

2025年臺灣國際科學展覽會

優勝作品專輯

作品編號	090029
參展科別	醫學與健康科學
作品名稱	Production of Nano-Composite Artificial Bone Tissue Using Bioceramic Synthesis from Bio-Waste
得獎獎項	四等獎

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關鍵詞	<u>Tissue scaffold, nano silica, dicalcium silicate, starch consolidation, PEEK, biocompatibility, Staphylococcus aureus, strength tests</u>
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作者照片



Production of Nano Composite Artificial Bone Tissue Through Bioseramic Synthesis Derived from Bio-Waste

Abstract

Certain specially structured ceramics, which can be used as biomaterials to replace bone, have recently started being utilized in the medical field.

The aim of this study is to produce high-bioactivity silica from corn cob waste, a widely available organic material in nature, and combine it with calcium oxide (CaO) obtained by grinding organic mussel shell waste with high bioactivity. This combination is intended to synthesize dicalcium silicate ($2\text{CaO} \cdot \text{SiO}_2$) to develop an alternative tissue scaffold with high bioactivity, capable of replacing bone, for existing titanium alloys. The goal is to incorporate this scaffold into PEEK (polyether ether ketone), a novel tissue scaffold material, at varying percentages to create a next-generation innovative bone substitute material.

An additional objective is to demonstrate through biocompatibility tests that the produced ceramic-polymer biocomposite exhibits antibacterial activity against *Staphylococcus aureus*.

In the study, starch-based consolidation was used to create tissue scaffolds containing dicalcium silicate.

This method is both cost-effective and easy to implement. PEEK material, known for its excellent chemical, mechanical, and thermal properties, offers high strength and can be manufactured using CAD/CAM systems. Dicalcium silicate material, blended with PEEK in proportions ranging from 1% to 12%, was processed using a twin-screw extruder to produce homogeneous filaments. These filaments were further used to fabricate samples with 60% bone-like density using CAD/CAM systems. The antibacterial effects of these samples against *Staphylococcus aureus* bacteria were investigated through biocompatibility tests.

The study concluded that filaments homogenized with 1% to 12% PEEK using a twin-screw extruder have the potential to become a next-generation bone substitute material.

Results from SEM-EDX analysis conducted with a scanning electron microscope indicated that the synthesized ceramic contained Si, O, Mg, and C in amounts sufficient to produce a biomaterial capable of replacing bone. It was concluded that the material is biocompatible and suitable for use as artificial bone tissue.

Keywords: Tissue scaffold, nano silica, dicalcium silicate, starch consolidation, PEEK, biocompatibility, *Staphylococcus aureus*, strength tests

Objective:

Silicon (Si), the most abundant element on Earth, possesses numerous beneficial properties in living organisms (Ehrlich H., 2019). Certain surface-reactive ceramics stand out for their ability to bond with connective tissues and function as bone substitutes (Hench LL, Bioceramics).

This study aims to utilize the abundant waste materials from corn (*Zea mays*) and farmed mussels (*Mytilus edulis*, *Mytilus galloprovincialis*). Natural silica will first be produced from corn cobs, while calcium carbonate powder obtained from ground mussel shells will be synthesized with the prepared natural silica to produce dicalcium silicate bioceramic. This material will then be combined with polyether ether ketone (PEEK) in proportions ranging from 1% to 12%. The goal is to develop a next-generation bone substitute material with enhanced strength and to produce a nano-composite artificial bone tissue. Furthermore, biocompatibility tests will be conducted on the produced tissue to evaluate its potential for biomedical applications.

1. Introduction

Corn (*Zea mays*), belonging to the tribe Maydeae of the family Poaceae (grasses), is a plant that provides the highest yield among all cool and warm climate cereals. It is a C4 plant that efficiently utilizes solar energy and produces the most dry matter per unit area. Corn holds a significant place in global agriculture (Vartanlı & Emeklier, 2007).



Figure 1. Corn Plant (*Zea mays*)

Globally, corn ranks second after wheat in terms of production volume among cereal crops cultivated over large areas.

TABLE 1 : Global Corn Production and Consumption TABLO 1 : Dünya'da Mısır Üretimi ve Tüketimi										
m. tons	2010/11	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18	2018/19 (est.)	2019/20 (f'cast)
Opening Stocks	184,6	177,8	197,2	211,9	271,9	301,6	324,9	367,1	339,3	322,7
Production	849,4	907,3	900,9	1031,8	1061,2	1023,5	1134,5	1091,6	1129,7	1112,2
Imports	94,1	99,3	99,6	121,7	125,1	136,3	137,4	153,3	164,7	168,8
Total Availability	1034,0	1085,2	1098,1	1243,7	1333,1	1325,1	1459,4	1458,7	1469,0	1434,9
Food	104,2	108,5	109,4	112,9	118,2	113,8	123,2	126,3	128,5	128,9
Feed	480,0	496,7	493,9	551,2	593,9	569,7	635,0	652,5	674,9	675,9
Industrial	241,7	250,5	250,1	269,6	277,9	281,0	292,4	302,7	302,2	308,3
Other	30,3	32,2	32,8	38,2	41,6	35,7	41,8	37,8	40,7	37,9
Total Consumption	856,2	887,9	886,2	971,8	1031,6	1000,1	1092,4	1119,3	1146,3	1151,0
Exports	94,1	99,3	99,6	121,7	125,1	136,3	137,4	153,3	164,7	168,8
Ending Stocks	177,8	197,2	211,9	271,9	301,6	324,9	367,1	339,3	322,7	284,0
a) Respective marketing years. Wheat and coarse grains. Excluding rice										
Source/ Kaynak: International Grain Council – Uluslararası Tahıl Konseyi (2020)										

Table 1. Global Corn Production and Consumption Volume (1000 tons) Source: 2020 International Grains Council Report

Corn cob ash contains >60% silica by mass, along with small amounts of metallic elements, making it an economically viable raw material for the production of silicates, silica, and silica nanoparticles. Corn cob ash is obtained as a fine powder after combustion, requiring minimal grinding, and is considered the most cost-effective source of silica (Velmurugan, 2015).



Figure 2. Corn Cobs (Dried)

The Mediterranean mussel and the blue mussel are species of mussels belonging to the Mytilidae family (Edible Mussels). They are found in almost all seas (Demirsoy, 2001). Today, both species are widely used worldwide (Gönülal, Balcıoğlu, 2002). The natural populations of mussels have an average density of approximately 24,000 mussels per square meter in the densest beds (FAO, *Mytilus galloprovincialis*).



Figure 3. Mediterranean Mussel
(*Mytilus galloprovincialis*)



Figure 4. Blue Mussel (*Mytilus edulis*)

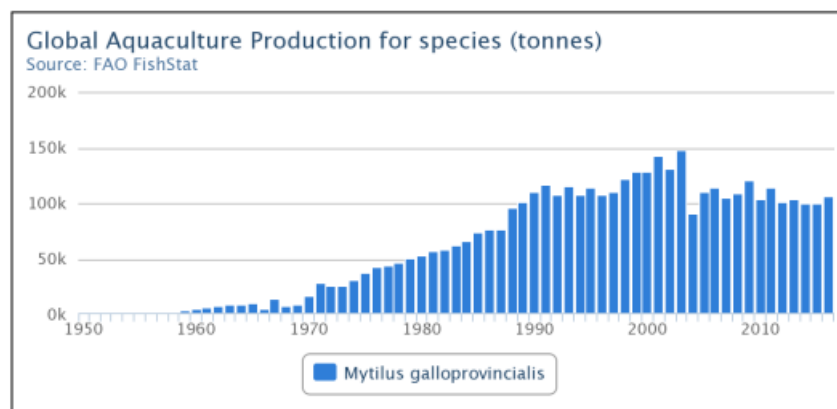


Figure 5. Global Aquaculture Production Volumes of *M. galloprovincialis* Over the Years (tons)
According to FAO FishStat Data (k = 1000)

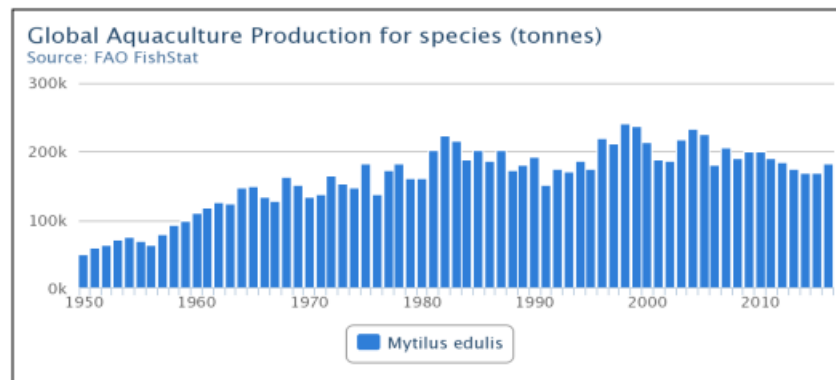


Figure 6. Global Aquaculture Production Volumes of *M. edulis* Over the Years (tons) According to FAO FishStat Data (k = 1000)

After harvesting, shellfish products (meat from shellfish) may undergo a refinement process to improve their appearance and increase their value. Mussels are typically cooked in water or steam, and their shells are discarded. Between 75% and 90% of all shellfish produced consists of shells. These shells are composed of approximately 95% CaCO_3 , with the remainder consisting of organic materials (Hamester, Balzer, & Becker, 2012).

Biotechnology can prove to be a practical approach for finding new alternatives to reduce pollution and reutilize the discarded shells (FAO, *Mytilus galloprovincialis*).



