e-ISSN: 2637-0611

Available online at
https://journal.uitm.edu.my/ojs/index.php/COS

Compendium of Oral Science 12 (2) 2025, 89 - 103

Compendium of Oral Science

Bone Regeneration in Periodontal Therapy: Exploring the Compressive Strength and Structural Characteristics of Calcium Sulfate-Chitosan Composites

Ikhwan Hakimi Mohamad[1,2], Nik Zarina Nik Mahmood[3], Farha Ariffin[1*]

[1]*Centre of Studies for Periodontology Dentistry, Faculty of Dentistry, Universiti Teknologi MARA Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia*
[2]*Periodontics Unit, School of Dental Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, 16150 Kota Bharu, Kelantan, Malaysia*
[3]*Faculty of Dentistry, SEGI University, 9, Jalan Teknologi PJU 5, Kota Damansara, 47810, Petaling Jaya, Selangor, Malaysia*

ARTICLE INFO

Article history:
Received 11 November 2024
Revised 08 January 2025
Accepted 04 March 2025
Online First
Published 01 September 2025

Keywords:
calcium-sulfate chitosan
periodontal regeneration
periodontal ligament fibroblast
compressive strength
surface morphology
biomaterial

DOI:
10.24191/cos.v12i2.8833

ABSTRACT

Objectives: This study aims to investigate the mechanical properties and structural characteristics of innovative calcium sulfate-chitosan (CS-CHT) biomaterials on human periodontal ligament fibroblasts (PDLF) to advance periodontal regeneration.

Materials and Methods: The biomaterials were prepared by mixing calcium sulfate with distilled water and chitosan, followed by molding into cylindrical samples. Human periodontal ligament fibroblasts (PDLF) were grown in a complete medium until they reached 90% confluency, then seeded onto the CS-CHT biomaterial. The compressive strength of the samples was tested using a universal testing machine, with measurements taken at 24 hours, 48 hours, 72 hours, and one week. Scanning electron microscope (SEM) imaging was used to analyze the structural characteristics.

Results: The results showed that the compressive strength of CS-CHT biomaterials increased over time, surpassing that of CS alone after one week, with average compressive strengths ranging from 2.93 MPa to 5.61 MPa. SEM images revealed a crystalline arrangement and textured surface with pores ranging from 6.0 µm to 89.5 µm. Successful adhesion of PDLF to the biomaterials was observed, with cytoplasmic extensions visible in the SEM images. There were no significant differences between the two groups at various time points based on the independent t-test.

[1*] Corresponding author. *E-mail address*: drfarha@uitm.edu.my

Conclusions: In conclusion, CS-CHT biomaterials exhibit suitable mechanical properties and structural characteristics for bone regeneration in periodontal therapy. The increasing compressive strength and successful adhesion of PDLF indicate their potential for clinical application in periodontal treatments. This study provides valuable insights into the development of effective biomaterials for bone and periodontal regeneration, highlighting the promise of CS-CHT in advancing dental tissue engineering.

1. INTRODUCTION

Alveolar bone loss is a primary clinical feature and consequence of periodontitis, along with bleeding on probing and increased probing pocket depth. Currently, conventional scaling and root debridement techniques can effectively slow disease progression and alleviate symptoms. However, these treatments alone are insufficient for fully addressing tissue and bone loss (Y. Liang et al., 2020). If untreated, the loss of periodontal attachment can lead to abscess formation, tooth mobility, and ultimately, tooth loss (Jeffcoat, 1993).

Bone graft treatments for alveolar bone defects have shown promising improvements in bone height, probing depth, and soft tissue attachment (Reynolds et al., 2003). However, current bone substitutes have notable limitations: autografts can cause pain and donor site morbidity, allografts carry a risk of disease transmission, xenografts often have less favorable healing outcomes, and synthetic alloplastic materials like calcium phosphate and hydroxyapatite provide limited bone regeneration. Additionally, regenerative biomaterials must be mechanically strong enough to withstand external pressures, such as masticatory forces, during healing, requiring mechanical strength comparable to human alveolar bone.

At present, no scaffold has been universally accepted as the "gold standard" in regenerative periodontal therapy. An ideal scaffold should meet comprehensive criteria for effective bone regeneration, including biocompatibility, bone induction and conduction, antibacterial properties, blood vessel formation, and sufficient mechanical strength. The ongoing pursuit of an ideal scaffold—one that combines mechanical robustness with properties like growth factor stimulation, osteogenic and angiogenic potential, and antimicrobial effects—underscores the critical need for innovative research in this field.

