**Production Of 3D Printed Suture Anchoring Screw Based on Biopolymer Coated by Chitosan/HAp Using Dip Coating Method to Enhance Bioactivity**

Muhamad Bahrul Ulum1, a), Muhammad Rizky Aulia Susilo1, b), Riska Ayu Ramadhana Putri1, c), Adzrofil Tsabitah Basyasyah1, d), Alfidah Nur Denya1, e), and Achmad Dwitama Karisma1, f)

1Department of Industrial Chemical Engineering, Faculty of Vocational Studies, Institut Teknologi Sepuluh Nopember, Surabaya, 60111, Indonesia

Author Emails  
f) Corresponding author: [dwitama@its.ac.id](mailto:dwitama@its.ac.id)

*a)*[*ulummuhamadbahrul680@gmail.com*](mailto:ulummuhamadbahrul680@gmail.com) *b)*[*rizkyauliasusilo@gmail.com*](mailto:rizkyauliasusilo@gmail.com)

*c)*[*risyuramadhana211@gmail.com*](mailto:risyuramadhana211@gmail.com)

*e)*[*alfidanurdenyaa@gmail.com*](mailto:alfidanurdenyaa@gmail.com)

*d)*[*adzrofilits@gmail.com*](mailto:adzrofilits@gmail.com)

**Abstract**. A medical tool utilized in orthopedic surgery operations is the suture anchor screw. Stainless steel or titanium are the materials typically used to make suture anchors, and these materials are tough for the body to absorb. Suture anchors made of biopolymer material are, therefore, one way to solve this issue. Nevertheless, suture anchors made of biopolymer materials still have certain bioactivity issues; thus, the dip coating technique is required to address this issue. Dip coating has emerged as a highly pertinent and intriguing coating technique for additional study in the medical field. Chitosan can increase the antimicrobial activity. As hydroxyapatite, or HAp, is utilized to promote bone regeneration, it also possesses certain antibacterial properties like those of chitosan. There are three variations of hydroxyapatite mass coating in PLA 3D printing, which results in a significant difference compared to the other two variations that have no hydroxyapatite coating at all. FTIR spectroscopy can identify a substance that contains a hydroxyl group (OH-). The substance or surface can be composed of PLA, hydroxyapatite, or chitosan. The FTIR test itself shows that there is an amine group (NH) owned by chitosan and a phosphate group (PO4) owned by HAp. The C=O cluster owned by the PLA is not visible because it is perfectly coated. The results of the EDX test of coating variations of samples A and C are by the Ca/P ISO 13175-3 standard with an optimal range of 1.59 – 1.72. In the compressive strength test, it can be said that the coating plays a very important role in the increase in compressive strength. The addition of HAp as a reinforced filler is very influential as an increase in compressive strength. The more HAp, the stronger the suture anchoring screw produced. Scaffolds with HAp solution have antibacterial activity against *Staphylococcus aureus* bacteria with an inhibitory zone range of 3.24 mm.

# Introduction

Several incidents occur while performing daily tasks that can result in injury. The most frequently injured body parts include the tendons, ligaments, muscles, and bones. A rotator cuff injury is a result of damage to the group of muscles and tendons that surround and support the shoulder joint. Repetitive motions or severe shoulder injuries are the usual causes of this condition. The risk of this injury can be raised by engaging in repetitive upper arm movements or lifting large weights1. According to data, rotator cuff injuries are a widespread issue, particularly for athletes and people with occupations requiring a lot of upper arm movement.

