different calcium sources ($Ca(OH)_2$ and sea shell) were selected at the proper crystallinity features to add the powder mixture of 40 g. The ratio of powder/liquid was prepared as 2:1. Molds, in accordance with ASTM F 451 standard, were filled with the mixture after hand mixing process. Specimens were removed from molds after one hour and compression tests were performed at room temperature after 24 hours.

Each product was coded by initial letters of the raw materials (C: Calcium hydroxide and S: Sea shell), agitation time of hydroxyapatite and addition amount of hydroxyapatite to the bone cement composition. For instance, "C-48-0.5" indicated the product bone cement composition using the calcium source of $Ca(OH)_2$ at agitation time of 48 hours and addition amount was 0.5 g.

2.4. Characterizations of the synthesized products

The synthesized samples were identified with a Philips PANalytical Xpert Pro diffractometer (XRD) analyses by using a Cu-Ka tube at 45 kV and 40 mA in the 2θ range of 20 – 80°. For the characterization of synthesized hydroxyapatites and bone cements, a Perkin Elmer Spectrum One FT-IR with the universal diamond/Zn attenuated total reflectance (ATR) sampling accessory was used in a wavenumber range of 4000 – 650 cm^{-1}. Thermal decomposition of bone cements synthesized from the calcium sources of sea shell and $Ca(OH)_2$ were studied by thermal analyses (TG-DTA) techniques. For thermal analysis, a Perkin Elmer Diamond DTA/TG was used. Thermal behaviors of samples were in the temperature range of 50 – 550°C and heating rate of 10°C/min in platinum crucibles under a nitrogen atmosphere. The surface morphologies of samples were examined under a scanning electron microscope (SEM, CamScan Apollo 300 Field-Emission) at 20 kV and at the magnification scales between 250 and 20.000. The compressive strength of bone cements with hydroxyapatite additive were determined according to ASTM F 451 standard. Bone cement specimens were molded in steel molds (Ø=6.0±0.1 mm, L=12.0±0.1 mm) for one hour and end of specimen grounded with P240 silicon carbide abrasive in accordance with the standard. After 24 hours, compressive strength test was performed by constantly increasing load with simultaneous recording of load versus deformation using Hounsfield tensile test machine. A constant speed of 20 mm/min was applied. The average compressive strength was calculated from results of 5 specimens.

3. RESULTS AND DISCUSSION

3.1 XRD Results

The calcium sources used in experiments were calcium hydroxide and sea shell. According to XRD results, calcium hydroxide was identified with the powder diffraction file (pdf no.) of "01-078-0315, ($Ca(OH)_2$)" and XRD score of 91. Sea shell was identified with the pdf no. of "01-076-0606, ($CaCO_3$)" and XRD score of 77 —when all the peak intensities (%) and peak locations are matched perfectly with the reference card, the XRD score of the analysed mineral is equal to 100—.

After the synthesis of hydroxyapatite, products were determined as "$Ca_5(PO_4)_3(OH)$" with pdf no. of 00-024-0033. XRD scores of synthesized hydroxyapatite samples are given in Table 1. In Table 1, XRD scores increased with the increasing aging time until 48 hours, and the highest XRD scores are obtained at the agitation time of 48 hours for both of calcium sources. The XRD results indicated that both sources of $Ca(OH)_2$ and $CaCO_3$) can be used in hydroxyapatite preparation. The XRD patterns of synthesized hydroxyapatite samples at the agitation time of 48 h for both of calcium sources are presented in Figure 1.

Table 1. XRD results of the synthesized hydroxyapatite

Raw material source	Aging time (h)	Product Code	Chemical Formula	XRD Scores
$Ca(OH)_2$	4	00-024-0033	$C_5(PO_4)_3(OH)$	82
$Ca(OH)_2$	24	00-024-0033	$C_5(PO_4)_3(OH)$	83
$Ca(OH)_2$	48	00-024-0033	$C_5(PO_4)_3(OH)$	84
$Ca(OH)_2$	72	00-024-0033	$C_5(PO_4)_3(OH)$	81

Sea shell ($CaCO_3$)	4	00-024-0033	$C_5(PO_4)_3(OH)$	80
Sea shell ($CaCO_3$)	24	00-024-0033	$C_5(PO_4)_3(OH)$	80
Sea shell ($CaCO_3$)	48	00-024-0033	$C_5(PO_4)_3(OH)$	88
Sea shell ($CaCO_3$)	72	00-024-0033	$C_5(PO_4)_3(OH)$	83