Calcium sulfate (gypsum) is a naturally occurring mineral used as a bone substitute since 1982, valued for its biocompatibility, osteoinductive properties, and safety as a graft material. Chitosan, derived from chitin, is biocompatible, biodegradable, non-toxic, and exhibits antimicrobial activity. Research on calcium sulfate-chitosan (CS-CHT) biomaterials is recent but promising, showing non-cytotoxic effects on stem cells from human-exfoliated deciduous teeth and antibacterial effects on *S. mutans* and *S. sobrinus.*

There are limited studies on the compressive strength and detailed structural characteristics of CS-CHT biomaterials and their interaction with periodontal ligament fibroblasts. The objectives of this study are to evaluate the mechanical and structural characteristics of Calcium Sulfate-Chitosan (CS-CHT) biomaterials and to examine the viability of periodontal ligament fibroblasts in relation to CS-CHT biomaterials.

https://doi.org/10.24191/cos.v12i2.8833

2. MATERIALS AND METHODS

2.1 Material preparation

Baseline ratio of calcium sulfate mixed with distilled water at 2.5g: 1.9mL (Figure 1) and Calcium sulfate-chitosan (CS-CHT) with distilled water at 2.5g: 0.29g: 1.9mL (Figure 2) based on the experiment conducted on SHED (Low et al., 2015). Material mixed at ambient room temperature and 60 + 5% relative humidity.

Fig.1. Calcium Sulfate (Left) & Chitosan (Right)

Fig. 2. Mixing of Calcium Sulfate & Chitosan with Distilled Water

2.2 Periodontal Ligament Fibroblasts

Commercially available human periodontal ligament fibroblasts (ScienCell, USA) were cultured in T25 flasks using a complete medium composed of Dulbecco's Modified Eagle Medium High Glucose with stable Glutamine and Sodium Pyruvate (Capricorn Scientific, Germany), supplemented with 20% fetal bovine serum (Thermo Scientific) and 1% penicillin-streptomycin (Thermo Scientific). The flasks were observed daily under a light microscope, and the medium was refreshed after washing with phosphate-buffered saline (PBS). The fibroblasts were allowed to grow until they reached at least 90% confluency, as shown in Figure 3. Cell culture and passaging continued until passages 6 and above were obtained.

https://doi.org/10.24191/cos.v12i2.8833

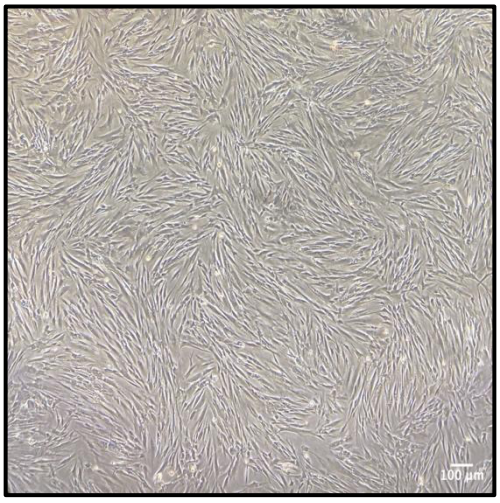

Fig. 3. PDLF at 10x Magnification Reaching at least > 90% confluency (after 5-7 days) before being used for experiment. The scale bar represents 100µm

2.3 Mold and materials preparation

A prefabricated cylindrical mold with an internal diameter of 8mm and a length of 9mm (8 x 9 mm) was used in this experiment, as shown in Figure 4 (Al Qahtani & Binsufayyan, 2011; Low et al., 2015). The mold was placed on a flat glass slab, and petroleum jelly was applied at the base to prevent movement. A metal plugger was used to ensure adequate packing and compression of the materials, avoiding air bubbles.