Figure 7. Mussel Shells (*Mytilus galloprovincialis* and *Mytilus edulis*)

Tissue scaffolds, one of the three fundamental components of tissue engineering, not only provide a suitable adhesion surface for cells but also ensure mechanical strength. They help establish interaction with surrounding tissue to respond to physiological and biological changes and contribute to the regeneration of the actual extracellular matrix (ECM).

The selection of materials for scaffold production is crucial. The material must be biocompatible, meaning it should not cause undesirable tissue reactions when implanted in the body. It should also possess surface chemistry that enhances cell adhesion and functionality. Once cells reach the capacity to form new ECM, the scaffold is no longer needed. Therefore, it is essential to produce the scaffold from a biodegradable material that can degrade within the body environment without losing biocompatibility or releasing toxic by-products.

Additionally, the scaffold must have a porous structure to allow the passage of cells and nutrients.

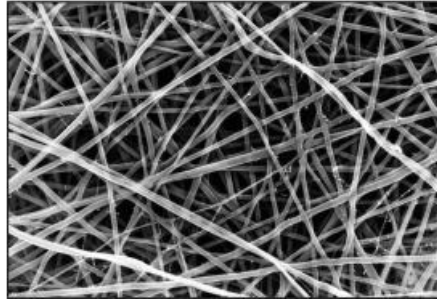


Figure 8. Tissue Scaffold

PEEK

Polyether-ether-ketone (PEEK) and poly-aryl-ether-ketone polymers belong to the family of high-temperature polymers. PEEK is a semi-crystalline polymer formed by the bonding of ketone and ether functional groups (Stawarczyk et al., 2015) and is known as a high-performance thermoplastic polymer that can substitute metallic components. Due to its elastic modulus, which is similar to cortical bone, PEEK plays a significant role as a viable alternative to traditional implant materials like titanium in orthopedics and traumatology.

Parts manufactured from the high-temperature plastic PEEK can operate at temperatures up to 260°C (480°F) and have a melting point of approximately 341°C (646°F). PEEK plastic is commonly used in hot water or steam environments and retains its physical properties, such as flexibility and tensile strength, to a large extent. Polyether-ether-ketone is often regarded as one of the more expensive engineering thermoplastics. However, experienced users recognize its added value as it enables the production of lighter, stronger, and more durable parts in demanding conditions.

Synthetic thermoplastic polymer PEEK has become a popular component in clinical orthopedic and spinal applications. However, its practical use is limited by certain constraints. While PEEK is biocompatible, chemically stable, radiolucent, and possesses an elastic modulus similar to normal human bone, it is biologically inert, which hinders effective integration with adjacent bone tissue upon implantation.

Recent efforts have focused on enhancing the bioactivity of PEEK to improve the bone-implant interface. Two main strategies have been employed to overcome PEEK's inert nature:

1. **Surface Modification:** Activating PEEK via surface treatment, either alone or in combination with a surface coating.
2. **Bioactive PEEK Composites:** Preparing bioactive PEEK composites by impregnating bioactive materials into the PEEK substrate.

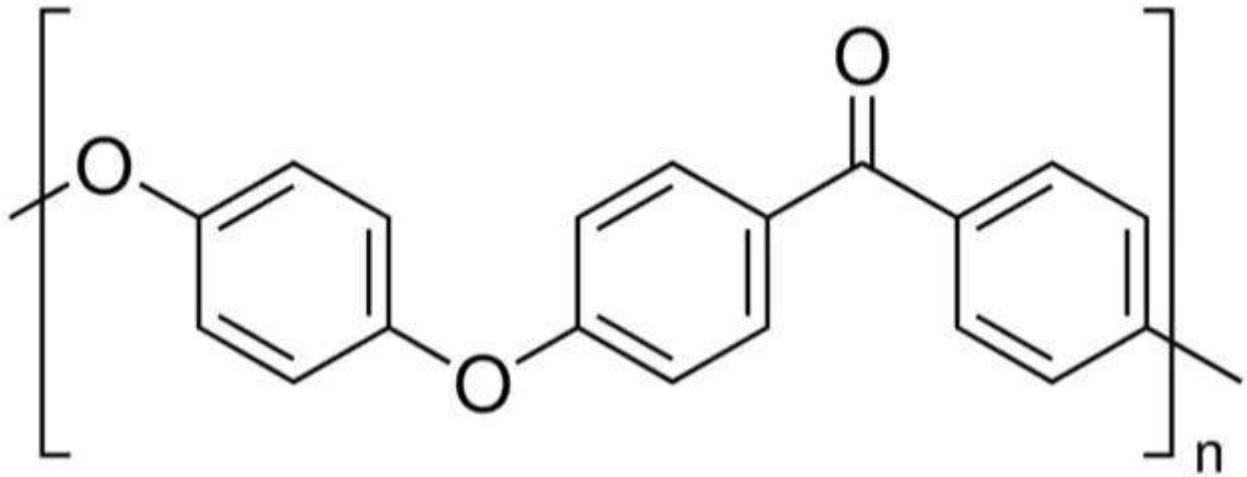


Figure 1. Molecular Formula of PEEK (CH₂O)_n

Additive Manufacturing Method:

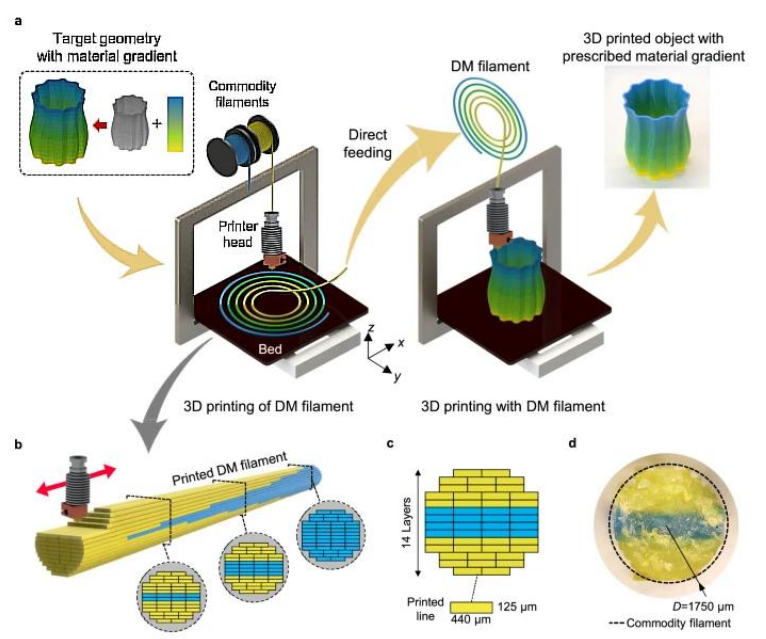
Additive manufacturing (AM) technology involves the process of transforming digital data into physical objects. In the first step of the process, the desired part is modeled using computer-aided design (CAD/CAM) software. The three-dimensional model is then sliced, converted into STL format, and subsequently printed.



Figure 2. Additive Manufacturing Method

Material Extrusion (Fused Deposition Modelling – FDM):

This method is commonly preferred for polymer materials. As a result, thermoplastic materials are typically used as the primary material, and these are most often in filament form.



PEEK Filament Production

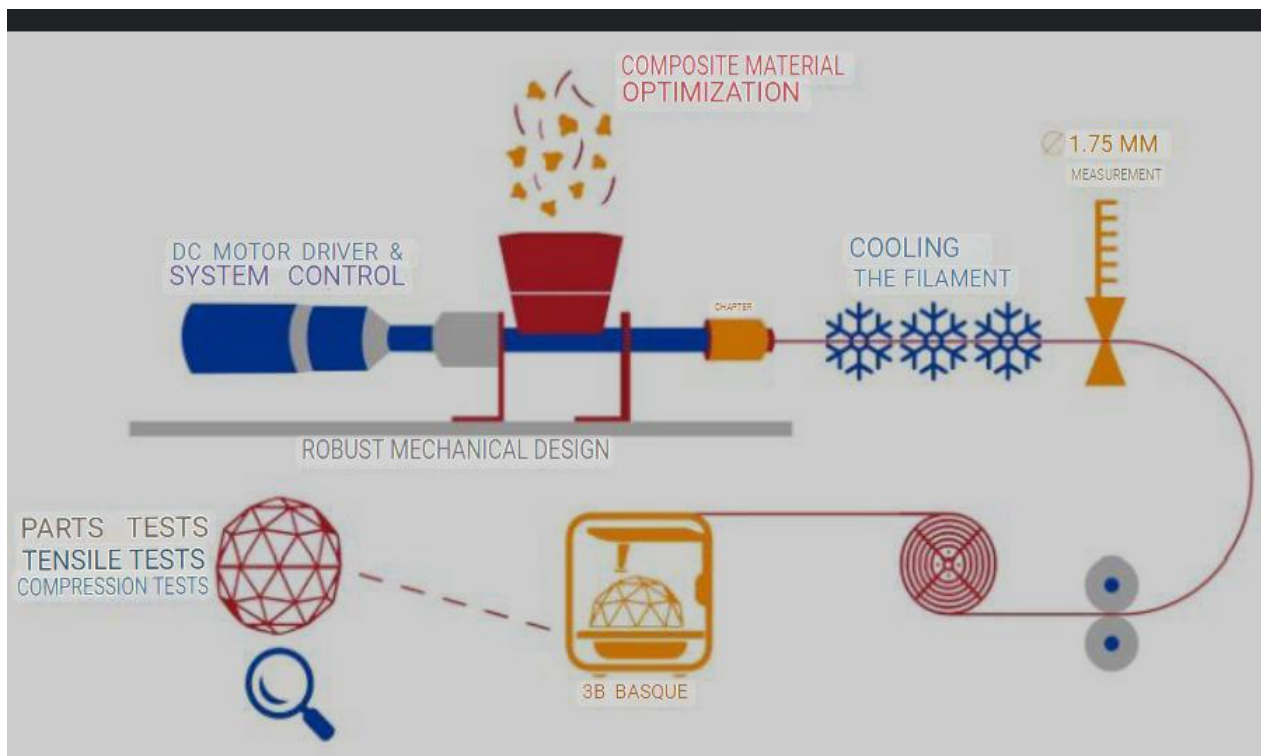


Figure 4. PEEK Filament Production and Usage Stages

Obtaining a Homogeneous Mixture of Two Materials Production of Twin-Screw Filament Extruder