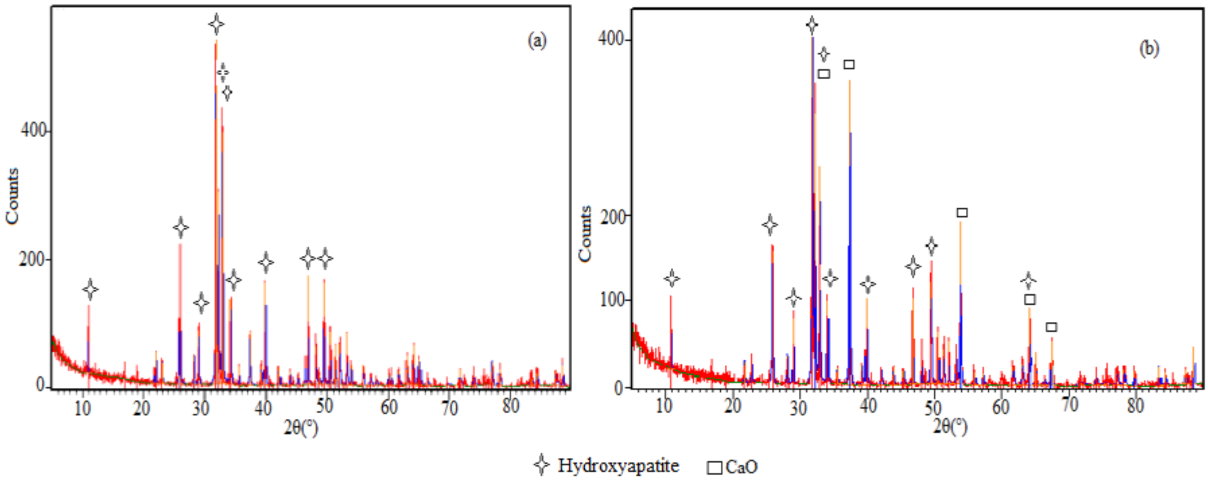

Figure 1. XRD patterns of synthesized hydroxyapatite with 48 h aging
a. with $Ca(OH)_2$, **b.** with Sea shell ($CaCO_3$)

The hydroxyapatite samples, which has the highest XRD scores for both C and S sets, added to bone cement. The XRD patterns of bone cement with hydroxyapatite additive (0.5, 1, 1.5 and 2 g amounts) are shown in Figure 2 and Figure 3. All peaks related to bone cement with 0.5, 1, 1.5 and 2 g hydroxyapatite additive (from both C and S) were in accordance with each other and peaks of reference bone cement. According to the XRD results, hydroxyapatite addition didn't have any adverse effect to bone cement composition.

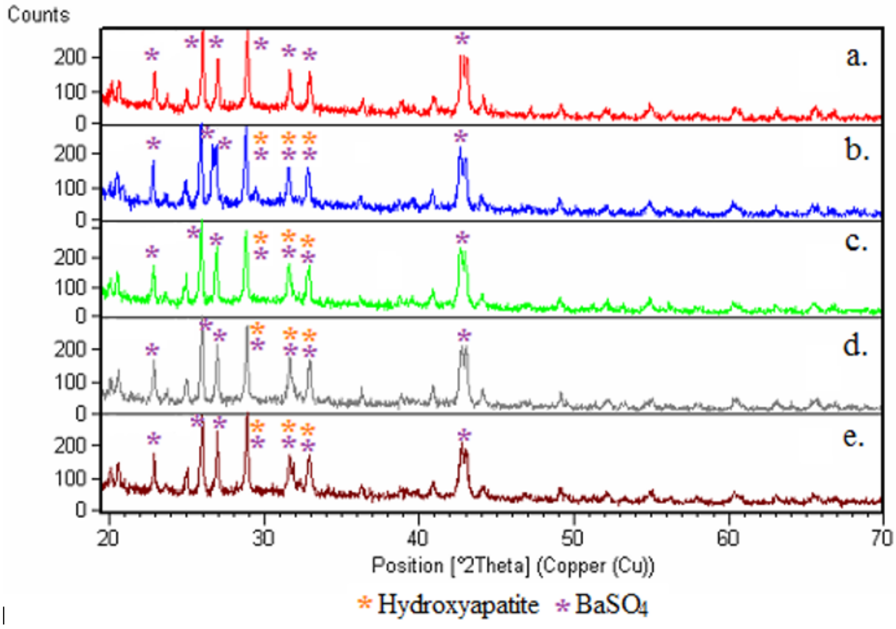

Figure 2. XRD patterns of the bone cements, a. Reference, b. C-48-0.5, c. C-48-1, d. C-48-1.5, e. C-48-2