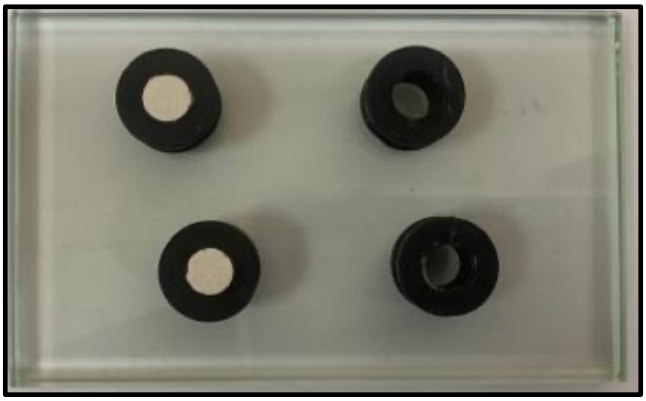

Fig. 4. Pre-fabricated cylindrical mold with ID of 8mm and height of 9mm

The materials were mixed using specific ratios: for calcium sulfate, a ratio of 2.5g to 1.9mL of distilled water was used, and for CS-CHT, a ratio of 2.5g calcium sulfate to 0.29g chitosan to 1.9mL distilled water was used (Low et al., 2015). The mixing was done at ambient room temperature with 60 ± 5% relative humidity. After mixing, the materials were transferred to the mold and gently packed or vibrated, depending on viscosity, to eliminate air bubbles. Four cylindrical samples (8 x 9 mm) per group were prepared (2

https://doi.org/10.24191/cos.v12i2.8833

groups), and the experiment was performed in quadruplicate to obtain an average compressive strength value (Figures 5 & 6).

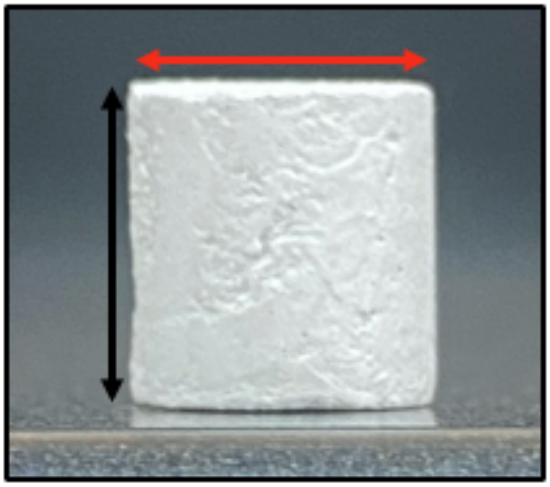

Fig. 5. Cylindrical sample with 8mm diameter (red arrow) and 9mm height (black arrow)

Fig. 6. Calcium sulfate samples (4 from right) and CS-CHT samples (4 from left)

2.4 Compressive strength test

Every sample was kept for a full day at or below 37 degrees Celsius. For this experiment, a universal testing machine (Shimadzu Co. Ltd., Japan) was utilized, and the materials were compressed between two opposing metal plates as shown in Figure 7. Materials were first positioned in the middle of the lower metal plates. Subsequently, the upper plates were lowered until they were almost in contact with the samples. After that, the samples were subjected to a load at a rate of one millimeter per minute. The specimens' compressive strength was measured at the point of fracture (Low et al., 2015; Subhi et al., 2018) The compressive strength of every material was determined using the formula below:

$$C = \frac{4P}{\pi D^2}$$

P = max load applied in Newton

D = mean diameter of the specimen in mm

https://doi.org/10.24191/cos.v12i2.8833

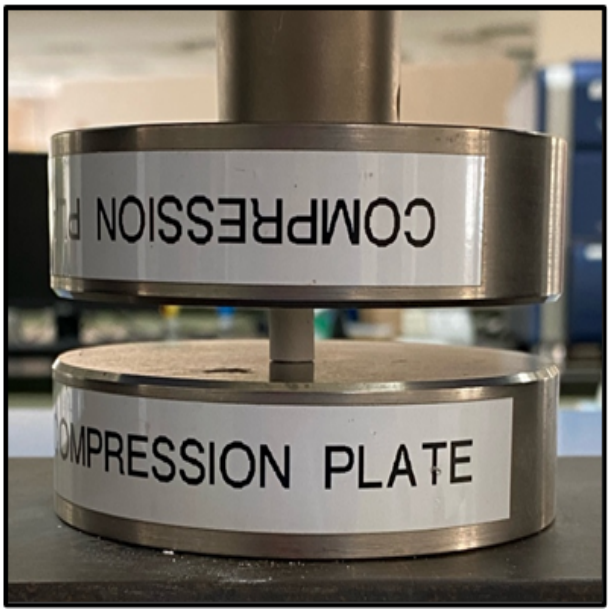

Fig. 7. The sample was compressed in between 2 flat plates until fracture

The experiment was further repeated at 48 hours, 72 hours, and 1 week to investigate the effect of time and the compressive strength of the biomaterials (Aleem et al., 2018; Silva et al., 2012; Vyas et al., 2019). At various time points, we aim to demonstrate the long-term stability of these materials over a duration of up to one week.