In overcoming rotator cuff problems, the use of suture anchoring screws in orthopedic surgical procedures has become an important requirement in the restoration of bones and body tissue. A suture anchoring screw is a medical tool used in orthopedic surgical procedures2. This tool is used to repair or bind soft tissue to bones. Typically, suture anchor screws are used in surgical procedures to repair tendon damage, such as in sports injuries or certain medical conditions. However, currently available products still have limitations in terms of bioactivity, namely the product's ability to interact safely with human body tissue3. Currently, bio-composite anchor screw sutures are being developed to overcome problems that may arise from commonly used metal anchor screw sutures. The metals commonly used are titanium and stainless steel. Although metal suture anchors provide rigid strength and have a long service life, their use has disadvantages, such as being prone to damage to the bone, and if further surgery occurs, it will be more difficult because the suture anchor must be removed to prevent potential metal ion toxicity to the surrounding tissue. Therefore, an alternative solution is needed besides conventional metal suture anchoring screws, which have quite a large potential for toxicity. One of the proposed solutions is to use suture anchoring screws made from biopolymer as the basic material. Biopolymers are materials that come from natural sources and are environmentally friendly and not harmful to the body. The materials used in making anchor screw sutures are PLA (polylactide acid) and PCL (polycaprolactone).

Usually, making suture anchor screws from biopolymer uses 3D printing of the fused deposition modeling (FDM) type, or another name, fused filament fabrication (FFF), because the basic material for this type of 3D printing process is a kind of filament rolled on a roller. PLA filament is a polymer material made from lactic acid, which is the result of the fermentation of natural sugars such as corn or starch. PLA has biodegradable properties, meaning it can be decomposed naturally by microorganisms over a certain period. The advantage of using a suture anchor screw made from PLA is that, because the basic material is biodegradable, there is no need for a second surgery to remove the screw after the healing procedure. Apart from that, PLA also has good abilities in supporting the healing process of bones and soft tissue. Meanwhile, polycaprolactone filament is a type of biodegradable filament polymer that can decompose itself in the body. However, if a suture anchor made from biopolymer is directly used for injury surgery, its bioactivity or tendency to blend naturally with body tissue without infection or rejection from the body is still not perfect. Therefore, additional measures are needed to increase the bioactivity properties of suture anchors made from biopolymer materials. In terms of increasing bioactivity, the dip coating method is a suitable method to improve these properties 4.

In the medical world, dip coating has become a coating method that is very relevant and interesting for further research. This method has great potential for improving the quality of medical devices and providing additional protection to their surfaces. Dip coating can be used to coat various types of medical devices, such as orthopedic implants, hearing aids, and other medical devices. By using dip coating, the surface of medical devices can be given a protective layer that is resistant to infection, corrosion, and chemical reactions5. This is very important for preventing infections and improving patient safety. In addition, dip coating can also increase the bioactivity of medical devices, thereby reducing the risk of rejection by the patient's body. With further research, this method can continue to be developed to create more effective and safer protective layers in medical devices. Thus, dip coating has great potential for improving the quality of medical devices and providing significant benefits for patients6 Substances that are suitable to be used as coating materials for biopolymer-based suture anchors include chitosan and hydroxyapatite. Chitosan is a substance that can increase antibacterial properties. Meanwhile, HAp (hydroxyapatite) is a substance that is suitable for use in increasing bone regeneration ability. By combining 3D printing technology, biopolymer materials, and chitosan/HAp dip coating, it is hoped that we can produce suture anchoring screw products that have better bioactivity properties.

# Materials and Method

To make the suture anchoring screw, the materials and equipment used are Poly-lactic Acid (PLA) filament, hydroxyapatite (HAp), glacial acetic acid (CH3COOH), chitosan (CS), and FDM 3D printer (Creality Ender-3). The suture anchoring screw 3D design was created, then it was printed from PLA filament using FDM 3D printer. The mixture of HAp and chitosan solution was used as the coating of the suture anchoring screw. HAp and chitosan were mixed in the 2% acetic acid solution with various compositions using a magnetic stirrer at the temperature of 100°C until it reached homogeneity. Then, the suture anchoring screw was coated with the HAp/CS/CH3COOH mixture using the dip coating method. After the dip coating process, the suture anchoring screw was dried, and its characteristic was analyzed. In this study, the material characteristic, tensile strength, and antibacterial activity was obtained.

