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Hydroxyapatite of cow bone used as filler to enhance compressive strength characteristics in composite restoration

Abstract

Objective: The goal of the current study is to ascertain whether hydroxyapatite from cow bones could potentially be used as a filler to improve the compressive strength characteristics of composite fillings. **Material and Methods:** The composite filling material used in the test group was self-processed and comprised filler derived from hydroxyapatite of cow bones, which were synthesized via an alkaline hydrothermal method. A composite material manufactured at a factory with silica filler served as the control group. Each group was formed into 16 slabs, each measuring 2 mm thick and 6 mm in diameter. The slabs were polymerized using light curing for 20 seconds. A compression testing machine was used to conduct the compressive strength test, and statistical analysis was performed on the collected data. **Results:** The average compressive strength value of the test group was greater than the control group, with a statistically significant difference. **Conclusion:** When employed as a filler, hydroxyapatite from cow bones can enhance a composite filling's mechanical qualities, specifically its compressive strength.

Keywords: composite, filler, hydroxyapatite

Introduction

Based on improvements and innovations in dental material science and technology, dental materials evolve in tandem with world situations. One of the restorative materials that is frequently utilised in dentistry is composite. It has a number of benefits, such as matching the colour of the teeth, only requiring minor tooth preparation, and being completed in a single visit. On the other hand, the low durability and high cost of the raw materials are two drawbacks of composite.^{1,2}

The composite resin composition comprises an organic polymer matrix, inorganic filler, coupling agent, and initiator-activator. Frequent fillers are quartz, zirconia borosilicate glass, lithium, barium aluminum silicate, and strontium or zinc glass. Inorganic fillers reduce shrinkage due to polymerization, thermal expansion, and water absorption and strengthen the composite matrix by increasing hardness, strength, and resistance to wear. Research on composite resins continues to be carried out to obtain composite resins with high biocompatibility with tissue. One way to get a composite resin with good biocompatibility properties is to replace the commonly used filler material with hydroxyapatite (HA). Apart from synthetics, HA can also be obtained from natural materials such as vertebrate bones or animals because HA is the main component of hard tissue in vertebrates.³⁻⁶

Efficient and economical use of livestock waste will prevent devastating environmental pollution, aesthetic value, and various health problems for human life. The presence of livestock manure and bone waste in Indonesia is relatively high due to the high total consumption of beef, chicken, and pork in Indonesia, which reached 3,572 and 4,092 kg/capita/year in 2009 – 2010 and is predicted to continue to increase. The potential for biological waste, such as cow bones in Indonesia, is quite significant, while cow bones have a relatively high calcium content, around 85.84%. The high calcium content can be used to synthesize hydroxyapatite as a composite filler material.⁷⁻⁸

This research uses composite resin with a filler containing hydroxyapatite from cow bone, which theoretically has good mechanical properties. Apart from that, the beef bones used are natural and halal ingredients.

The composite used must have good durability so that the restoration can last longer. Thus, the

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clinical application of composite resin must consider various mechanical properties, such as compressive strength, especially for posterior tooth restorations with large chewing loads. Based on the background described above, this study aims to evaluate the compressive strength properties of artificial composites with fillers using natural hydroxyapatite from cow bone.

Material and Methods

This research protocol has been reviewed and approved by the ethics commission of the Indonesian Muslim University under approval number 205/A.1/KEPK-UMI/XII/2023.

Preparation for Removing Protein and Fat from Cow Bones

Cow bones are cleaned with a water jet from dirt and residual substances such as cartilage, joints, ligaments, and other tissues attached to the bones. Clean bones are boiled in distilled water for 4 hours (the boiled water is changed every hour) to facilitate the removal of bone marrow and tendons. Bone fat was removed by placing the bones in an autoclave for 30 minutes, followed by immersion in a 1:1 methanol-chloroform solution. The bones were then washed clean and dried in an oven at 100 °C for 24 hours to avoid the formation of soot on the material's surface during the heating treatment. The dried specimens were cut into cubic prism shapes (10 mm × 10 mm × 10 mm) and then calcined in an oven/kiln at a temperature of 900 °C using a temperature rate of 5 °C / min at ambient conditions. The temperature is maintained for 2 hours at the same degree to remove organic substances. Next, cool it until it reaches room temperature.

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Synthesis of Bovine Bone Hydroxyapatite

The alkaline hydrothermal process was carried out on bone pieces by adding 20% NaOH solution, followed by an autoclave process for 30 minutes, and continued with stirring using a magnetic stirrer at a temperature of 100°C for 5 hours. The crushed bones are turned into a fine powder, filtered, and washed until the pH is neutral. The hydroxyapatite precipitate formed was left for 24 hours at room temperature, and then the precipitate was dried in an oven at 60°C.