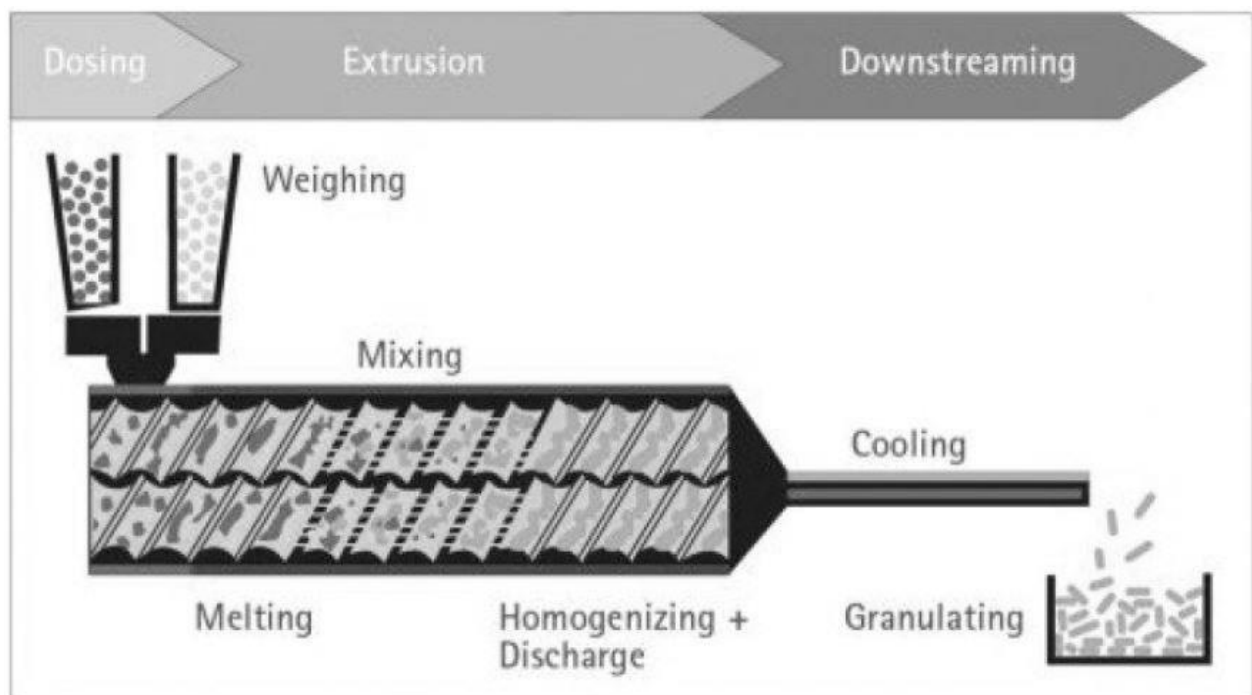


Figure 5. Homogenization and Granulation of Two-Material Mixture

For reference only

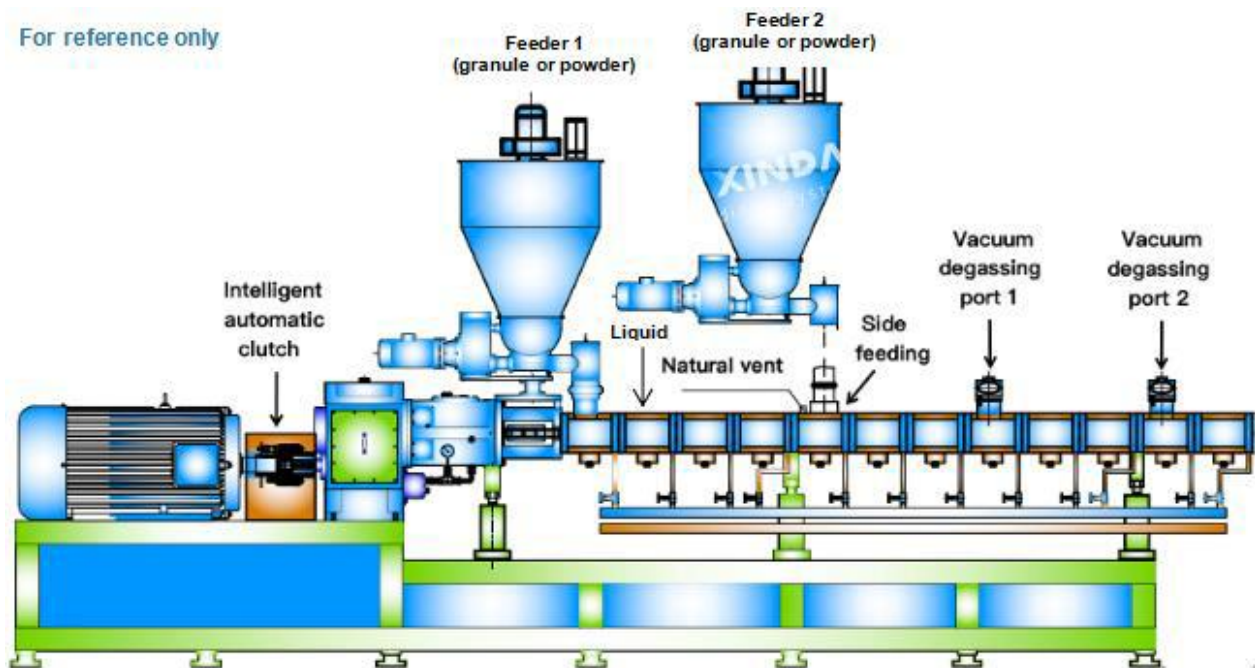


Figure 6. Conversion Stages of Granulated Two-Material Mixture into Filament

Treatment of Prosthetic Joint Infection with Implant Retention and Its Association with Commonly Encountered *Staphylococcus aureus*

Prosthetic joint infection (PJI) often requires a combination of medical and surgical treatment. While revision surgery is widely regarded as the gold standard surgical procedure, debridement, antibiotics, and implant retention with irrigation represent a highly attractive alternative.

Debridement, Antibiotics, and Implant Retention (DAIR) is an appealing treatment option for PJI. It is less burdensome than revision surgery for both the surgeon and the patient. Compared to revision surgery, DAIR is less time-consuming, technically easier to perform, and causes less physiological damage, thereby facilitating recovery.

Despite the wide range of recommendations presented in the literature, it is universally agreed that treatment should only be attempted for well-fixed, properly positioned, and stable prostheses (i.e., prostheses worth salvaging).

Staphylococcus (*Staphylococcus*) derives its name from the Greek words *staphyle* ("grape cluster") and *coccus* ("grain"), as these Gram-positive bacteria from the family *Staphylococcaceae*, order *Bacillales*, appear as grape-like clusters under a microscope.

The natural habitat of *Staphylococcus aureus* in humans is the skin and nasopharynx. It can cause a wide range of infections affecting skin and soft tissues, endovascular regions, and internal organs. *S. aureus* continues to be a significant pathogen responsible for high morbidity and mortality in both community and hospital settings.

The organism can spread from a superficial site to internal organs via the bloodstream, creating metastatic infection foci. In hospitalized patients, the primary sites of infection include surgical wounds and implanted medical devices. In the latter case, the bacteria can colonize the implanted device, causing localized damage or spreading across the surface of the device.

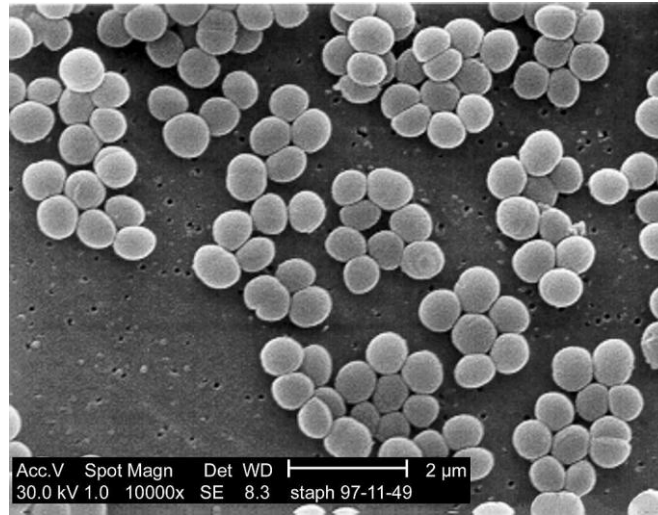


Photo A - Electron Microscope Image of *Staphylococcus aureus* Colonies

2. MATERIALS AND METHODS

Material Acquisition

Corn cobs were obtained from farmers engaged in corn cultivation, while mussel shells were collected from waste produced by mussel vendors and from dead mussels washed ashore.