# result and discussion

**FIGURE 1** shows the FTIR analysis result of the suture anchoring screw coated by various HAp/CS/CH3COOH compositions. There are three variations of mass of hydroxyapatite-coated on the suture anchoring screw (sample A, B, C), which might result in a significant difference compared to the other two variations (sample D and E) that are without hydroxyapatite content. From **FIGURE 1**, FTIR spectroscopy shows that the substance or its surface may consist of PLA7, hydroxyapatite8, or chitosan9. At consecutive wavenumbers of 3749 cm-1, 3675 cm-1, 3675 cm-1, and 3652 cm-1, the coating variations of 0; 0.6; 1; and 1.4 grams of HAp clearly show the presence of NH2 groups10. This suggests that Chitosan has coated all four variables. The variations in coating and the differences in coating of 1.4, 1, and 0.6 grams of HAp show the presence of HAp groups that are only found in hydroxyapatite. These groups can be obtained at wavenumbers between 300 and 3400 cm-1. The changes of 1.4, 1, and 0.6 grams of HAp coating made the PO4 group stand out clearly in the 1050–560 cm1 wavenumber range11, which is only found in hydroxyapatite.

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**FIGURE 1** FTIR Result of Suture Anchoring Screw Coated by Chitosan/HAp Using Dip Coating Method

**TABLE 1** shows the ratio of Ca/P values in hydroxyapatite that has been coated in suture anchoring screw consecutively in samples A, B, and C are 1.695, 1.880, and 1.661. The data shows that only samples A and C met the Ca/P standardization of ISO 13175-3 with an optimal range of 1.59 – 1.72. The theoretical value of the most optimal Ca/P ratio of hydroxyapatite is 1.6712. Sample A and C have met the Ca/P ratio of medical standards, while sample B shows there is an excess of calcium or a lack of phosphorus. There are several influences if the Ca/P ratio is coating adhesion, which can reduce adhesion delamination and decrease coating stabilization. Furthermore, if the Ca/P ratio does not meet the requirement of the standard, it can reduce the ability to stimulate bone growth and integration with biological tissues, and the last influence is the durability of the coating.

TABLE 1 EDX Test Results For Verifying the Ratio of Ca/P Values

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Variation HAp : CS (g) Element | % mol | | (Ca/P) |
| A | 1.4 ; 0.125 ; 10 ml Acetic Acid 2 % | *P K* | 0.589 | 1.695 | |
| *CaK* | 0.999 |
| B | 1 ; 0.125 ; 10 ml Acetic Acid 2 % | *P K* | 0.424 | 1.880 | |
| *CaK* | 0.798 |
| C | 0.6 ; 0.125 ; 10 ml Acetic Acid 2 % | *P K* | 0.706 | 1.661 | |
| *CaK* | 1.173 |

**FIGURE 2** shows the results of the compressive strengthfor uncoated variables, 0g HAp 0.125 Chitosan 10 mL Acetic Acid 2%, 0.6g HAp 0.125 Chitosan 10 mL Acetic Acid 2%, 1g HAp 0.125 Chitosan 10 mL Acetic Acid 2%, 1.4g HAp 0.125 Chitosan 10 mL Acetic Acid 2% respectively are 60.08 MPa, 181.90 MPa, 185.62 MPa, 196.42 MPa, and 218.93 MPa. The addition of HAp and chitosan affected the increase of compressive strength, which the result exceeded the compressive strength standard for biopolymer-based suture anchoring screws, which is around 60-70 MPa. The variable of 1.4g HAp, 0.125g Chitosan 10 mL acetic acid 2% is the variable that has the maximum compressive strength, i.e. 218.93 MPa. This result shows that coatings from HAp and Chitosan can increase compressive strength13. The hydroxyapatite function as a reinforced filler to the chitosan polymer matrix, so the higher amount of reinforced filler, the compressive strength of the suture anchoring screw also increases14. The impact of increased compressive strength is to reduce the risk of mechanical failure during installation or use, provide a greater margin of safety, and have good stability under high load conditions15.