Manufacture of composite filling materials

Filler is made by mixing hydroxyapatite from cow bone extract and a coupling agent (in the form of trimethoxy (propyl) silane) in a mixing container and stirring using a magnetic stirrer until homogeneous for 48 hours, then drying using filter paper and drying in an oven at 80°C for 30 minutes, then at 100°C for the next 30 minutes (stored in container a). It is making the matrix in container b by mixing UDMA (urethane methacrylate, mixture with isomers), TEGDMA (tri ethylene glycol methacrylate), and HEMA (2-hydroxyethyl methacrylate). Next, in container c, mix the inhibitor in the form of butylated hydroxytoluene, the initiator in the form of camphorquinone, and the accelerator in the form of DMAEMA (dimethylaminoethyl methacrylate). In a different container, the three types of materials from containers a, b, and c were mixed in a ratio of 1 wt%: 1 wt%: 1 wt%. The mixture is stirred slowly until it is homogeneous to form a paste.

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Creating Composite Plates and Testing their Compressive Strength

Each of the 16 test and control group samples was made into a round plate with a thickness of 2 mm and a diameter of 6 mm, which was then polymerized using LED light curing (Xlite II LED curing unit, Flight Dental System, China) with a light intensity of 1700 mW/cm² for 20 seconds. Next, the sample was soaked in a closed container containing liquid NaCl to maintain humidity according to conditions in the mouth and kept for 24 hours at 25°C in an incubator. All samples were tested for compressive strength using a Universal Testing Machine (UTM; TISY Co., Ltd., Taichung, Taiwan) with a speed of 1 mm/min and a maximum load of 200 kgf.

Data Analysis

The compression test results in this study were collected and processed using SPSS software version 17.0 (SPSS inc., Chicago, IL, USA), and analyzed using the Mann Whitney Test. The *p* value < 0.05 indicates statistical significance.

Results

This research was conducted to determine the compressive strength of composite resin containing cow bone's hydroxyapatite filler. The hydroxyapatite compound is extracted from cow bones using a wet method, hydrothermal alkaline. Next, the hydroxyapatite extract obtained is used as a filler to manufacture composite backfill material whose compressive strength will be tested.

Microscopic images of the test group and control group are shown in Figure 1 and obtained from a CCD camera installed on the Vickers Hardness testing Machine close to the indenter. This picture was obtained in previous research which tested the hardness of this composite material. The image received shows the diagonal of the indenter facing the filling surface, which the machine has pressurized. The microscopic image shows that the chemical structure in the test group is hexagonal, while in the control group, it is irregular.

Table 1 shows the values of the compression test results for the test and control groups. The test group that used a composite with filler from cow bone hydroxyapatite showed a higher average compressive strength than the control group and a *p*-value <0.05, which indicated there was a significant difference between the two groups.

Discussion

Based on the microscopic image, the filler's different chemical structures and shapes may cause the different microscopic images between the test and control groups. Silica, the filler from the control group, is shaped like a chain and looks irregular. In contrast, hydroxyapatite, the filler from the test group, has a hexagonal shape formed from calcium, phosphate, and hydroxyl atoms.⁹⁻¹¹ This hexagonal structure allows the substitution of many other ions to obtain optimal multiple benefits without changing the symmetry.¹² This crystal shape is the same as enamel hydroxyapatite crystals.¹²

A good restoration will show strength against chewing in the oral cavity over a long period and sufficient strength and resistance to fracture.¹³ One of the essential mechanical properties of restorative material is compressive strength, which should resemble the tooth's ability to withstand chewing forces.^{13,14} When chewing, teeth will receive an average compressive force of around 100 N to 150 N.¹⁵ In this study, composite materials containing hydroxyapatite filler can receive an average compressive force nine times higher than teeth.

One of the filler materials used in resin is hydroxyapatite, which shows various advantages, including good mechanical properties and lower shrinkage compared to resins that do not use hydroxyapatite.¹⁶ However, resin with hydroxyapatite has several weaknesses that also affect the

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mechanical strength and durability of the composite as a restorative material, so efforts are being made to synthesize hydroxyapatite filler with a nanoparticle size that is capable of showing a high surface-to-volume ratio and better dispersion which in the end will provide better mechanical strength too.¹⁶

Several studies have shown that the light intensity and polymerization time of composites using light cure can influence the restoration's physical properties and clinical performance.¹⁷⁻²⁰ This research was polymerized using a light cure LED with high light intensity, which may influence the composite material's compressive strength. The high light intensity reduces the curing time, thereby making restoration procedures more efficient.¹⁹ This study used a polymerization time of 20 seconds, which in other studies showed compressive strength better than 10 seconds.^{18,19} Longer polymerization times (more than 40 seconds) can reduce compressive strength because increasing temperature causes a decrease in crystals, ultimately affecting the polymer's strength.¹⁸ This study used cow bone hydroxyapatite as a filler. It is well known that filler has a potential to improve the mechanical characteristics of composite fillings. Calcium (Ca) and phosphate (PO₄³⁻) are components of hydroxyapatite. According to stoichiometric calculations, animal bones typically contain a higher ratio of calcium to phosphate than synthetic materials.²¹ Consequently, it is possible to state that the composite filling's compressive strength value can be raised by hydroxyapatite.