2.1 Extraction of Silica (SiO₂) from Corn Cobs

2.1.1 Burning of Corn Cobs

Corn cobs were dried in sunlight and pre-burned on an open flame to reduce their volume. Subsequently, to remove organic components from the ash, the cobs were burned in a furnace at 650°C for 3 hours with a heating rate of 10°C per minute.



Figure 9. Pre-Burning Process of Corn Cobs



Figure 10. Burning of Corn Cobs in the Furnace

2.1.2 Chemical Processing of Corn Cob Ash

Initially, 100 grams of corn cob ash was mixed with 800 grams of 3M NaOH in a sealed beaker. The mixture was stirred at a constant speed using a magnetic stirrer for 4 hours at a stable temperature of 70–71°C to produce sodium silicate. The solution was then filtered using filter paper, and the residue was washed several times with deionized water. Finally, the residue was left to dry at room temperature.



Figure 11. Mixing of Corn Cob Ash with NaOH Solution



Figure 12. Filtration of the Solution



Figure 13. Reduction of pH Value of the Residue

The water from the stored solution was drained, and the residue was dried in an oven at 75°C for 24 hours. The residue was then washed several times with deionized water and dried again in an oven at 100°C for 24 hours to obtain silica.

2.2 Preparation of Mussel Shells for Artificial Bone Tissue Production

Initially, the mussel shells were washed with deionized water. To remove any surface residues, the shells were then placed in a pot with enough deionized water to cover them and boiled for 10 minutes.

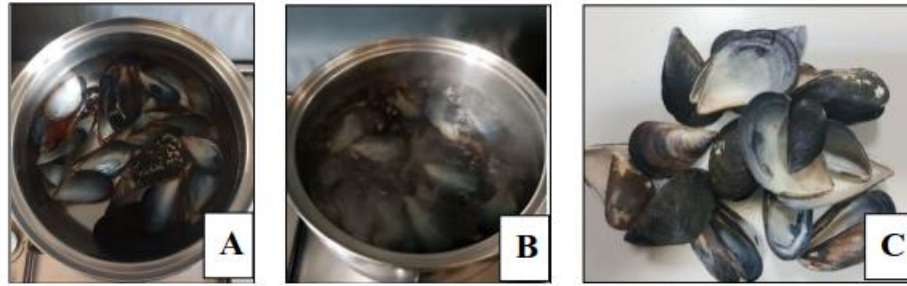


Figure 14. Cleaning of Mussel Shells
(A) During washing, (B) During boiling, (C) After cleaning

The mussel shells were first pre-ground to reduce particle size, then further ground using a milling process.

For the milling process, an amount of deionized water equal to the mass of the mussel shells was added, along with 0.008 times the solid mass of Defloplant (Dolopix CE64). The shells were milled at 300 rpm for 1 hour.



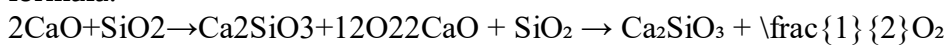
Figure 15. Pre-Grinding Process



Figure 16. Mill Used for Grinding and Mixing Processes

2.3 Production of Tissue Scaffold

To produce tissue scaffolds with varying pore sizes, the formation of Ca_2SiO_3 was calculated using the formula:



The SiO_2 used in the formula was derived from corn cobs, while the CaO was obtained from the high CaCO_3 content of mussel shells. During the synthesis of Ca_2SiO_3 , CaCO_3 decomposes as follows:



To achieve a porous structure, the starch casting method was employed.

Using this information, a product formulation was developed.

Table 2. Product Formulation (KK = Solid Mass [Mass of CaCO₃ + SiO₂])

Product Quantities	CaCO ₃	SiO ₂	Pure Water	Defloplant	Starch
Group 1	33.362g (0.3 mol)	10.014g (0.15 mol)	31.41g (Solid ratio according to KK: %58)	0.347g (0.008% of KK)	2.168g (5% of KK)
Group 2	33.362g (0.3 mol)	10.014g (0.15 mol)	34.081g (Solid ratio according to KK: %56)	0.347g (0.008% of KK)	6.506g (15% of KK)
Group 3	33.362g (0.3 mol)	10.014g (0.15 mol)	36.949g (Solid ratio according to KK: %54)	0.347g (0.008% of KK)	13.012g (30% of KK)

Product Quantities	CaCO ₃	SiO ₂	Pure Water	Defloplant	Starch
Group 1	33.362gr (0.3 mol)	10.014gr (0.15 mol)	31.41gr (solid ratio according to CC: 58%)	0.347gr (0.008 of KK)	2,168gr (5% of CC)
Group 2	33.362gr (0.3 mol)	10.014gr (0.15 mol)	34.081gr (solid ratio according to CC: 56%)	0.347gr (0.008 of KK)	6,506gr (15% of CC)
Group 3	33.362gr (0.3 mol)	10.014gr (0.15 mol)	36.949gr (solid ratio according to KK: 54%)	0.347gr (0.008 of KK)	13,012gr (30% of CC)

In this study, tissue scaffolds were produced using the starch consolidation method. By increasing the amount of starch in the composition, it is possible to enhance the porosity of the samples. In this method, starch serves both as a shaping component and as a pore-forming agent. The starch consolidation method is highly suitable for producing samples with different geometries and has been the focus of numerous studies in recent years as a promising shaping technique.

As shown in **Table 2**, three different groups were prepared with starch amounts constituting 5%, 15%, and 30% of the solid mass (KK) to achieve varying pore sizes. For successful casting, the material must not be excessively solid (to avoid difficulty in pouring) or overly liquid (to prevent prolonged drying in the oven). To maintain workability, the solid content was reduced by 2% for 15% starch and by 4% for 30% starch due to the increased starch amounts.

Initially, water and Defloplant were mixed using a magnetic stirrer for 5 minutes. The resulting solution was then combined with the other materials and mixed in a mill with 150 grams of beads at 300 rpm for 45 minutes. The prepared material was cast into two different molds.



Figure 17. Mold Used in the Casting Process

The casting process involved adding specific amounts of material from each group into the molds. The material was dried in an oven at 80°C until solidification began. After this, another material group was added, and the drying process was repeated.

In this study, to mimic the structure of bone tissue, the order of material groups in the molds was as follows:

- In one mold: Group 1 → Group 2 → Group 3
- In the other mold: Group 3 → Group 2 → Group 1

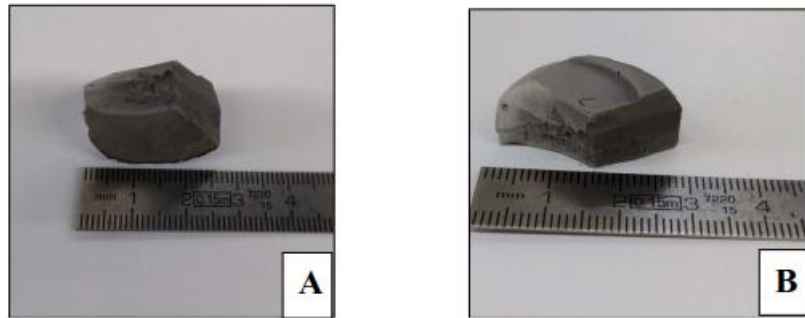


Figure 18. Artificial Bone Tissues Obtained Before Sintering (A, B)

In this study, sintering was performed at 1200°C for 1 hour, and the obtained electron images demonstrated the production of a scaffold structure containing pores at different ratios. The XRD analysis conducted identified dicalcium silicate and calcium oxide phases.

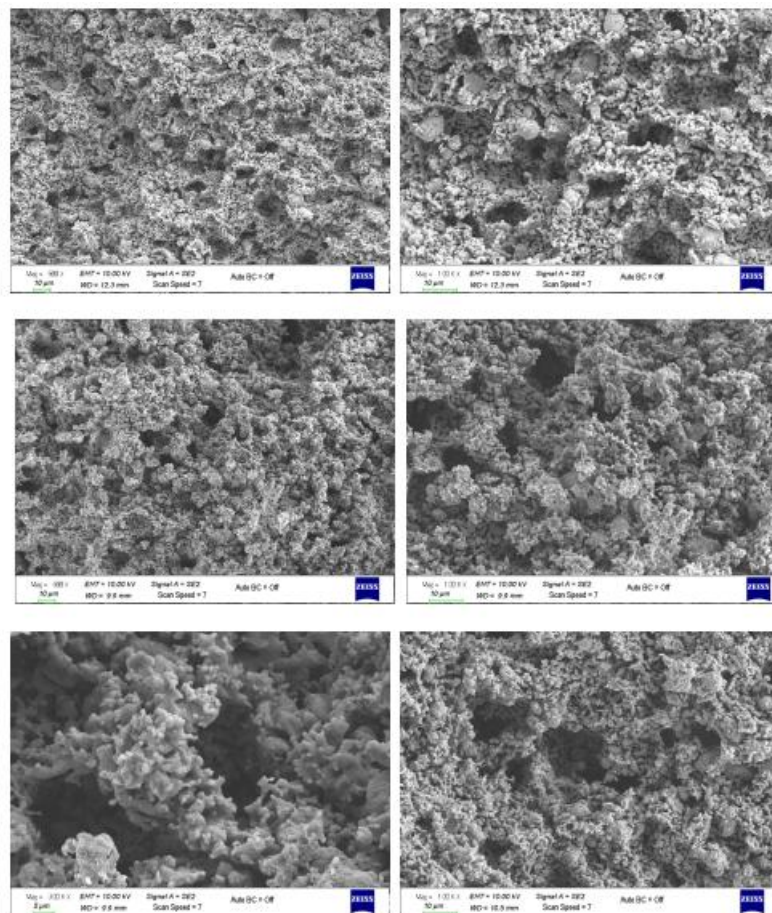


Figure 19. Microstructure Obtained After Sintering

2.4. Incorporation of Dicalcium Silicate Material into PEEK Material

Dicalcium silicate material was incorporated into PEEK material at weight ratios ranging from 1% to 12%. Measurements were conducted using a precision balance. The procedure used for the mixture is as follows:

1. For the 1% Mixture Sample:

- 99 grams of PEEK and 1 gram of dicalcium silicate were mixed using a twin-screw extruder filament machine to produce 100 grams of PEEK + dicalcium silicate filament.
- The obtained filament, in 8-gram portions, was used with a PEEK printer to produce a total of 9 tensile test specimens, with 3 specimens for each of the 3 different internal fill densities.
- Production consisted of:
 - 3 solid lower layers,
 - 3 specimens with 60% internal fill density,
 - 3 solid upper layers,resulting in a total of 9 layers.
- The tensile strength tests will be conducted by taking the arithmetic mean of the 3 specimens produced for each identical configuration.