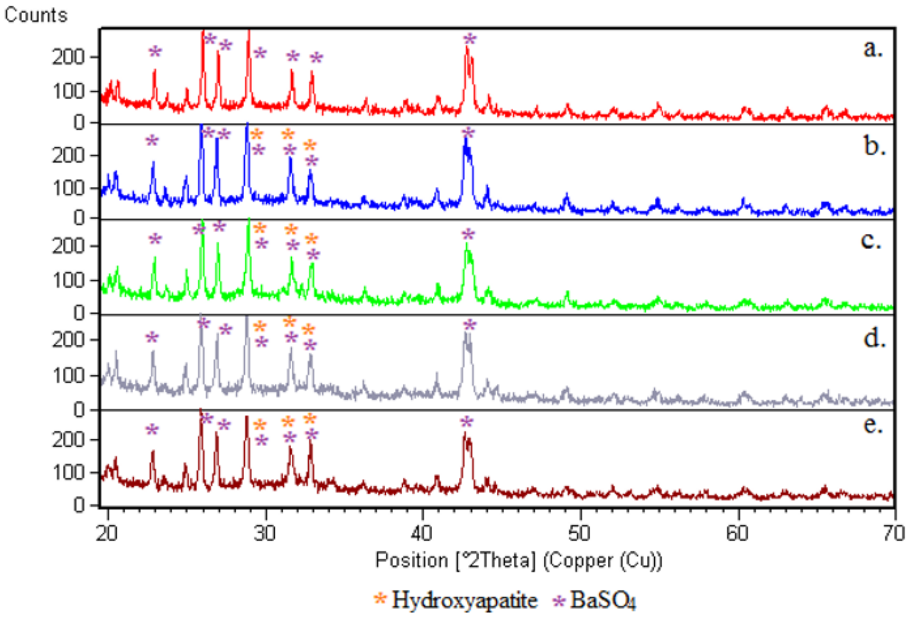

Figure 3. XRD patterns of the bone cements, a. Reference, b. S-48-0.5, c. S-48-1, d. S-48-1.5, e. S-48-2

3.2. FT-IR results

FT-IR spectra of synthesized hydroxyapatite from $Ca(OH)_2$ and sea shell are shown in Figure 4. For the synthesized hydroxyapatite from $Ca(OH)_2$, bands due to the OH stretching were at 3572 cm^{-1} and 2982 cm^{-1}. Bands observed at 1085 and 1028 cm^{-1} are assigned to asymmetric stretching vibration mode of PO_4^{2-} groups. Symmetric stretching vibration mode bands of PO_4^{2-} occurred at 961 cm^{-1} and also the bands observed at around 672cm^{-1} are assigned to the bending vibration mode of OPO. For the synthesized hydroxyapatite from sea shell, bands due to the OH stretching were at 3572 cm^{-1} and 2976 cm^{-1}. Bands observed at 1087 and 1022 cm^{-1} are assigned to asymmetric stretching vibration mode of PO_4^{2-} groups. Symmetric stretching vibration mode bands of PO_4^{2-} occurred at 961 cm^{-1} and also the bands observed at around 673cm^{-1} are assigned to the bending vibration mode of OPO. The obtained characteristic band values for the C and S sets are good agreement with the literature. [6].

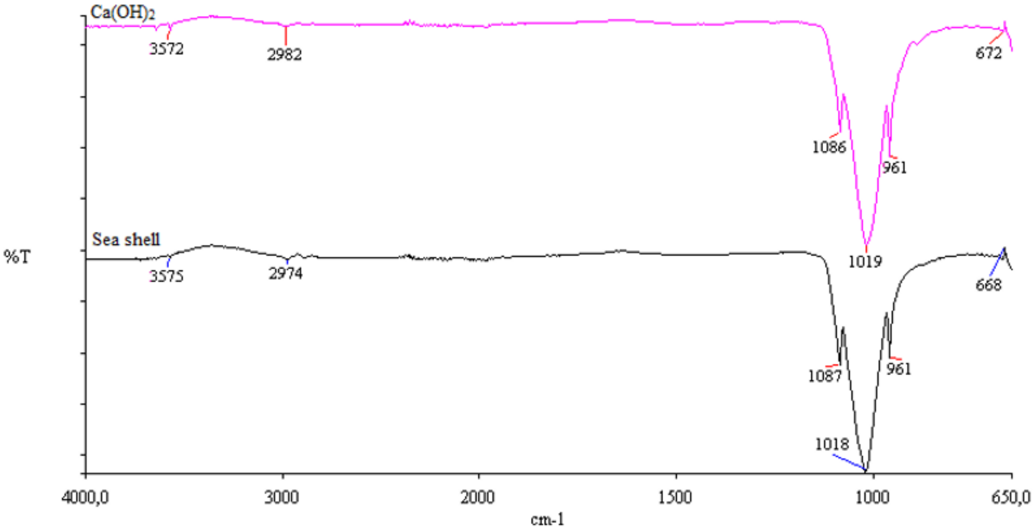

Figure 4. FT-IR spectra of synthesized hydroxyapatite using $Ca(OH)_2$ with 48 h aging and sea shell with 48 h aging

The FT-IR spectra comparison of bone cement with hydroxyapatite additive (0.5, 1, 1.5 and 2 g amounts) are shown in Figure 5 and Figure 6.