Conclusion

When utilised as a filler, hydroxyapatite from cow bones may improve the composite filling material's mechanical qualities, such as its compressive strength, which will ultimately render the restoration more resilient to chewing.

Acknowledgment

The author expresses gratitude to the dean of FKG UMI Prof. drg. Mohammad Dharma Utama., Ph.D., Sp. Pros (K) for his assistance during the research process.

Conflict of Interest

The author stated no conflict interest.

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Figure

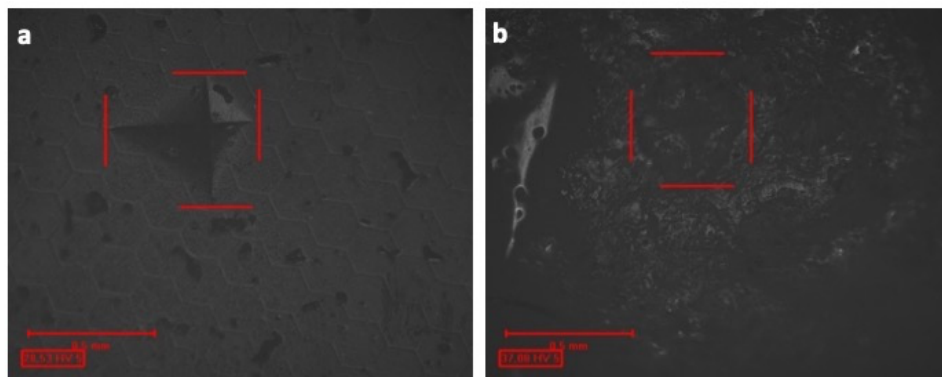


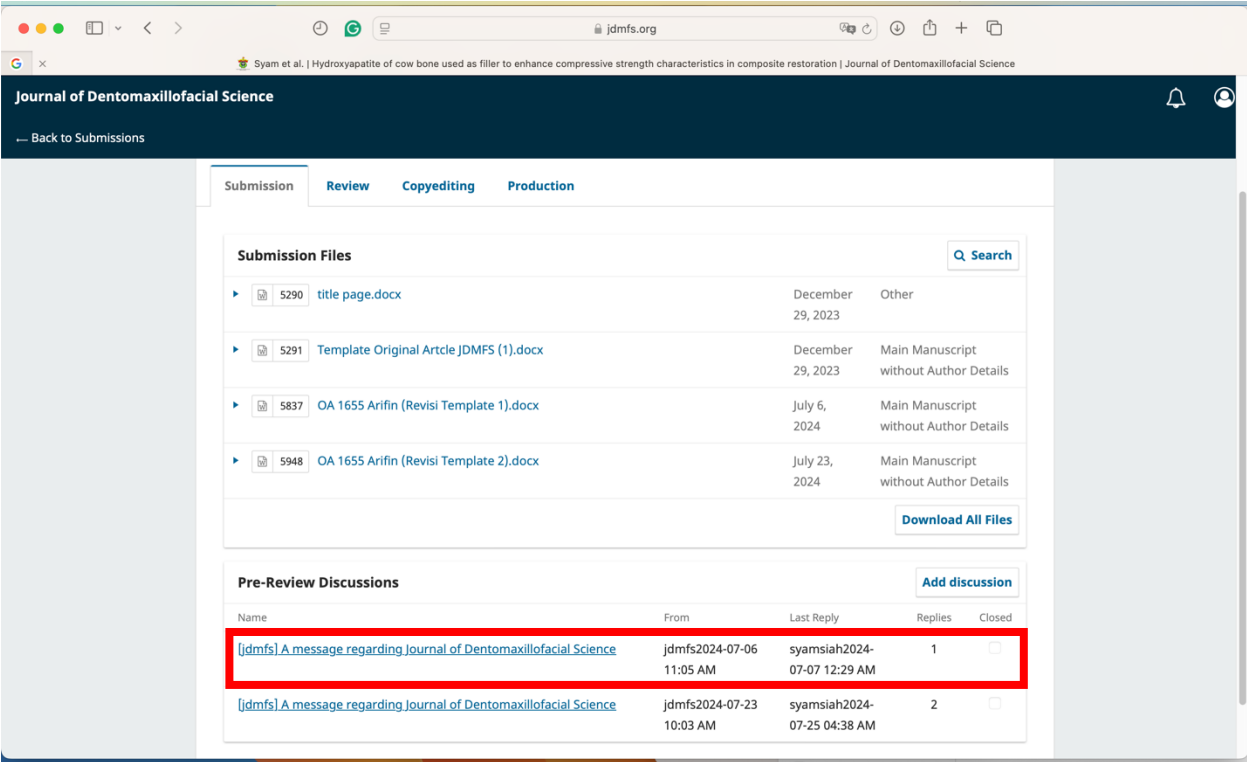
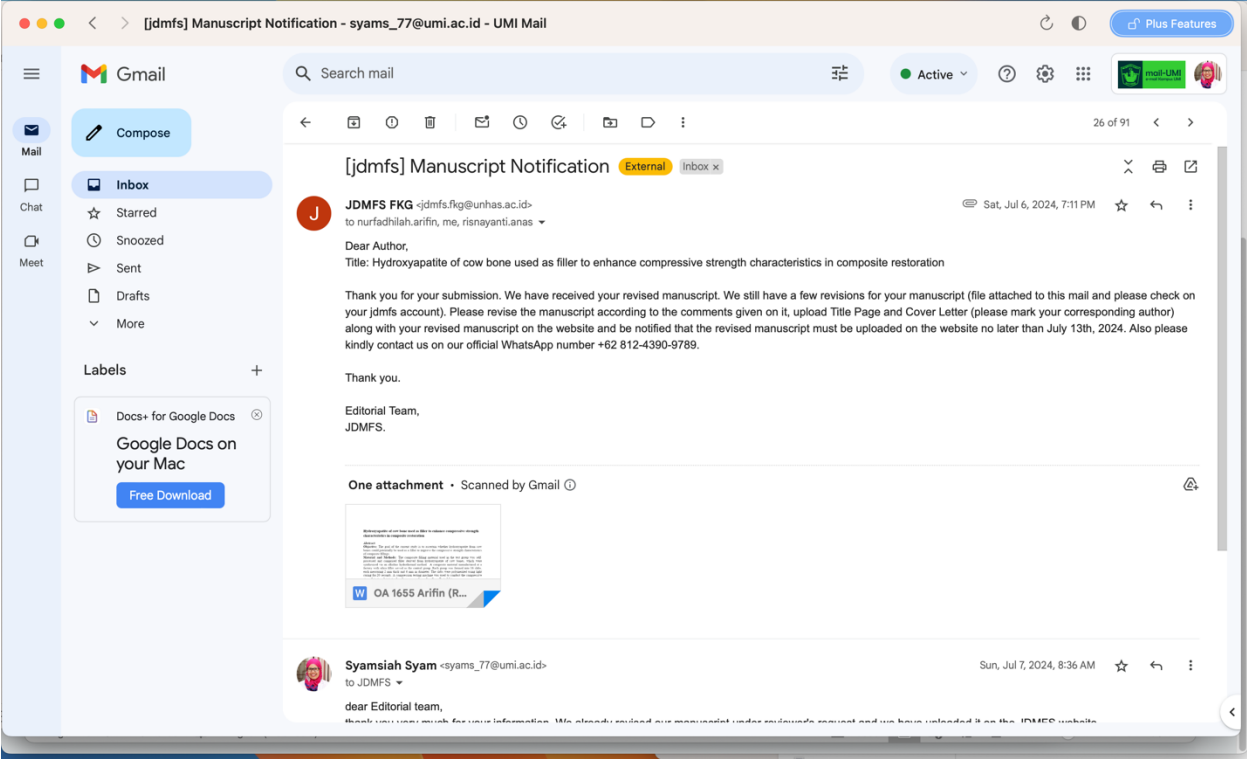
Figure 1. Microscopic view of the composite surface of the: (a) test group and (b) control group