2.5 Structural analysis of CS-CHT

The CS and CS-CHT biomaterials were formed in an 8.0 x 2.0 mm mold. Once set, the materials were removed for structural analysis and assessment of periodontal ligament fibroblast attachment using a scanning electron microscope. Eight samples (4 calcium sulfate and 4 CS-CHT) were placed in a 24-well plate to equilibrate with the media. Initially, the samples were soaked in normal saline for 1 hour to reduce hydrophobicity and improve cell attachment (Wang et al., 2016). Subsequently, the materials were washed twice with phosphate-buffered saline (PBS) and sterilized with 70% ethanol for 10 minutes (Landis et al., 2006). The ethanol solution was then removed, and the materials were washed three times with PBS before being shaken at 1000 rpm for 1 hour. The materials were then dried overnight (Landis et al., 2006).

The next day, the samples were soaked in growth media and incubated for 1-2 days (Saunders et al., 2022). Afterward, the growth media was removed, and cell seeding was performed to achieve 50,000 cells per well, along with the samples. The samples were incubated for another 2-3 days to allow cell proliferation. Before SEM analysis, all media were removed from the samples. They were gently washed with PBS before being immersed in 4% glutaraldehyde solution for 1-2 hours. Following this, the materials were washed again three times with PBS and shaken to remove excess fixatives. A series of dehydration processes were then performed using ethanol washes: 70% ethanol for 30 minutes, 80% ethanol for 30 minutes, and 100% ethanol overnight. After drying, the materials were coated with 0.22µm gold particles, and the attachment of PDLF to CS and CS-CHT was analyzed at 5.0-8.0x magnification. Descriptive statistical analysis was used to describe the findings.

https://doi.org/10.24191/cos.v12i2.8833

3. Results And Statistical Analyses

3.1 Compressive strength of CS-CHT

The compressive strength values were measured at intervals of 24 hours, 48 hours, 72 hours, and 1 week. The average values are displayed in Figure 8. To compare the means between these two groups at different time points, an independent t-test was used to determine the level of significant difference. An alpha value of 0.05 was set to establish the level of significance.

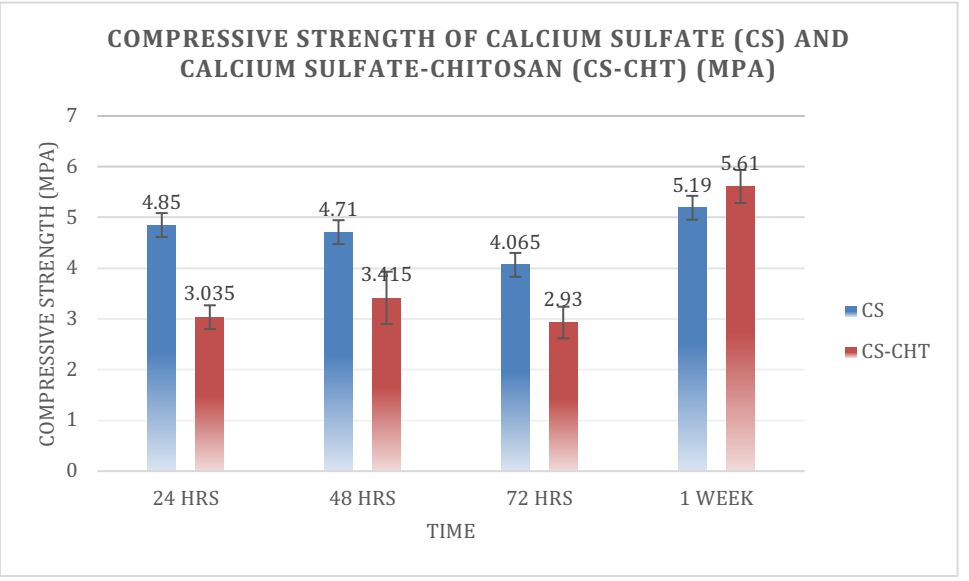

Fig. 8. Compressive strength of calcium sulfate (left-blue bar) and calcium sulfate-chitosan (right-orange bar) measured at 24 hours, 48 hours, 72 hours, and 1 week (n=4).