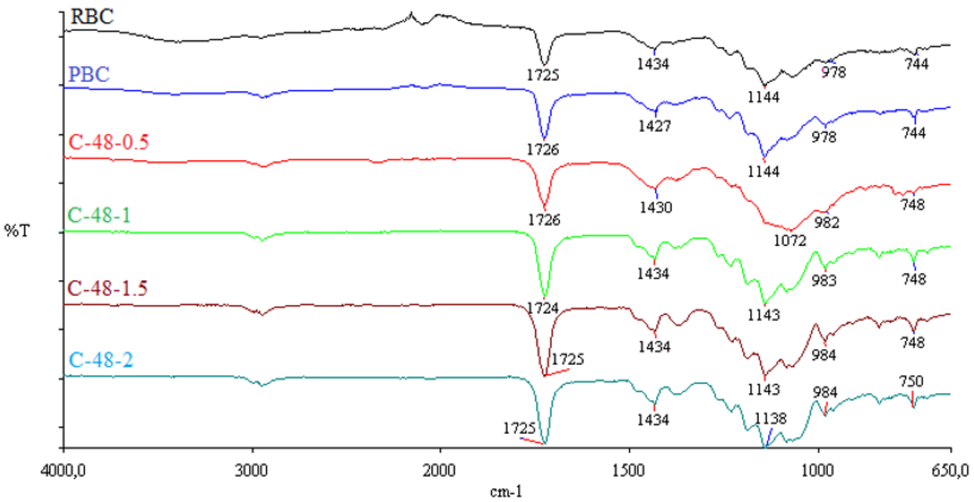

Figure 5. FT-IR spectra of the **a.** Reference bone cement (RBC), **b.** Produced cement without hydroxyapatite (PBC), **c.** C-48-0.5 **d.** C-48-1 **e.** C-48-1.5 **f.** C-48-2

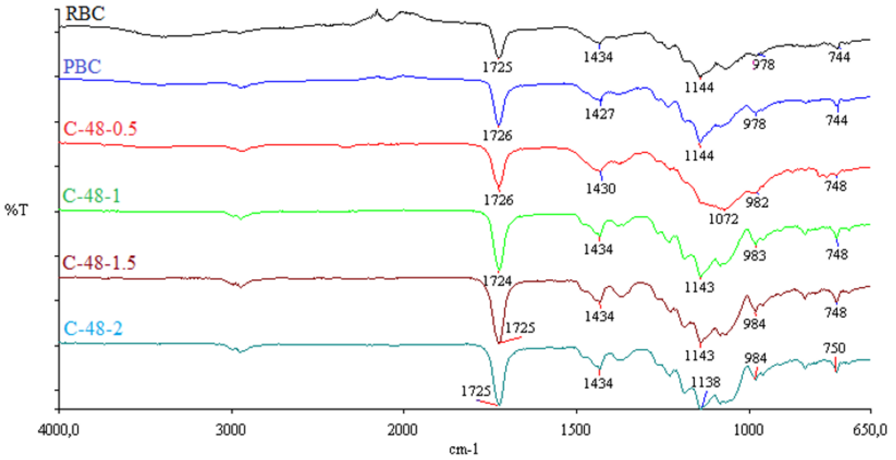

Figure 6. FT-IR spectra of the **a.** Reference bone cement (RBC), **b.** Produced cement without hydroxyapatite (PBC), **c.** S-48-0.5 **d.** S-48-1 **e.** S-48-1.5 **f.** S-48-2

All peaks related to bone cement with 0.5, 1, 1.5 and 2 g hydroxyapatite additive were in accordance with each other's and peaks of reference bone cement. The FT-IR results of hydroxyapatite added bone cements indicate that characteristic bands of bond cements are constant and hydroxyapatite successfully bonded to polymeric cement.

3.3. Surface Morphologies of Samples

The SEM morphologies of hydroxyapatite produced using $Ca(OH)_2$ with different aging times are given Figure 7. As it is seen from Figure 7, the agglomerated angled particle were obtained in use of $Ca(OH)_2$ as calcium source. The particle sizes of hydroxyapatite produced with 4 h aging were between 246-818 nm. The average particle size was determined as 475 nm. The particle sizes of hydroxyapatite produced with 24 h aging were found to be between 136-318 nm. The average particle size was determined as 246 nm. The particle sizes of hydroxyapatite produced with 48 h and 72 h aging were between 255-655 and 230-473 nm and the average particle size was determined as 350 nm and 340 nm, respectively.