2. For the 2% Mixture Sample:

- 98 grams of PEEK and 2 grams of dicalcium silicate were mixed using a twin-screw filament machine to produce 100 grams of PEEK + dicalcium silicate filament.
- The obtained filament, in 8-gram portions, was used with a PEEK printer to produce a total of 9 tensile test specimens, with 3 specimens for each of the 3 different internal fill densities.
- Production consisted of:
 - 3 solid lower layers,
 - 3 specimens with 60% internal fill density,
 - 3 solid upper layers,resulting in a total of 9 layers.
- The tensile strength tests will be conducted by taking the arithmetic mean of the 3 specimens produced for each identical configuration.

The same procedure was repeated for dicalcium silicate materials at 3%, 4%, ..., and 12% ratios. The resulting filaments were processed into 1.75 mm filament and used for production as shown in **Figure 7**.

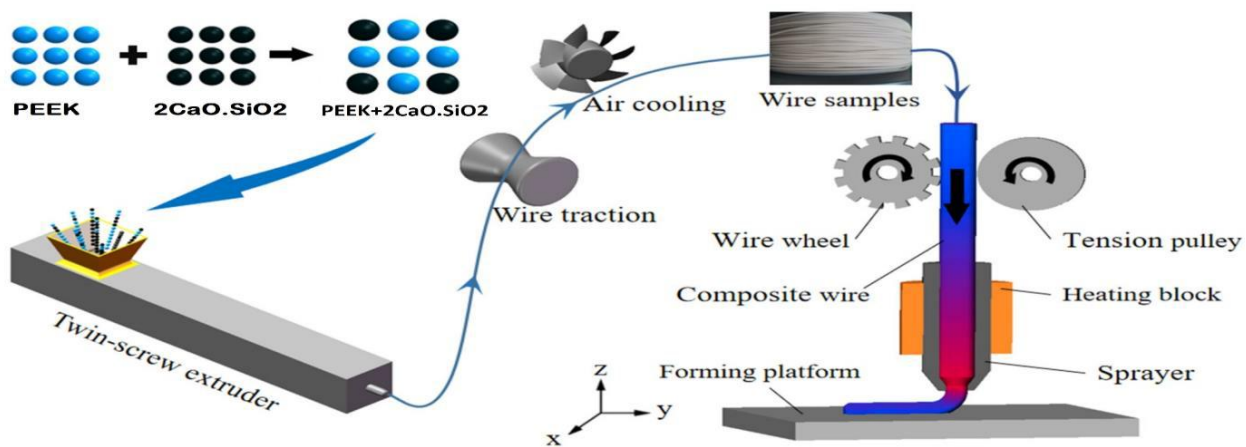


Figure 7 - Procedure for Incorporating Dicalcium Silicate into PEEK, Filament Production, and FDM Specimen Manufacturing

Twin-screw extruders are machines used in R&D studies to homogeneously mix thermoplastic materials with other polymers, additives, colorants, and stabilizers, resulting in new products. The main components of extruders include the motor, gearbox, barrel assembly, and support frame.



Photo 2 - Twin-Screw Extruder Machine



Photo 3 - 1.75 mm Filament

Filament samples were modeled according to ISO 524-4 standards using 3D printers for tensile strength tests.

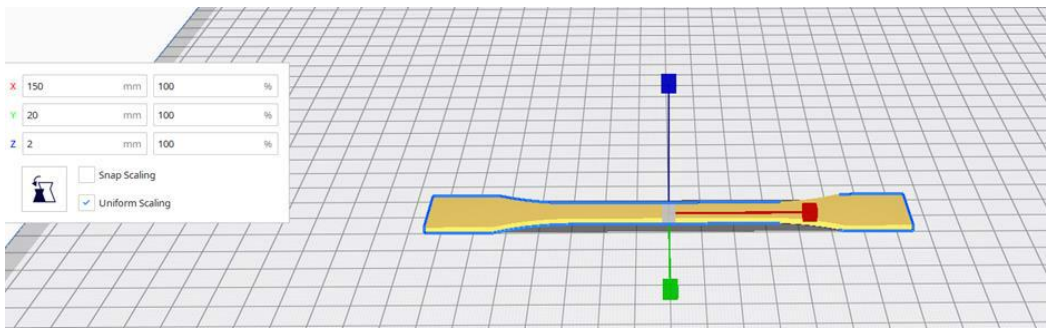


Figure 8 - BS_EN_ISO_527-4_Type_1_2mm (150 mm x 20 mm x 2 mm)

ISO 527-4:2023 (Plastics - Determination of tensile properties - Part 4: Test conditions for isotropic and orthotropic fiber-reinforced plastic composites)

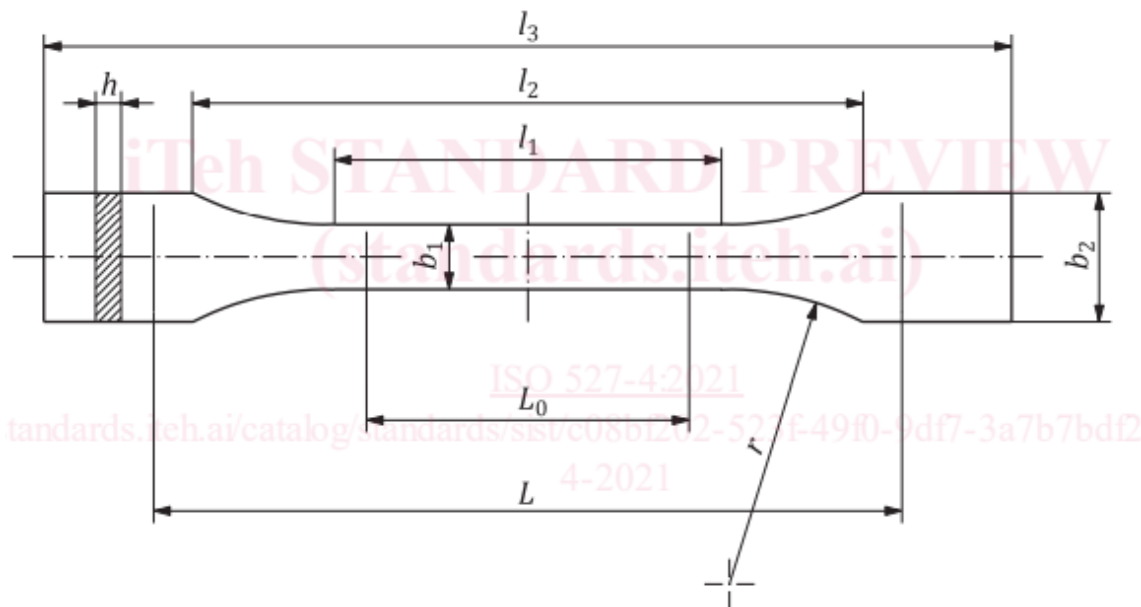


Table - ISO 527-4 Type 1,2 mm

Symbol	Name	Dimensions in millimetres
l_3	Overall length ^a	≥ 150
l_1	Length of narrow parallel-sided portion	$60,0 \pm 0,5$
r	Radius ^b	≥ 60
b_2	Width at ends	$20,0 \pm 0,2$
b_1	Width of narrow portion	$10,0 \pm 0,2$
h	Thickness	2 to 10
L_0	Gauge length (recommended for extensometers)	$50,0 \pm 0,5$
L	Initial distance between grips	115 ± 1

For PEEK-based prints with dicalcium silicate added in proportions ranging from 1% to 12%, 100 grams of 1.75 mm filament was produced for each composition. Three specimens were produced for each sample.

The produced filaments were used for 3D printing with the **IEMAI MAGIC HT-M** device.



Figure 9 - IEMAI MAGIC HT-M Device

The printing details for all prints are as follows:

Kullanılan 3D Yazıcı : IEMAI HT
Kullanılan Nozzle Çapı : 0.4 mm
Nozzle Sıcaklığı : 410 °C
Tabla Sıcaklığı : 150 °C
Çember Sıcaklığı : 100 °C
Katman Yüksekliği : 200 mikron
Alt ve üst katı katman Sayısı: 3
Kabuk Sayısı : 3
İç Doluluk : % 60

The ISO 527-4 tensile specimens produced by the device were attached to a **Devotrans CKS-III** Tensile, Compression, and Bending Test Machine for testing. Three consecutive tests were performed for each sample, and the arithmetic mean was calculated.