Table

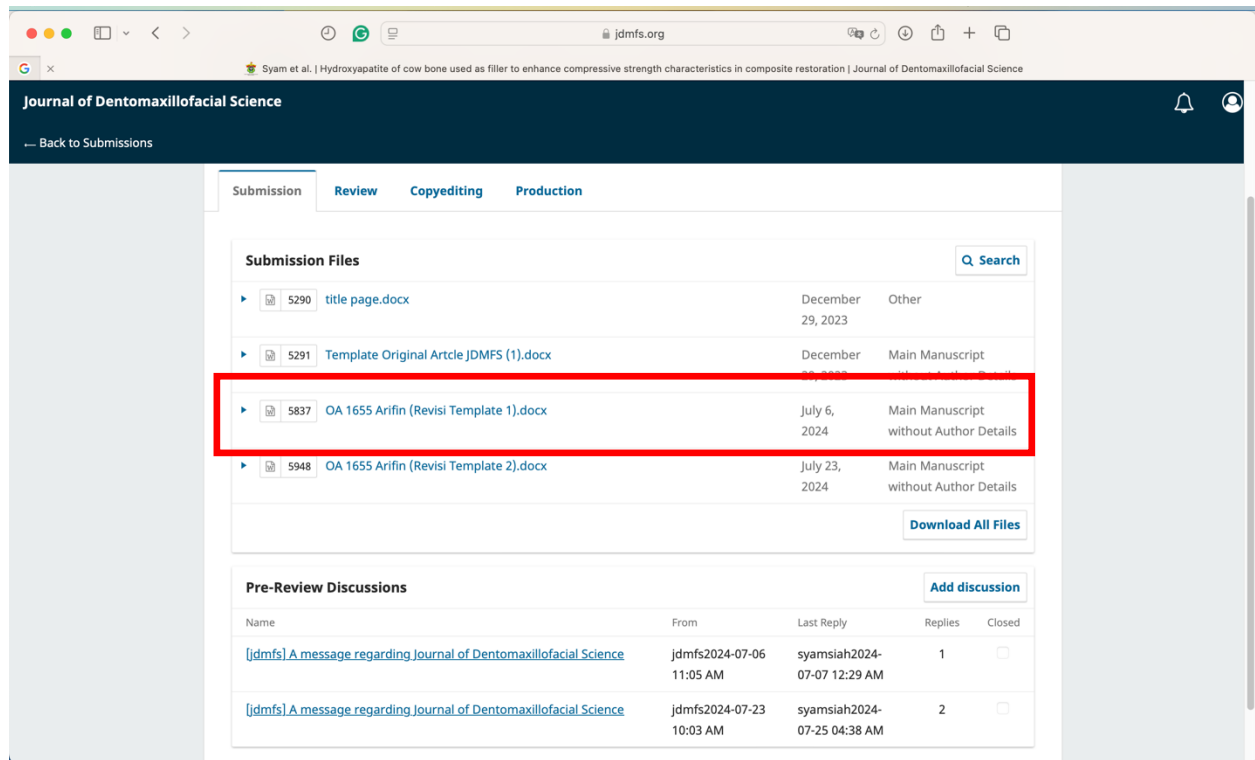
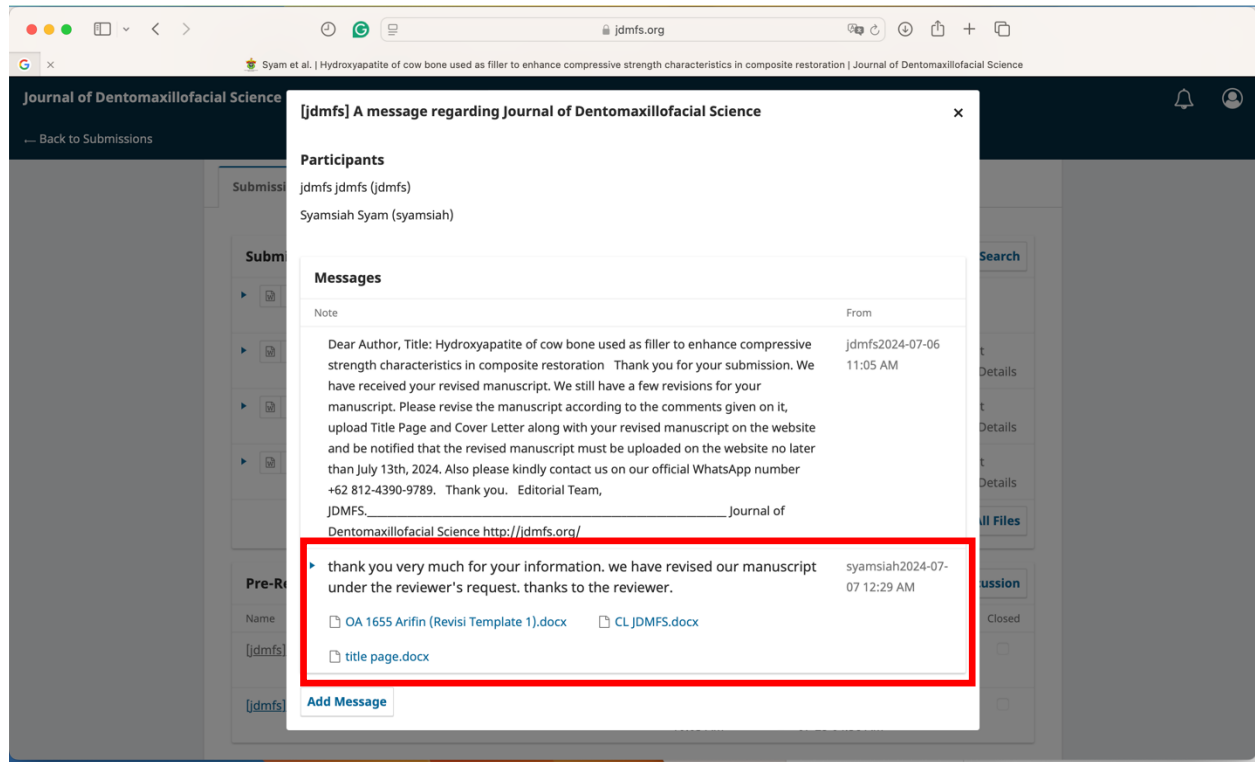
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Group	n	mean (N)	SD (min-max)	<i>p</i>
Control	16	2310.25	1262.49 (376.5-4802.9)	0.007
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2.Bukti Konfirmasi Review dan Hasil Review Pertama (6 Juli 2024)