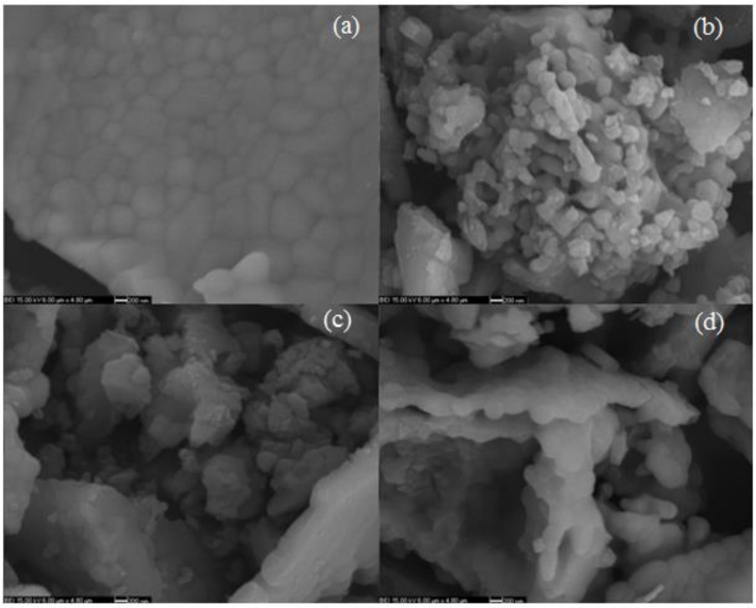

Figure 7. SEM images of hydroxyapatite produced from produced from $Ca(OH)_2$;
a. Produced with 4 h aging **b.** Produced with 24 h aging **c.** Produced with 48 h aging **d.** Produced with 72 h aging

The SEM morphologies of hydroxyapatite produced using sea shell with different aging times are given Figure 8. As it is seen from Figure 8, generally agglomerated and rounding particles were synthesized. The particle sizes were decreased and agglomeration was increased with the increasing aging time. The particle sizes of hydroxyapatite produced with 4 h aging were between 236-490 nm. The average particle size was determined as 388 nm. The particle sizes of hydroxyapatite produced with 24 h aging were found to be between 335-975 nm. The average particle size was determined as 540 nm. The particle sizes of hydroxyapatite produced with 48 h and 72 h aging were between 270-1150 and 316-686 nm and the average particle size was determined as 650 nm and 450 nm, respectively.

In the comparison of Figure 7 and 8, agglomeration of particle increased in the use of $CaCO_3$. This result will be essential in the potential applications of modification of hydroxy apatite added bone cement.

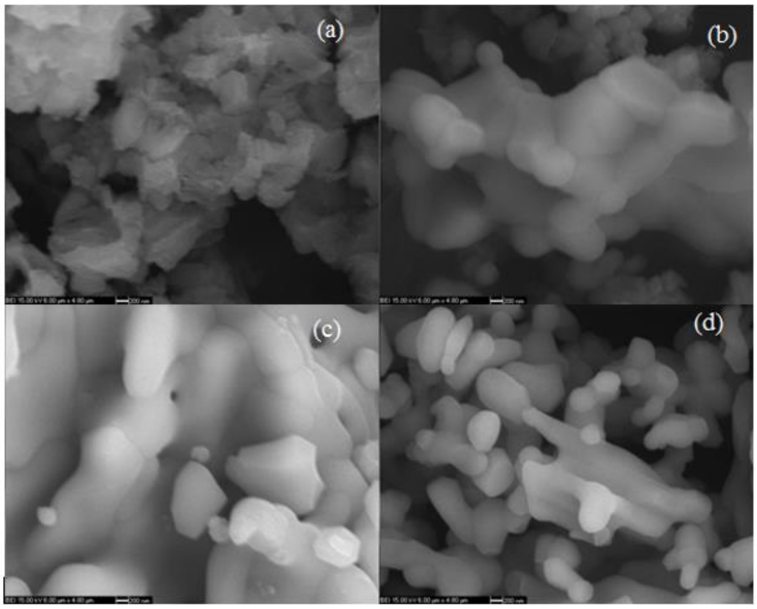

Figure 8. SEM images of hydroxyapatite produced from produced from sea shell ($CaCO_3$);
a. Produced with 4 h aging **b.** Produced with 24 h aging **c.** Produced with 48 h aging **d.** Produced with 72 h aging

The SEM morphologies of bone cements are given Figure 9. The particle sizes of reference bone cement were between 21-75 µm. The average particle size was determined as 42 µm. The particle sizes of produced bone cement without hydroxyapatite were found to be between 14-76 µm. The average particle size was determined as 31 µm. The particle sizes of bone cements consisting of 0.5 g synthesized hydroxyapatite from $Ca(OH)_2$ with 48 h aging and consisting of 0.5 g synthesized hydroxyapatite from sea shell with 48 h aging were between 50-88 µm and 25-88 µm and also the average particle size was determined as 74 µm and 48 µm, respectively.