The tensile test is a fundamental material science test in which a specimen is subjected to uniaxial tensile forces until it fractures. The results obtained from the test are used for material selection, quality control, and predicting material behavior under other forces in various applications.

A test specimen prepared in accordance with the standards is placed in the test machine, and force is applied. The elongation in the material is measured using an extensometer. Based on the elongation and load values, a stress-strain curve is generated. The tensile test determines the material's strength, ductility, and stiffness.



Figure 10 - Devotrans CKS-III Tensile, Compression, and Bending Test Machine

Technical Specifications of the Device:

- **Maximum load:** 20 kN, with an additional 10 N precision load cell.
 - **Measurement accuracy for longitudinal strains:** $\pm 0.002\%$.
 - **Measurement range:** Independent of the specimen size.
 - Ability to **transfer stress values** to the tensile testing device in both analog and digital formats.
 - Capability to **generate graphs** using specialized software.
 - **Templates** available for various test settings and applications.
 - **Standard measurement speed:** 70 Hz.
 - Capability to measure strain **between two lines or markers**.
-

2.5 Antibacterial Activity Testing of PEEK Material with Dicalcium Silicate Additive Against *Staphylococcus aureus*:

For antibacterial activity testing, 5 mg of each PEEK material containing 1% to 12% dicalcium silicate was weighed and sterilized under UV light for 60 minutes.

To prepare the bacterial culture, a single colony of *Staphylococcus aureus* (ATCC 25923) was inoculated into nutrient broth medium and incubated at 37°C with shaking for 16 hours. The resulting bacterial culture was diluted using nutrient broth to a concentration of **10⁸ CFU/ml**.

- **Test setup:**
 1. Onto each prepared sample, 1 ml of the diluted *Staphylococcus aureus* culture was added.
 2. The samples were incubated at 37°C for 5 hours at 250 rpm.
 3. A bacterial culture without any sample was incubated under the same conditions and used as a control.
- **Post-incubation procedure:**
 1. After the incubation period, 100 μ l samples were taken from the bacterial cultures.
 2. These samples were spread onto petri dishes containing nutrient agar using the streaking method.
 3. The dishes were incubated at 37°C for 16 hours.

Finally, the bacterial counts on each petri dish were compared with the control, and the effect of the produced filaments on bacterial growth was evaluated.

3. RESULTS

3.1. Yield Calculation for Silica Production from Corn Cobs

Table 2. Mass Loss During Silica Extraction from Corn Cobs

Process	Mass Loss (%)
Combustion Process	98.75%
NaOH Treatment	87.5%
HCl Treatment	18.7%

The secondary electron images (SEI) of silica obtained from corn cobs at different magnifications using a scanning electron microscope (SEM) are presented in **Figure 21**. As observed, the production of ceramic particles with very fine grain sizes, in the nanometer range, has been successfully achieved. It was determined that the grain size is significantly smaller than 100 nm.

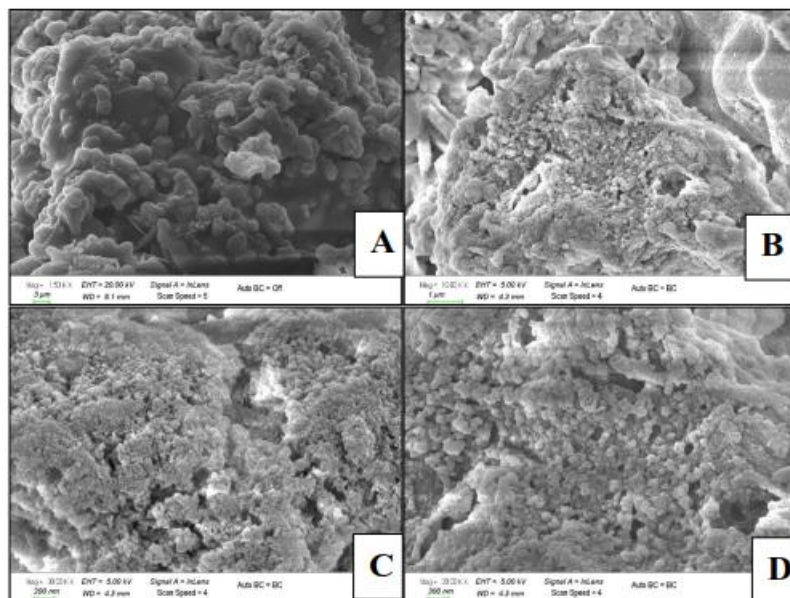


Figure 20. SEM Analysis of Silica Obtained from Corn Cobs at Different Magnifications

The EDX analysis results of the produced powders are presented in **Figure 21**. As shown, the ceramic synthesized from corn cobs contains the elements **Si**, **O**, **Mg**, and **C**.

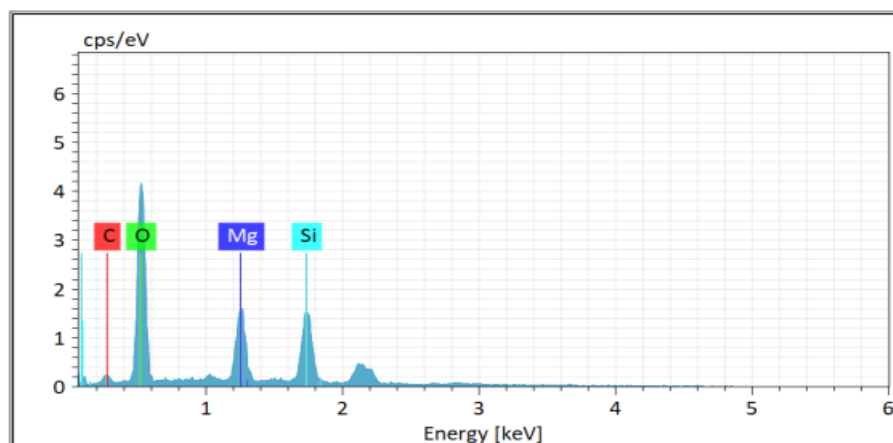


Figure 21. Results of the SEM-EDX Analysis of the Obtained Silica

Mixing of Dicalcium Silicate with PEEK Material



Table: Dicalcium Silicate and PEEK Blending Ratios

Dicalcium Silicate (g)	PEEK (g)	Total (g)
1	99	100
2	98	100
3	97	100
4	96	100
5	95	100
6	94	100
7	93	100
8	92	100
9	91	100
10	90	100
11	89	100
12	88	100

Control Group: 100 g PEEK

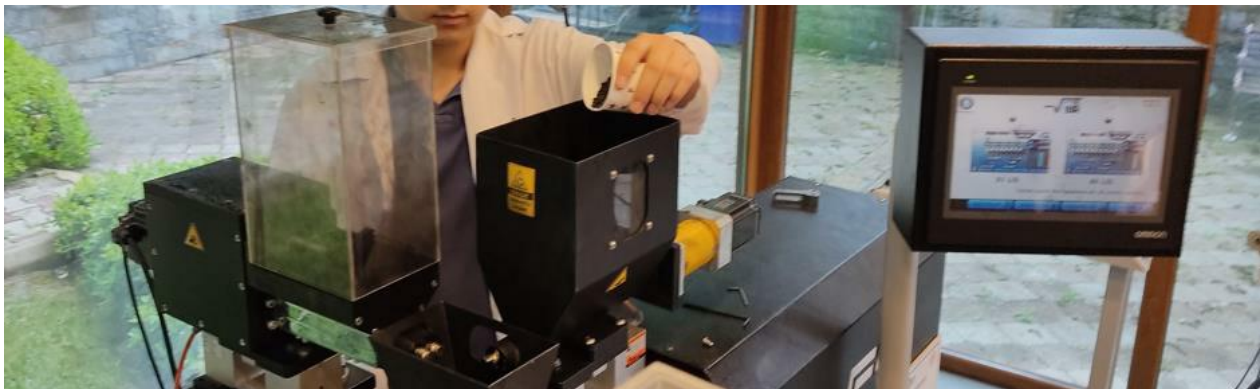


Figure 11 - Preparation of Material Mixtures by Weight

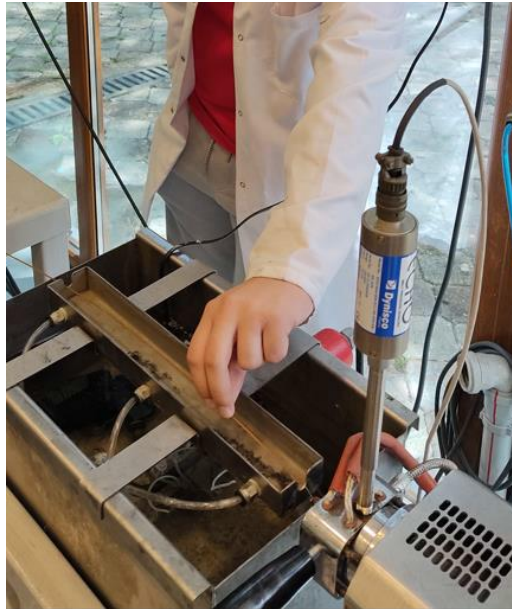


Figure 12 - Conversion of Material Mixtures into 1.75 mm Filament After Blending and Passing Through a Twin-Screw Extruder