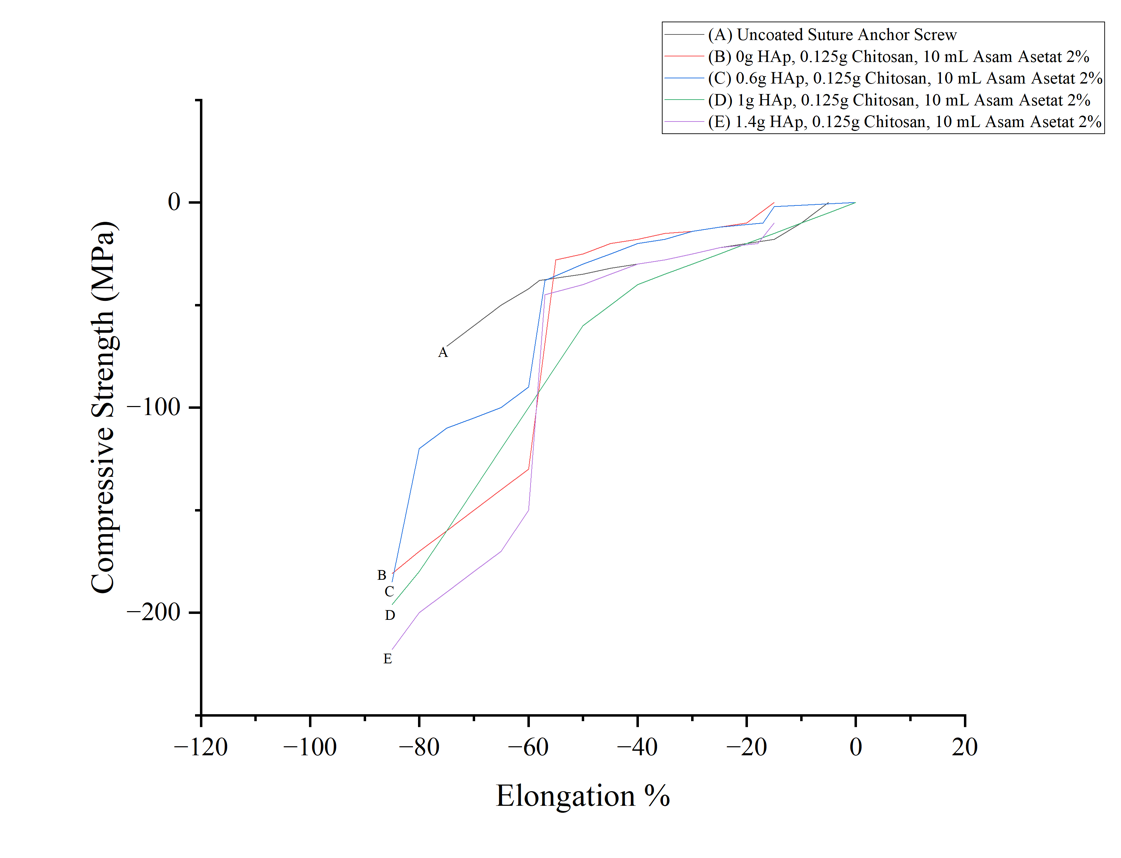