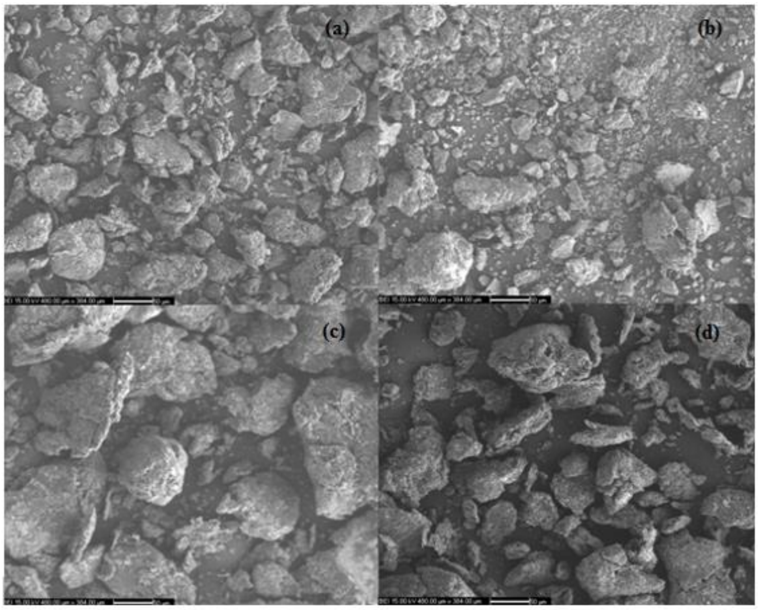

Figure 9. SEM images of bone cement **a.** Reference **b.** Produced without hydroxyapatite **c.** C-48-0.5 **d.** S-48-0.5

3.4. Compressive strength results

The compression test was performed according to ASTM F 451 standard. The comparison of compressive strength results for reference commercial bone cement, bone cement without addition and hydroxyapatite added bone cement samples are given in Table 2.

Table 2. Compression strength results of bone cement with hydroxyapatite additive

Sample Name	Average Max. Strength (MPa)
Reference	74.38
Bone cement without additive	86.79
C-48-0.5	105.93
C-48-1	103.35
C-48-1.5	84.18
C-48-2	92.35
S-48-0.5	103.38
S-48-1	86.97
S-48-1.5	78.55
S-48-2	79.37

According to compression strength test results, the addition of hydroxyapatite caused the reinforcing effects to compressive strength of bone cement. The obtained results are in good agreement with the hydroxyapatite added bone cements [18 – 20]. The results indicate that both of the calcium sources ($Ca(OH)_2$ and $CaCO_3$) can be preferred for the increase mechanical strength of polymeric bone cements. The compressive strengths of hydroxyapatite added bone cements were measured as 105.93 MPa for C-48-0.5 and 103.38 MPa for S-48.0.5, respectively.

3.5. Thermal Decomposition Behaviour of Samples

Thermal curves of reference commercial bone cement, bone cement without addition and hydroxyapatite added bone cement samples are presented in Figure 10. Obtained mass losses and temperatures range were in compatible with reference bone cement. Thermal analyses results are given Table 3.