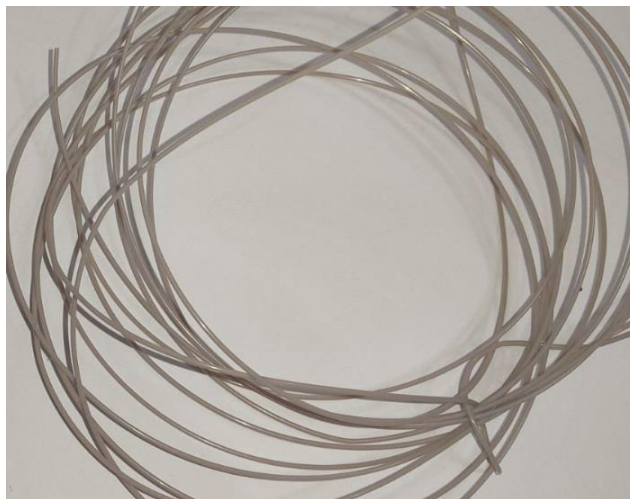


Figure 13 - 1 g Dicalcium Silicate + 99 g PEEK Formed into 1.75 mm Filament

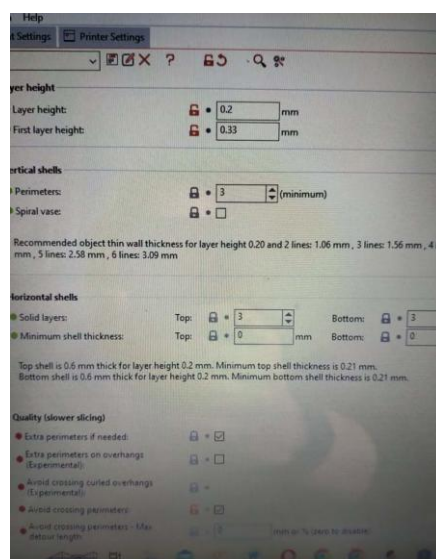


Figure 14 - Screenshot of 3D Printer Settings

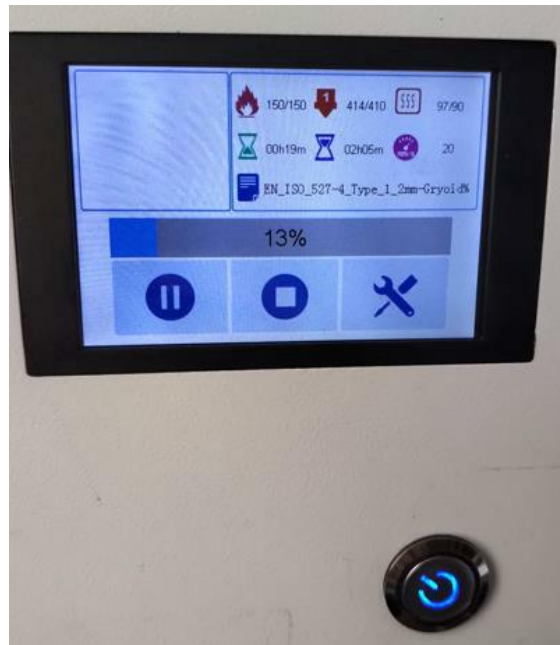


Figure 15 - 3D Printer Nozzle and Bed Temperature

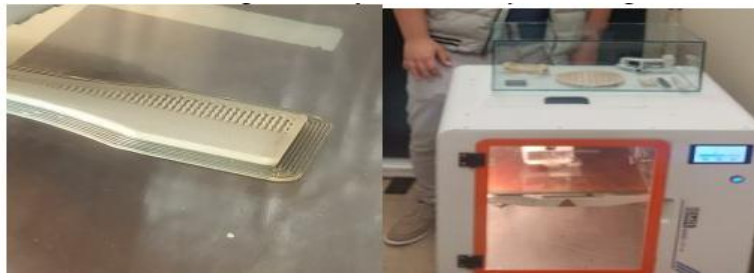


Figure 16 - 3D Printer in Operation During Printing



Figure 17 - ISO 527-4 Tensile Test Specimen

Three specimens were produced for each sample.
During 3D printing, five different patterns were used for the internal infill.

The pattern combinations are as follows:

- Cubic
- Triangles

- Gyroid
- Concentric
- Honeycomb

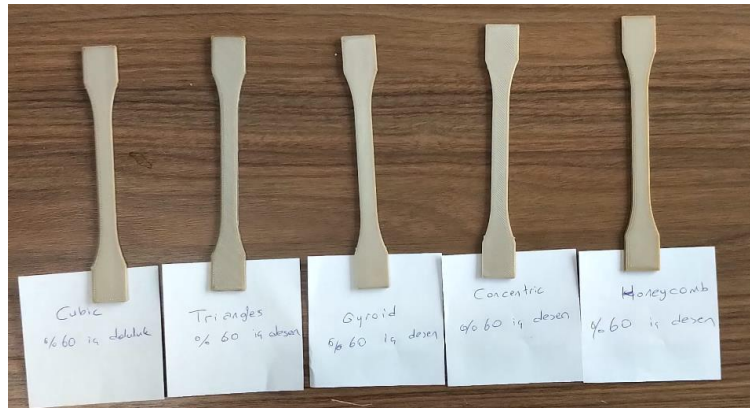


Figure 18 - Tensile Test Specimens Produced with Different Infill Patterns

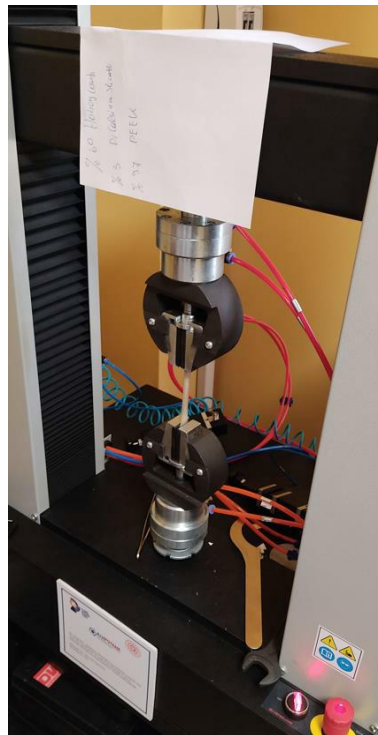
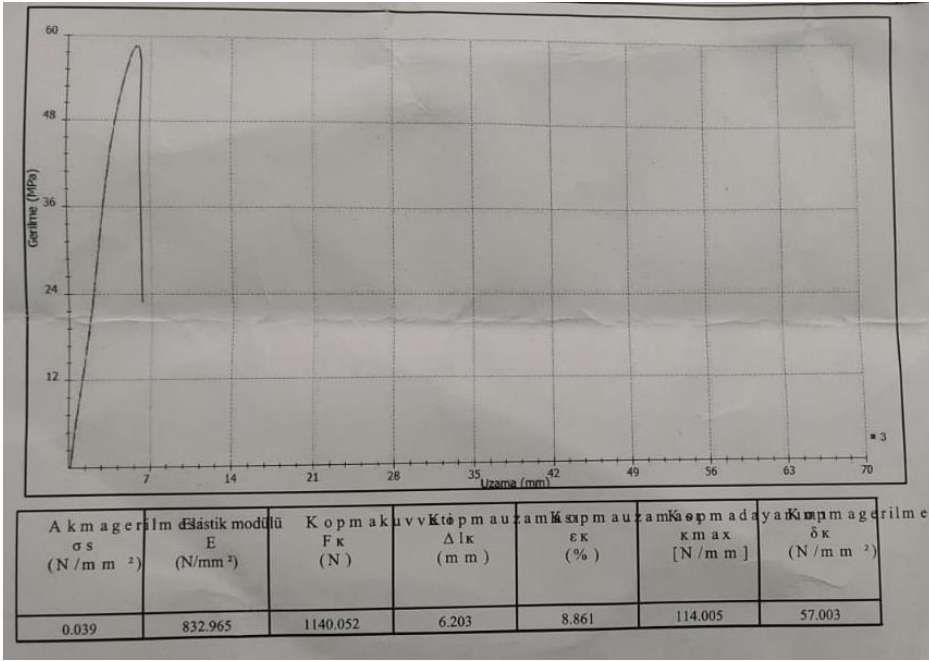


Figure 19 - Devotrans CKS-III Tensile, Compression, and Bending Test Machine + 60% Infill Honeycomb Pattern 3% Dicalcium Silicate + 97% PEEK ISO 527-4 Tensile Specimen

Test Results:

1. Control Group: 100% PEEK

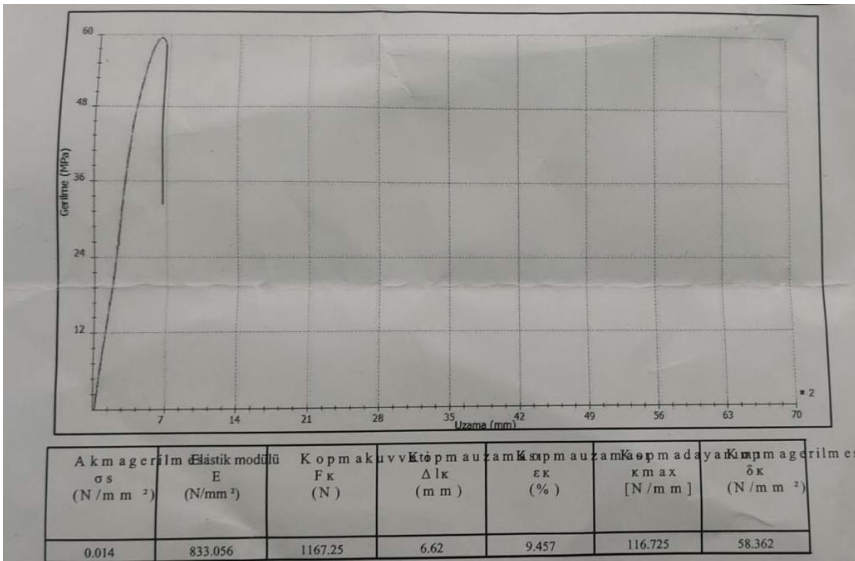


Test Results:

1. Control Group: 100% PEEK

- **Breaking Stress:** 57.003 N/mm²

2. 1% Dicalcium Silicate + 99% PEEK



Test Results:

2. 1% Dicalcium Silicate + 99% PEEK Material Breaking Stress

The breaking stress of the 1% Dicalcium Silicate + 99% PEEK material with a cubic pattern is 58.362 N/mm². (Each measurement was performed three times, and the arithmetic mean was calculated and recorded in the table below.)