The average compressive strength of calcium sulfate (CS) and calcium sulfate-chitosan (CS-CHT) biomaterials ranged from 2.93 MPa to 5.61 MPa over the experimental period. Within the first 24 hours, the compressive strengths were 4.85 MPa for CS and 3.04 MPa for CS-CHT. After 48 hours, the compressive strength of CS decreased to 4.71 MPa, while that of CS-CHT increased to 3.42 MPa. Both materials reached their lowest compressive strengths at 72 hours, with values of 4.07 MPa for CS and 2.93 MPa for CS-CHT. Interestingly, after one week, the compressive strength of CS-CHT biomaterials surpassed that of the CS group, with both materials achieving their highest compressive strength values within the experiment's timeframe. An independent t-test indicated no significant differences in compressive strength between the two groups at the various time points throughout the study, as shown in Table 1.

https://doi.org/10.24191/cos.v12i2.8833

Table 1. The differences between CS alone and CS-CHT at different time points using an independent t-test. (n=4)

Biomaterials	Compressive Strength (MPa)	Two-sided p-value ($p < 0.05$)
CS (24 Hrs)	4.85	0.376
CS – CHT (24 Hrs)	3.04	
CS (48 Hrs)	4.71	0.100
CS – CHT (48 Hrs)	3.42	
CS (72 Hrs)	4.07	0.458
CS – CHT (72 Hrs)	2.93	
CS (1 Week)	5.19	0.390
CS – CHT (1 Week)	5.61	

3.2 Structural analysis

The scanning electron microscope (SEM) images in Figures 9 and 10 display the structures of calcium sulfate (CS) and calcium sulfate-chitosan (CS-CHT) biomaterials, respectively. Descriptive analysis was used to interpret these findings. The images were captured at magnifications of 1.5kx and 3.0kx. Figures 9 and 10 reveal the crystalline arrangement of CS and CS-CHT, showing a random mingling of crystals, which creates a diverse array of biomaterial patterns.

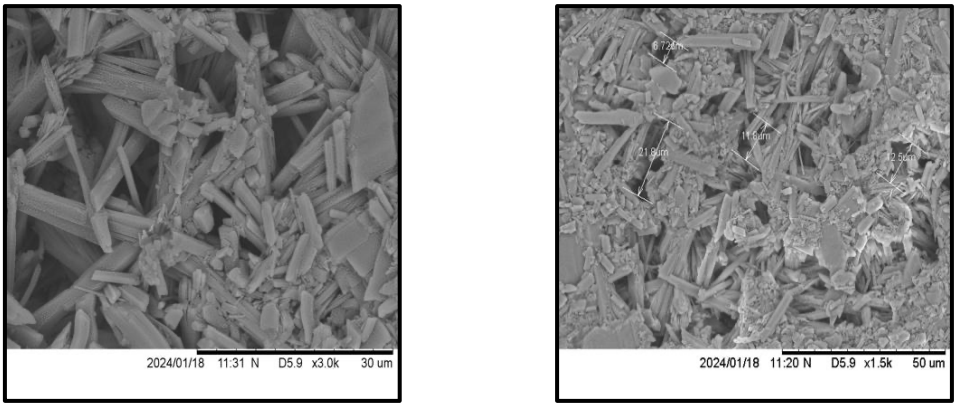

Fig. 9. Scanning Electron Microscope (SEM) Imaging of Calcium sulfate (CS) captured at x3.0k (left) and x1.5k (right) magnification

https://doi.org/10.24191/cos.v12i2.8833

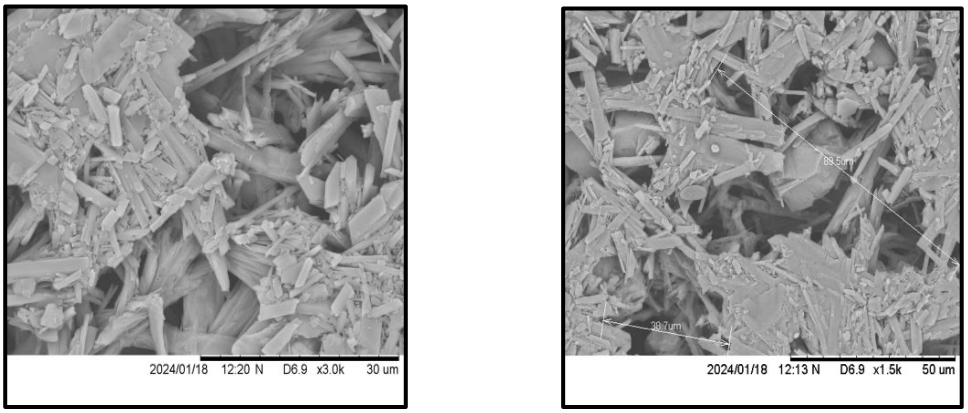

Fig. 10. Scanning Electron Microscope (SEM) Imaging of calcium sulfate-chitosan (CS-CHT) captured at x3.0k and x1.5k magnification

Notably, the variations in the shapes and sizes of calcium sulfate and chitosan contribute to a textured surface for both materials, as evident in the figures. Additionally, both CS and CS-CHT biomaterials were found to have pores of varying sizes, ranging from 6.0 µm to 89.5 µm, indicating structural diversity.