**3. Bukti Konfirmasi Submit Revisi Pertama,
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Abstract

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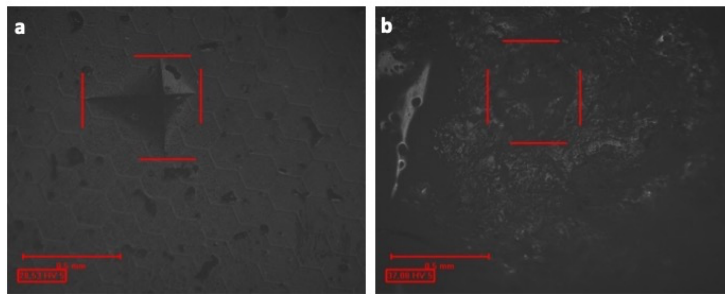


Figure 1. Microscopic view of the composite surface of the: (a) test group and (b) control group.

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One of the filler materials used in resin is hydroxyapatite, which shows various advantages, including good mechanical properties and lower shrinkage compared to resins that do not use hydroxyapatite.¹⁶ However, resin with hydroxyapatite has several weaknesses that also affect the mechanical strength and durability of the composite as a restorative material, so efforts are being made to synthesize hydroxyapatite filler with a nanoparticle size that is capable of showing a high surface-to-volume ratio and better dispersion which in the end will provide better mechanical strength too.¹⁶

Several studies have shown that the light intensity and polymerization time of composites using light cure can influence the restoration's physical properties and clinical performance.¹⁷⁻²⁰ This research was polymerized using a light cure LED with high light intensity, which may influence the composite material's compressive strength. The high light intensity reduces the curing time, thereby making restoration procedures more efficient.¹⁹ This study used a polymerization time of 20 seconds, which in other studies showed compressive strength better than 10 seconds.^{18,19} Longer polymerization times (more than 40 seconds) can reduce compressive strength because increasing temperature causes a decrease in crystals, ultimately affecting the polymer's strength.¹⁸

This study used cow bone hydroxyapatite as a filler. It is well known that filler has a potential to improve the mechanical characteristics of composite fillings. Calcium (Ca) and phosphate (PO_4^{3-}) are components of hydroxyapatite. According to stoichiometric calculations, animal bones typically contain a higher ratio of calcium to phosphate than synthetic materials.²¹ Consequently, it is possible to state that the composite filling's compressive strength value can be raised by hydroxyapatite.

Conclusion

When utilised as a filler, hydroxyapatite from cow bones may improve the composite filling material's mechanical qualities, such as its compressive strength, which will ultimately render the restoration more resilient to chewing.

Acknowledgment

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Conflict of Interest

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Hydroxyapatite of cow bone used as filler to enhance compressive strength characteristics in composite restoration

Abstract

Objective: The goal of the current study is to ascertain whether hydroxyapatite from cow bones could potentially be used as a filler to improve the compressive strength characteristics of composite fillings.

Material and Methods: The composite filling material used in the test group was self-processed and comprised filler derived from hydroxyapatite of cow bones, which were synthesized via an alkaline hydrothermal method. A composite material manufactured at a factory with silica filler served as the control group. Each group was formed into 16 slabs, each measuring 2 mm thick and 6 mm in diameter. The slabs were polymerized using light curing for 20 seconds. A compression testing machine was used to conduct the compressive strength test, and statistical analysis was performed on the collected data.

Results: The average compressive strength value of the test group was greater than the control group, with a statistically significant difference.

Conclusion: When employed as a filler, hydroxyapatite from cow bones can enhance a composite filling's mechanical qualities, specifically its compressive strength.

Keywords: composite, filler, hydroxyapatite

Introduction

Based on improvements and innovations in dental material science and technology, dental materials evolve in tandem with world situations. One of the restorative materials that is frequently utilised in dentistry is composite. It has a number of benefits, such as matching the colour of the teeth, only requiring minor tooth preparation, and being completed in a single visit. On the other hand, the low durability and high cost of the raw materials are two drawbacks of composite.^{1,2}