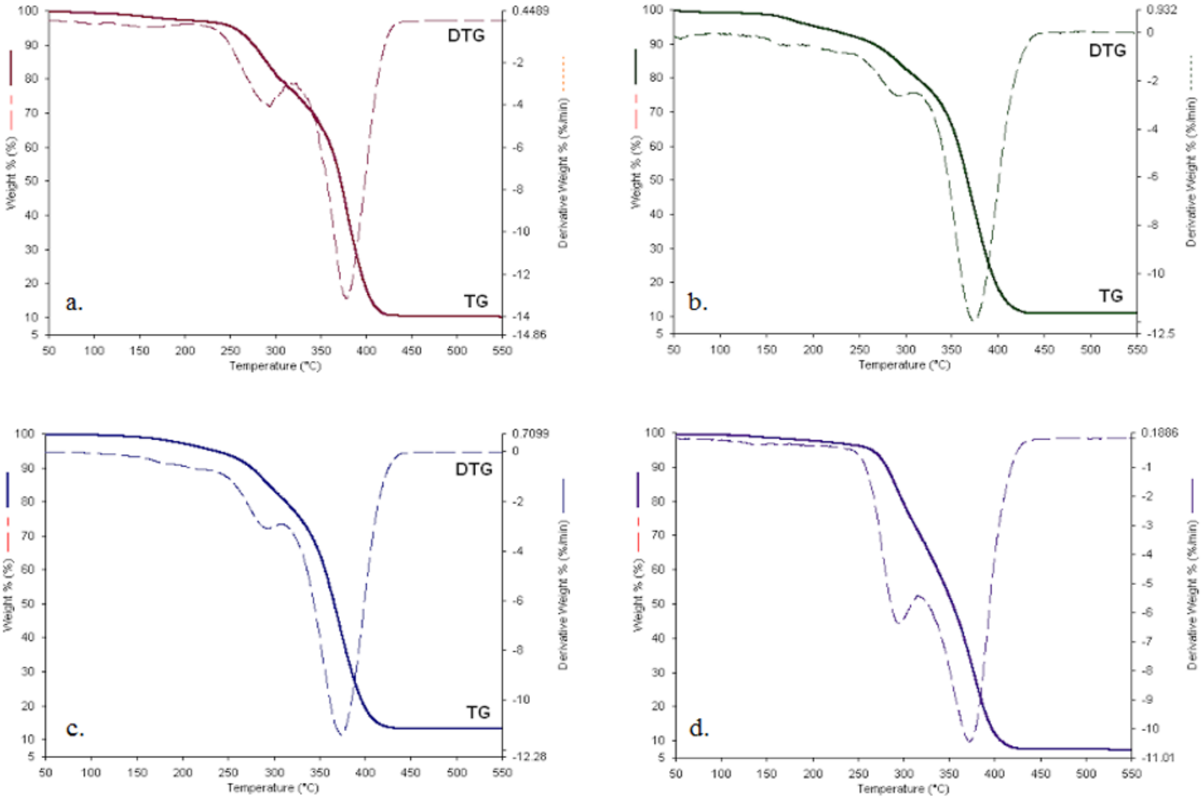

Figure 10. TG-DTG curves of bone cement **a.** Reference **b.** Produced without hydroxyapatite **c.** C-48-0.5 **d.** S-48-0.5

According to thermal analyses results, thermal decomposition of all samples occurred via a two-step process between the temperatures of 50 – 550°C. First step was approximately seen in the range of 200 – 317°C and mass losses were between 15.53 – 25.58%. The highest initial temperatures were seen at S-48-0.5. The second step of decomposition was occurred in the temperature range of 316.90 – 441.01°C. Approximately 63.09 – 70.05% of bone cement have left from the composition that can be explained with the removal PMMA from composition. Total mass losses were between 84.50 – 88.67%. Obtained thermal results of reinforced samples were consistent with the reference material.

Table 3. Thermal analyses result of bone cement samples

Step	**Temperature**	**Reference**	**Bone cement without additive**	**C-48-0.5**	**S-48-0.5**
1st step	Ti (°C)	202.37	211.27	222.40	247.09
	Tf (°C)	316.90	307.98	308.80	317.17
	Mass Loss (%)	20.14	15.53	16.68	25.58
2nd step	Ti (°C)	316.90	307.98	308.80	317.17
	Tf (°C)	435.4	441.01	440.92	432.82
	Mass Loss (%)	66.50	70.05	67.82	63.09
Total Mass Loss (%)		86.64	85.58	84.50	88.67

The thermal results of samples can be interpreted that the decomposition begin at higher temperature at the hydroxyapatite bone cements. To obtain a higher resist to decomposition of material, Sea shell ($CaCO_3$) can be preferred.

4. CONCLUSION

The addition of hydroxyapatite increased remarkably the compressive strength of bone cement. The hydroxyapatite used in these experiments, produced with two different calcium sources ($Ca(OH)_2$ and $CaCO_3$). There are minor changes were observed in the comparison of characteristic band values of hydroxyapatites synthesized by using the $Ca(OH)_2$ and $CaCO_3$.

Morphologies of reinforced samples were analyzed by SEM. The agglomeration increased with the increasing aging time. The angular particles were obtained in the use of $Ca(OH)_2$, whereas the round particle were synthesized in the of $CaCO_3$ as calcium source.

In addition of hydroxyapatite to bone cement retarded the decomposition of materials that may make the material useful in specific applications. Especially, the use of sea shell ($CaCO_3$) retard the initial temperature of first step of decomposition 36°C than the bone cement without bone cement.

Increasing of rate of hydroxyapatite into bone cement caused decreasing of viscosity of bone cement therefore caused decreasing of compressive strength of bone cement. Considering literature studies and reference bone cement, it was proved that the compressive strength of all bone cements with hydroxyapatite additive had higher strength. All of the synthesized samples have higher strength comparing to the reference sample. C-48-05’s strength resistance is 1.4 times greater than the reference.

The results showed that hydroxyapatite addition had not any adverse effects on characteristic features of bone cements.

KAYNAKLAR

[1] J. Zhang, C.S. Dai, J. Wei, J.W. Wen, “Study on the bonding strength between calcium phosphate/chitosan composite coatings and a Mg alloy substrate”, *Applied Surface Science*, vol. 261, pp. 276-286, 2012.

[2] Z. Khaled, Development of A New Generation of Bone Cements using Nanotechnology, Graduate Program in Engineering Science, School of Graduate and Postdoctoral Studies The University of Western Ontario, London, UK, 2009. [Online] Avaible: https://www.eng.uwo.ca/people/pcharpentier/docs/Khaled%20Thesis.pdf [Accessed May 13, 2020].