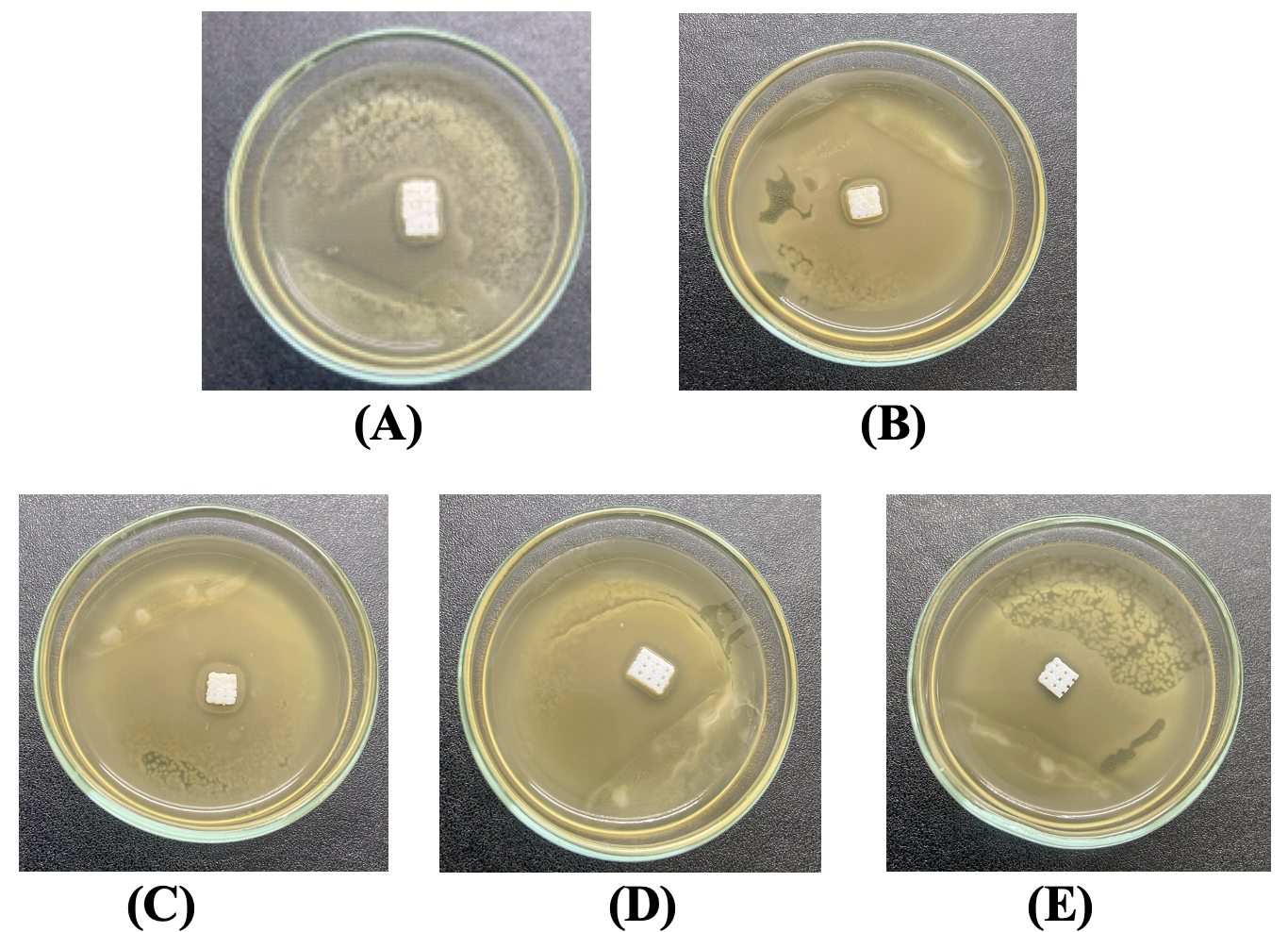