The composite resin composition comprises an organic polymer matrix, inorganic filler, coupling agent, and initiator-activator. Frequent fillers are quartz, zirconia borosilicate glass, lithium, barium aluminum silicate, and strontium or zinc glass. Inorganic fillers reduce shrinkage due to polymerization, thermal expansion, and water absorption and strengthen the composite matrix by increasing hardness, strength, and resistance to wear. Research on composite resins continues to be carried out to obtain composite resins with high biocompatibility with tissue. One way to get a composite resin with good biocompatibility properties is to replace the commonly used filler material with hydroxyapatite (HA). Apart from synthetics, HA can also be obtained from natural materials such as vertebrate bones or animals because HA is the main component of hard tissue in vertebrates.³⁻⁸

Efficient and economical use of livestock waste will prevent devastating environmental pollution, aesthetic value, and various health problems for human life. The presence of livestock manure and bone waste in Indonesia is relatively high due to the high total consumption of beef, chicken, and pork in Indonesia, which reached 3,572 and 4,092 kg/capita/year in 2009 – 2010 and is predicted to continue to increase. The potential for biological waste, such as cow bones in Indonesia, is quite significant, while cow bones have a relatively high calcium content, around 85.84%. The high calcium content can be used to synthesize hydroxyapatite as a composite filler material.^{9,10}

This research uses composite resin with a filler containing hydroxyapatite from cow bone, which theoretically has good mechanical properties. Apart from that, the beef bones used are natural and halal ingredients.

The composite used must have good durability so that the restoration can last longer. Thus, the clinical application of composite resin must consider various mechanical properties,

such as compressive strength, especially for posterior tooth restorations with large chewing loads. Based on the background described above, this study aims to evaluate the compressive strength properties of artificial composites with fillers using natural hydroxyapatite from cow bone.

Material and Methods

This research protocol has been reviewed and approved by the ethics commission of the Indonesian Muslim University under approval number 205/A.1/KEPK-UMI/XII/2023.

Preparation for Removing Protein and Fat from Cow Bones

Cow bones are cleaned with a water jet from dirt and residual substances such as cartilage, joints, ligaments, and other tissues attached to the bones. Clean bones are boiled in distilled water for 4 hours (the boiled water is changed every hour) to facilitate the removal of bone marrow and tendons. Bone fat was removed by placing the bones in an autoclave for 30 minutes, followed by immersion in a 1:1 methanol-chloroform solution. The bones were then washed clean and dried in an oven at 100 °C for 24 hours to avoid the formation of soot on the material's surface during the heating treatment. The dried specimens were cut into cubic prism shapes (10 mm × 10 mm × 10 mm) and then calcined in an oven/kiln at a temperature of 900 °C using a temperature rate of 5 °C / min at ambient conditions. The temperature is maintained for 2 hours at the same degree to remove organic substances. Next, cool it until it reaches room temperature.

Based on improvements and innovations in dental material science and technology, dental materials evolve in tandem with world situations. One of the restorative materials that is frequently utilised in dentistry is composite. It has a number of benefits, such as matching the colour of the teeth, only requiring minor tooth preparation, and being completed in a single visit. On the other hand, the low durability and high cost of the raw materials are two drawbacks of composite.

Synthesis of bovine bone hydroxyapatite and Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The alkaline hydrothermal process was carried out on bone pieces by adding 20% NaOH solution, followed by an autoclave process for 30 minutes, and continued with stirring using a magnetic stirrer at a temperature of 100°C for 5 hours. The crushed bones are turned into a fine powder, filtered, and washed until the pH is neutral. The hydroxyapatite precipitate formed was left for 24 hours at room temperature, and then the precipitate was dried in an oven at 60°C. Hydroxyapatite from bovine bone then subjected to FTIR analysis (FTIR-8400S Shimadzu, Japan) using wavelength of 500 – 4000 cm⁻¹ to confirm its hydroxyapatite compound.

Manufacture of composite filling materials

Filler is made by mixing hydroxyapatite from cow bone extract and a coupling agent (in the form of trimethoxy (propyl) silane) in a mixing container and stirring using a magnetic stirrer until homogeneous for 48 hours, then drying using filter paper and drying in an oven at 80°C for 30 minutes, then at 100°C for the next 30 minutes (stored in container a). It is making the matrix in container b by mixing UDMA (urethane methacrylate, mixture with isomers), TEGDMA (tri ethylene glycol methacrylate), and HEMA (2-hydroxyethyl methacrylate). Next, in container c, mix the inhibitor in the form of butylated hydroxytoluene, the initiator in the form of camphorquinone, and the accelerator in the form of DMAEMA (dimethylaminoethyl methacrylate). In a different container, the three types of materials from containers a, b, and c were mixed in a ratio of 1wt%: 1wt%: 1wt%. The mixture is stirred slowly until it is homogeneous to form a paste.

Creating composite plates and testing their compressive strength

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Each of the 16 test and control group (presented in Table 1) samples was made into a round plate with a thickness of 2 mm and a diameter of 6 mm, which was then polymerized using LED light curing (Xlite II LED curing unit, Flight Dental System, China) with a light intensity of 1700 mW/cm² for 20 seconds. Next, the sample was soaked in a closed container containing liquid NaCl to maintain humidity according to conditions in the mouth and kept for 24 hours at 25°C in an incubator. All samples were tested for compressive strength using a Universal Testing Machine (UTM; TISY Co., Ltd., Taichung, Taiwan) with a speed of 1 mm/min and a maximum load of 200 kgf.