[3] K. Serbetci, F. Korkusuz, N. Hasırcı, “Mechanical and Thermal Properties of Hydroxyapatite-Impregnated Bone Cement”, *Turkish Journal of Medical Sciences*, vol. 30, pp. 543-549, 2000.

[4] Y.H. Nien, C.L. Huang, “The mechanical study of acrylic bone cement reinforced with carbon nanotube”, *Materials Science and Engineering*, vol. 169, pp. 134-137, 2010.

[5] S. Marangoz, “Bone Cement”, *Totbid Jurnal*, vol. 10, pp. 103-108, 2011.

[6] E.M. Rivera, M. Araiza, W. Brostow, V.M. Castano, J.R.D. Estrada, R. Hernandez, J.R. Rodriguez, “Synthesis of hydroxyapatite from eggshells”, *Material Letters*, vol. 41, pp. 128-134, 1999.

[7] S.J. Lee, S.H. Oh, “Fabrication of calcium phosphate bioceramics by using eggshell and phosphoric acid”, *Material Letters*, vol. 57, pp. 4570-4574, 2003.

[8] C. Balázsi, F. Wéber, F. Kövér, Z. E. Horváth, A. Németh, “Preparation of calcium –phosphate bioceramics from natural resources”, *Journal of the European Ceramic Society*, vol. 27, pp. 1601-1606, 2007.

[9] S.V. Kenneth, X. Zhang, J.B. Massie, M. Wang, C. Kim, “Conversion of bulk seashells to biocompatible hydroxyapatite for bone implants”, *Acta Biomaterialia*, vol. 3, pp. 910-918, 2007.

[10] U. Ripamonti, J. Crooks, L. Khoali, L. Roden, “The induction of bone formation by coral-derived calcium carbonate/hydroxyapatite constructs”, *Biomaterials*, vol. 30, pp. 1428-1439, 2009.

[11] S. Jinawath, D. Polchai, M. Yoshimura, “Low-temperature, hydrothermal transformation of aragonite to hydroxyapatite”, *Materials Science and Engineering: C*, vol. 22, pp. 35-39, 2002.

[12] C.S. Chai, K.A. Gross, B.B. Nissan, “Critical ageing of hydroxyapatite sol–gel solutions”, *Biomaterials*, vol. 19, pp. 2291-2296, 1998.

[13] X. Yang, Z. Wang, “Synthesis of biphasic ceramics of hydroxyapatite and b-tricalcium phosphate with controlled phase content and porosity”, *Journal of Materials Chemistry*, vol. 8, pp. 2233-2237, 1998.

[14] G. Gergely, F. Weber, I. Lukacs, L. Illés, A.L. Tóth, Z.E. Horváth, J. Mihály, C. Balázsi, “Nano-hydroxyapatite preparation from biogenic raw materials”, *Central European Journal of Chemistry*, vol. 8, pp. 375-381, 2010.

[15] K. Serbetci, S. Orhun, F. Korkusuz, N. Hasırcı, “In Vivo Biocompatibility Of Hydroxyapatite Containing Bone Cement”, *Journal of Arthroplasty & Arthroscopic Surgery*, vol. 13, pp. 259-263, 2002.

[16] S.M. Zebarjad, S.A. Sajjadi, T.E. Sdrabadi, A. Yaghmaeı, B. Naderi, “A Study on Mechanical Properties of PMMA/Hydroxyapatite Nanocomposite”, *Scientific Research*, vol. 3, pp. 795-801, 2011.

[17] D., Verma, “Design of polymer-biopolymer-hydroxyapatite biomaterials for bone tissue engineering: through molecular control of interfaces”, Ph.D. Thesis, North Dakota State University, North Dakota, USA, 2008. [Online] Avaible: https://www.researchgate.net/publication/238026007_Design_of_polymer-biopolymer-hydroxyapatite_biomaterials_for_bone_tissue_engineering_Through_molecular_control_of_interfaces [Accessed May 13, 2020].

[18] A.H. Phakatkar, M.R. Shirdar, M.M. Taheri, S. Narayanan, T. Foroozan, T. Shokuhfar, “Novel PMMA bone cement nanocomposites containing magnesium phosphate nanosheets and hydroxyapatite nanofibers”, *Materials Science and Enginerring C*, vol. 109, no. 11049, pp. 1-10, 2020.

[19] Y. Che, S. Min, M. Wang, M. Rao, C. Quan, “Biological actvity of hydroxyapatite/poly(methylmethacrylate) bone cement wit different surface morphologies and modifications for induced ostogenesis”, *Journal of Applied Polymer Science*, vol. 48188, pp. 1-8, 2019.

[20] M.A. Ayatollahi, S.A. Mirmohammadi, H.A. Shirazi, “The tension-shear fracture behaviour of polymeric bone cement modified with hydroxiapatite nano-particles”, *Archieves of Civil and Mechanical Engineering*, vol.18, pp. 50-59, 2018.