FIGURE 2 Compressive strength test results for both the coating and non-coating samples

The antibacterial test treatment was carried out on the sample using the disc diffusion method with an incubation temperature of 37°C for 24 hours. The working principle of the agar diffusion method is the diffusion of antibacterial compounds into a solid medium in which the test microbes have been inoculated16. In this study, *S. Auerus* bacteria was used. The antibacterial activity of HAp and chitosan can be seen from the presence or the absence of clear zones around the solid media.



**FIGURE 3.** Antibacterial Activity Test Results of Thin Plate Samples (A) Sample A, (B) Sample B, (C) Sample C, (D) Sample D, and (E) Sample E, at 24-hour Observation

From **FIGURE 3**, It can be seen that each sample can inhibit the activity or growth of bacteria, which is characterized by the presence of a clear zone around the thin plate sample. This is because of the interaction between hydroxyapatite, which has the functional group of hydroxyapatite calcium oxide (CaO), which has an antibacterial activity17. Moreover, the interaction of *S.Aureus* bacteria with the antibacterial content of chitosan can form a thin layer around the cell due to the composition of phospholipids and carboxylic acids from the bacterial cell wall, which inhibits the absorption of nutrients18. The results of the inhibition zone measurement are shown in **TABLE 2**.

**TABLE 2** Results of Calculation of Inhibition Zones of Various Samples with S*.* aureus Bacteria

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Ratio (g) | Range of Inhibition Zones  After 24 hours | | Average (mm) | Length of the Inner Bacterial Living Zone | | Average  (mm) |
| HAp : CS |
|  | Longest (mm) | Shortest (mm) | Longest (mm) | Shortest (mm) |
| A | 1.4 : 0.125 | 2.11 | 1.91 | 2.01 | 1.07 | 0.89 | 0.98 |
| B | 1 : 0.125 | 2.42 | 2.27 | 2.345 | 1.7 | 1.3 | 1.5 |
| C | 0.6 : 0.125 | 3.54 | 2.94 | 3.24 | 2.16 | 2.02 | 2.09 |
| D | 0 : 0.125 | 1.9 | 1.8 | 1.85 | 0 | 0 | 0 |
| E | 0:0 | 1.33 | 1.23 | 1.28 | 0 | 0 | 0 |

**TABLE 2** shows that the sample with HAp content has antibacterial activity against *Staphylococcus aureus* bacteria by being covered by the presence of a clear zone around the thin plate with the average length of the inhibitory zone produced in the antibacterial activity test on all samples A, B, C, D, and E respectively was 2.01 mm, 2,345 mm, 3.24 mm, 1.85 mm, 1.28 mm. The higher the mass of HAp, the average length of the bacterial inhibition zone also decreases due to the agglomeration of HAp, which also affects the ability of HAp to bind chitosan. When HAp undergoes agglomeration, the specific surface area available to interact with chitosan decreases. Chitosan itself is an antibacterial substance that is stronger than HAp. Therefore, agglomeration can decrease the ability of HAp to bind chitosan strongly. HAp agglomeration is a collection of small HAp particles that have a larger size but are weaker and hollower than the HAp aggregate form19.

HAp is also brittle, so it requires substances such as chitosan to strengthen its adsorption properties and mechanical strength20. However, too much hydroxyapatite will only make the bacterial inhibition zone decrease. Too much agglomerated HAp can reduce the effective surface area that chitosan interacts with against bacteria, resulting in a smaller bacterial inhibition zone. There are bacterial zones that can live in the inhibition zone in samples A, B, and C; this is because the high concentration of a certain antibacterial substance can trigger the efflux pump phenomenon carried out by a bacterium to survive in the zone with high antibacterial substances. However, these lower concentrations failed to trigger the efflux pump effect, resulting in double bacterial life zones21.

**FIGURE 4** shows the simulated body fluid (SBF) analysis using FTIR. Based on **FIGURE 4,** it can be seen that samples A0, B0, and C0, which had PO4 content respectively, had peaks at wavenumber of 79.5 cm-1, 76 cm-1, and 80 cm-1, while coated suture anchoring screw sample before soaked into the SBF solution had lower peaks than A3, B3, and C3 with the peaks at wavenumber of 70 cm-1, 73 cm-1, and 69 cm-1, respectively. This is because the longer the duration of immersion into the SBF solution, the more the peak of the PO4 group will form more apatite layers. This is because the longer the duration of immersion in the SBF solution, the higher peak of the PO4 group will form more apatite layers22.