Table 1. Characteristics of composite resin used in control groups (manufactures' data)

Material	Manufacturer	Matrix composition	Filler type
Solare X	GC Corp., Tokyo, Japan	Camphorquinone photoinitiator, UDMA	Silica filler

Data Analysis

The compression test results in this study were collected and processed using SPSS software version 17.0 (SPSS inc., Chicago, IL, USA), and analyzed using the Mann Whitney Test. The *p* value < 0.05 indicates statistical significance.

Results

This research was conducted to determine the compressive strength of composite resin containing cow bone's hydroxyapatite filler. The hydroxyapatite compound is extracted from cow bones using a wet method, hydrothermal alkaline. Next, the hydroxyapatite extract obtained is used as a filler to manufacture composite backfill material whose compressive strength will be tested.

Microscopic images of the test group and control group are shown in Figure 1 and obtained from a CCD camera installed on the Vickers Hardness testing Machine close to the indenter. This picture was obtained in our previous research which tested the hardness of this composite material. The image received shows the diagonal of the indenter facing the filling surface, which the machine has pressurized. The microscopic image shows that the chemical structure in the test group is hexagonal, while in the control group, it is irregular.

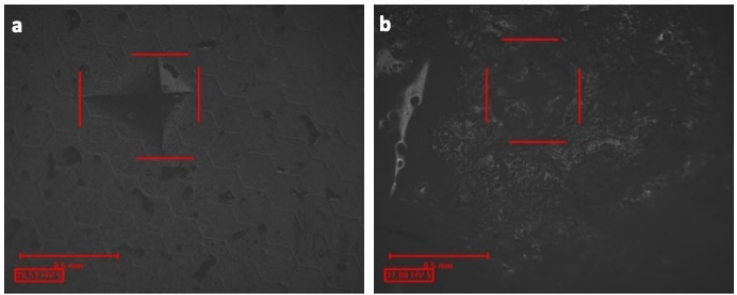


Figure 1. Microscopic view of the composite surface of the: (a) test group and (b) control group

The results of the FTIR analysis are shown in Figure 2. The results of the FTIR analysis of bovine bone hydroxyapatite were identified as having main components/functional groups

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consisting of hydroxyl (OH⁻), carbonate (CO₃²⁻), and phosphate (PO₄³⁻). These three groups are the main components in the formation of hydroxyapatite.

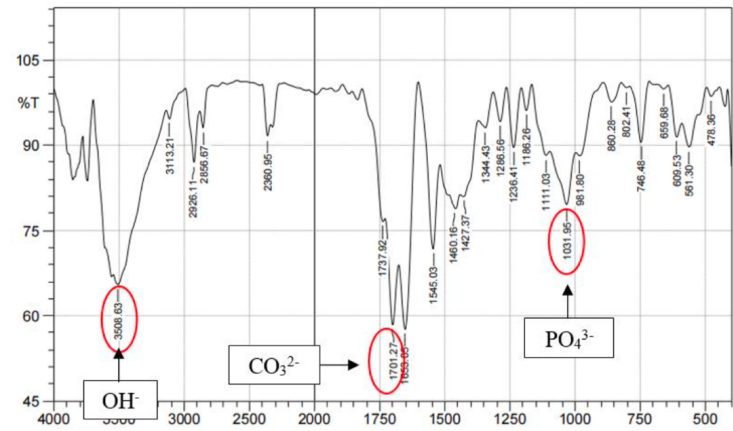


Figure 2. FTIR view of hydroxyapatite of bovine bone

Table 2 shows the values of the compression test results for the test and control groups. The test group that used a composite with filler from cow bone hydroxyapatite showed a higher average compressive strength than the control group and a *p*-value <0.05, which indicated there was a significant difference between the two groups.

Table 2. Compressive strength test results for test and control groups

Group	n	mean (N)	SD (min-max)	p
Control	16	2310.25	1262.49 (376.5-4802.9)	0.007
Test	16	4027.81	1442.76 (1309.7-4803.4)	

Discussion

Based on the microscopic image, the filler's different chemical structures and shapes may cause the different microscopic images between the test and control groups. Silica, the filler from the control group, is shaped like a chain and looks irregular. In contrast, hydroxyapatite, the filler from the test group, has a hexagonal shape formed from calcium, phosphate, and hydroxyl atoms.¹¹⁻¹³ This hexagonal structure allows the substitution of many other ions to obtain optimal multiple benefits without changing the symmetry.¹⁴ This crystal shape is the same as enamel hydroxyapatite crystals.¹⁴

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