Table: Breaking Stress of Dicalcium Silicate + PEEK Mixtures Based on Patterns

Dicalcium Silicate (g)	PEEK (g)	Infill Density (%)	Cubic (N/mm ²)	Gyroid (N/mm ²)	Triangles (N/mm ²)	Concentric (N/mm ²)	Honeycomb (N/mm ²)
1	99	60%	58.362	58.121	58.125	58.126	58.002
2	98	60%	58.364	58.125	58.209	58.235	58.060
3	97	60%	59.204	59.040	59.104	59.178	59.470
4	96	60%	59.598	59.529	59.520	59.421	59.528
5	95	60%	60.444	60.279	60.265	60.118	60.110
6	94	60%	61.340	61.284	61.267	61.440	61.148
7	93	60%	63.567	63.267	63.467	63.211	63.161
8	92	60%	65.268	65.125	65.265	65.818	65.284
9	91	60%	67.432	67.232	67.132	67.223	67.146
10	90	60%	68.006	67.107	67.108	67.206	67.406

Images of *Staphylococcus aureus*

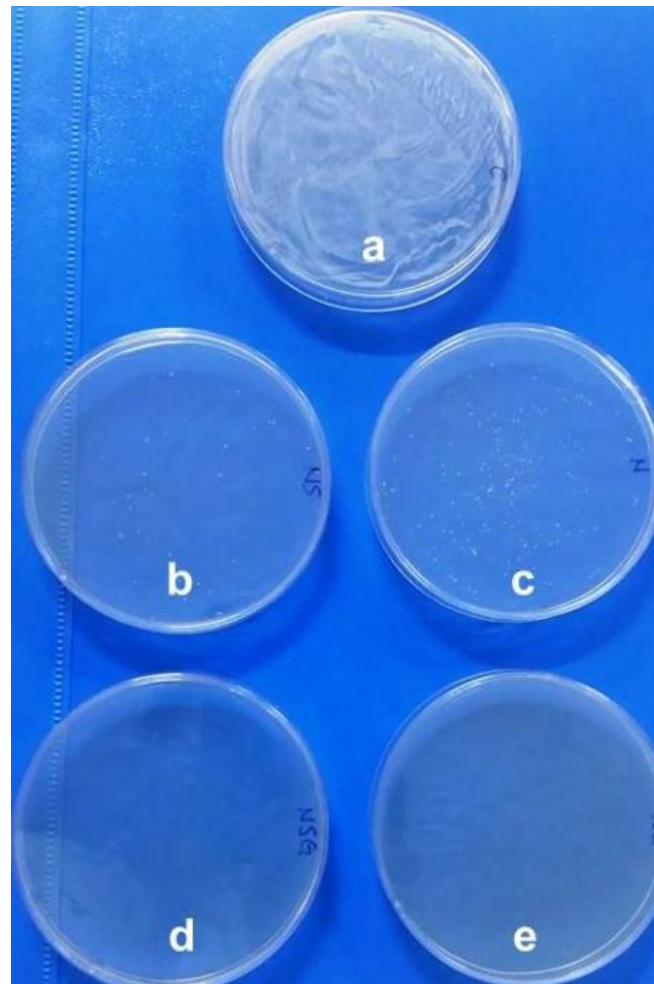


Figure 20 - Antibacterial Effects on *Staphylococcus aureus*

Table: Antibacterial Effects of Produced Dicalcium Silicate and PEEK Material Against *Staphylococcus aureus*

Sample Name	Type	Colony Count (CFU/ml)
a	Control Group	5.8×10^8
b	1% + 99% Mixture	1.7×10^3
c	4% + 96% Mixture	3.2×10^2
d	8% + 92% Mixture	-
e	12% + 88% Mixture	-

4. CONCLUSION AND DISCUSSION

In recent years, calcium silicate ceramics have been considered a candidate material for use as bone substitute biomaterials due to their excellent bioactivity. Calcium silicate-based bioceramics have shown significant potential for bone tissue regeneration in comprehensive studies because of their superior bioactivity compared to hydroxyapatite and their role in critical metabolic events associated with new bone formation.

In vivo and **in vitro** studies have demonstrated that calcium silicate ceramic materials form bone-like apatite layers in simulated body fluid (SBF) and chemically integrate with living bone tissue.

In this study, silica was first obtained from corn cobs for the production of calcium silicate-based scaffolds. It was then combined with calcium oxide (CaO) sourced from crushed mussel shells to successfully synthesize dicalcium silicate and layered scaffolds. The synthesis of dicalcium silicate was carried out at **1200°C for 1 hour**. The silica derived from corn cobs was found to have a grain size smaller than 100 nm, confirming the successful synthesis of a nanomaterial.

For the production of dicalcium silicate-based scaffolds, a starch-based consolidation method was used. This method is economical, easy to apply, and allows the production of materials with complex geometries and high porosity.

The results of SEM-EDX analysis of the synthesized ceramic silica revealed that the presence of **Si, O, Mg,** and **C** elements indicates its potential as a biocompatible artificial bone tissue biomaterial.

PEEK (Polyether Ether Ketone) has gained significant attention in recent years as a bone substitute material. Compared to titanium, PEEK is more economical and has the advantage of being producible using 3D printing. Typically reinforced with carbon, PEEK also exhibits favorable properties for use in bone replacement.

In this project, silica with high bioactivity was produced from the organic waste of corn cobs, and calcium oxide (CaO) was obtained from ground mussel shells (*Mytilus edulis* and *Mytilus galloprovincialis*). This unique production method has not been found in the literature. Combining these two natural materials, dicalcium silicate ($2\text{CaO} \cdot \text{SiO}_2$) was synthesized and incorporated at different percentages into PEEK to develop a novel, innovative bone substitute material with high bioactivity, which could serve as an alternative to existing titanium alloys.

The tensile specimens (ISO 527-4) produced from the filaments with varying dicalcium silicate content exhibited better breaking stress results in mechanical tests as the percentage of dicalcium silicate increased.

The biocompatibility tests indicated that the ceramic-polymer biocomposite materials produced exhibited antibacterial activity against *Staphylococcus aureus*. Furthermore, the antibacterial effect was found to increase as the natural dicalcium silicate content increased.

The incorporation of dicalcium silicate into PEEK improved its extrusion properties, making the production process easier and allowing the natural porous materials to better mimic bone. This resulted in a material that is not only better suited as a bone substitute but also more economical due to reduced production costs.

This new-generation material should be optimized for its production ease, customizability, and strength, making it a highly promising solution for bone substitution applications.

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【評語】 090029

1. Novelty and Significance:

This science project demonstrates novelty and significance in its approach to developing a next-generation bone substitute material. The use of organic waste materials, specifically corn cob and mussel shells, to synthesize dicalcium silicate for incorporation into PEEK is an innovative and sustainable approach. The project addresses the need for alternative biomaterials in orthopedic applications, potentially offering advantages over existing titanium alloys. The combination of a bioactive ceramic with a high-performance polymer like PEEK shows promise for creating a material with both biocompatibility and suitable mechanical properties. Furthermore, the investigation of antibacterial activity against *Staphylococcus aureus* adds an important dimension to the potential clinical applications of this material.

2. Strengths:

The study demonstrates several strengths in its methodology and approach. The use of starch-based consolidation for creating tissue scaffolds is highlighted as a cost-effective

and easily implementable method. The project employs a range of advanced manufacturing techniques, including twin-screw extrusion for homogeneous filament production and CAD/CAM systems for fabricating samples with bone-like density. The comprehensive characterization of the material, including SEM-EDX analysis, provides valuable insights into its composition and potential suitability as a bone substitute. The systematic approach to varying the dicalcium silicate content in PEEK from 1% to 12% allows for optimization of the composite's properties.

3. Weaknesses:

The lack of direct comparison with other contemporary bone substitutes, such as hydroxyapatite-based scaffolds, makes it difficult to assess the relative advantages of this new material. The study does not address the long-term stability and degradation behavior of the dicalcium silicate-PEEK composite, which is crucial for its function as a bone substitute. The high energy requirement for silica extraction from corn cobs raises questions about the overall sustainability and scalability of the process. There are also some unclear aspects in the methodology, such as the meaning of certain chemical equations and the specific methods used

for measuring *S. aureus* CFU/ml. The recommendation to use the agar-diffusion method for testing antibacterial activity suggests that the current methodology for assessing biocompatibility may not be optimal. Addressing these weaknesses and providing more comprehensive data on mechanical properties, long-term stability, and comparative performance would significantly strengthen the study's conclusions and potential impact in the field of biomaterials.