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**FIGURE 4** Simulated Body Fluid (SBF) FTIR Test Results

According to Ghomi et al. on the nanostructure of hydroxyapatite scaffolds that are inserted in SBF solution over a period, in the first 19 days of immersion time, the pH value showed an increase to 7.65 due to the exchange of Na+ and Ca2+ ions with H+ from SBF and then gradually decreased to 7.5 due to the consumption of OH- ions in the process of formation of the apatite layer on the scaffold 23. The consumption of OH- ions that form the apatite layer was also confirmed to result in an increase in the peak intensity of OH- in the FTIR test after being immersed in the SBF solution for a certain period. The concentration of calcium ions increases with increasing immersion time. This is due to the high bioactivity and absorption capacity of hydroxyapatite nanostructures (HAp). The higher intensity of PO4 formed after a certain immersion time, indicating that with the increase of immersion time, an apatite hydroxycarbonate (HCA) layer will be formed, which is also formed because CO32- from the HCO3- ions of the SBF solution moves to the scaffold surface23.

The composite, or in this suture anchoring screw coated by hydroxyapatite, has a good crystallinity so that its bioactivity properties are very good, proved by the increase in ions or apatite layers in the PO4 wavenumber region after being soaked in SBF solution for 3 days. Bioactivity is a property or activity where there is an increase in chemical ions from the body to biomaterials that are implanted into the body24.

**TABLE 3** Peak Transmittance of Simulated Body Fluid (SBF) FTIR Test Results

|  |  |
| --- | --- |
| SBF Immersion Sample | Peak Transmittance (%) |
| PO4 (1040-1090 cm-1) |
| A0 | 79.5 |
| A3 | 70 |
| B0 | 76 |
| B3 | 73 |
| C0 | 80 |
| C3 | 69 |
| D0 | No PO4 |
| D3 | No PO4 |
| E0 | No PO4 |
| E3 | No PO4 |

The D0 and D3 samples had no PO4 groups from HAp, indicating no significant difference in the addition of ions that occurred in PLA without HAp. According to the SBF analysis conducted by Faqhiri et al. with the presence of chitosan content, it shows that there were almost no structural changes after the SBF test, which showed that the polymer remained stable during the SBF test25.

The E0 and E3 samples had no PO4 groups of HAp, indicating no significant difference in ion addition to PLA without coating. According to Shuqiong Liu's research using FTIR, it was found that few ionic deposits formed if pure PLA was dissolved into an SBF solution. This shows that pure PLA has weak bioactivity, but the addition of HAp to PLA can increase the bioactivity that has been prepared due to the good conductivity of HAp26.

# CONCLUSION

There are three variations of hydroxyapatite mass coating in 3D-printed suture anchoring screws, which results in a significant difference compared to the other two variations that have no hydroxyapatite coating. FTIR spectroscopy can identify a substance that contains a hydroxyl group (OH-). The substance or surface can be composed of PLA, hydroxyapatite, or chitosan. The FTIR test itself shows that there is an amine group (NH) owned by chitosan and a phosphate group (PO4) owned by HAp. The C=O cluster owned by the PLA is not visible because it is perfectly coated. The results of the EDX test of coating variations of samples A and C are by the Ca/P ISO 13175-3 standard with an optimal range of 1.59 – 1.72.

In the compressive strength test, the coating plays a very important role in the increase in compressive strength. The addition of HAp as a reinforced filler is very influential as an increase in compressive strength. With the higher composition of HAp in the coating, the suture anchoring screwhas a higher strength.

Scaffolds with HAp solution have antibacterial activity against *Staphylococcus aureus* bacteria by being covered by the presence of a clear zone around thin plates with an inhibitory zone range of 3.24 mm. In addition, in the Simulated Body Fluid (SBF), the results were obtained that the mass variation of 0.6 grams of HAp, 0.125 grams of chitosan, and 10 mL of 2% acetic acid had a peak decrease from 80% to 69% after 3 days of immersion in SBF solution, indicates the formation of an apatite layer after immersion.

# Acknowledgments

Our gratitude goes to the relevant parties who have helped this research run smoothly, our institution, the Department of Industrial Chemical Engineering, the Faculty of Vocational Studies ITS, the research grant for the innovation and downstream research program of the ADB HETI fund – ITS (Grant no. 2160/PKS/ITS/2022), Program Kreativitas Mahasiswa-Research (PKM-RE) 2024 grant by Ministry of Education, Culture, Research, and Technology of Indonesia.

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