Figure 11 provides a visual representation of the biomaterials seeded with periodontal ligament fibroblasts (PDLF). Observations show successful adhesion of PDLF to the biomaterials, with cytoplasmic extensions from the fibroblasts, as indicated by the crimson arrow.

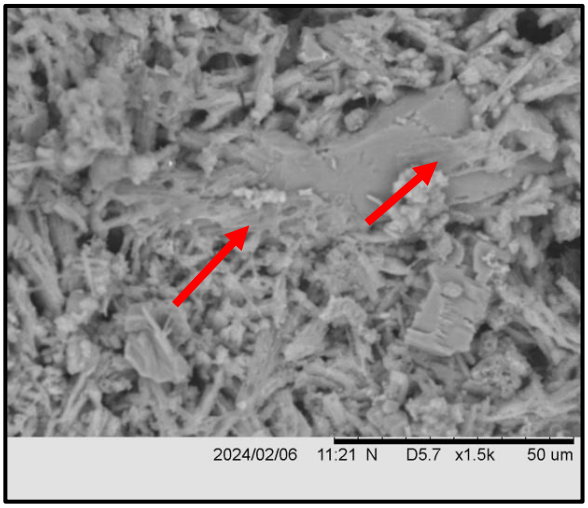

Fig. 11. The cytoplasmic projection of PDLF (red arrow) when seeded in CS biomaterials

https://doi.org/10.24191/cos.v12i2.8833

4. DISCUSSION

4.1 Compressive strength of CS-CHT

In this experiment, the mechanical strength of CS and CS-CHT biomaterials ranges from 2.93 MPa to 5.61 MPa. Interestingly, these values lie within the normal range of human cancellous bone which is 1.5 MPa to 45 MPa (Ginebra & Montufar, 2019). In addition, the values obtained from this experiment are in line with the previous study conducted by Low et al, which found that the compressive strength of Gypsum (Gyp) and Gypsum-chitosan (Gyp-CHT) biomaterials is in the range of 2.0 MPa to 5.2 MPa (Low et al., 2015). Furthermore, these results were further supported in different studies by Subhi et al which also found the compressive strength of Gyp and Gyp-CHT biomaterials to be around 2.63 MPa to 5.83 MPa (Subhi et al., 2018). However, these 2 previous studies only managed to measure the materials at one time point. It is important to highlight that, biomaterials in nature, are not static and always have the tendency to change including their compressive strength.

Previous studies have investigated the effect of dental material's compressive strength and time, especially in restorative dentistry. For example, a study by Sharma et al. found that the compressive strength of composite resins tends to fluctuate when tested at 3 different times (1 hour, 24 hours, and 7 days) (Sharma & Mishra, 2016). Another study by Dawood et al. also found differences in the compressive strength of amalgam when tested at 2 separate points. In this study, it was found that after 6 months, the compressive strength of amalgam tends to decrease compared to 24 hours (Dawood et al., 2019). These 2 examples have led to a similar conclusion that is, dental materials can alter with time and similar theories should be applied to periodontal regeneration.

Notably, no prior research has delved into the impact of time on the compressive strength of CS and CS-CHT materials. Intriguingly, particularly in the CS-CHT cohort, our investigation revealed a notable increase in compressive strength to 5.61 MPa after one week, surpassing the CS-only group, which exhibited a mean compressive strength of 5.19 MPa. This outcome aligns with previous studies by Low et al. (2015) and Subhi et al. (2018), which posit that the incorporation of chitosan into calcium sulfate/gypsum can bolster its mechanical properties (Low et al., 2015; Subhi et al., 2018).

Various factors can impact the compressive strength of calcium sulfate (i.e; gypsum), including the water-to-powder ratio, mixing technique, spatulation, and temperature during mixing, as highlighted in studies (Halawany, 2012; Prashant, 2011). Additionally, the shelf life of gypsum plays a significant role in determining its compressive strength. While it was initially assumed that the compressive strength of gypsum would decline after surpassing its shelf life, a study by (Kusumastuti et al., 2017) revealed an unexpected increase in compressive strength in the group stored for a longer duration. Surprisingly, gypsum can remain usable for up to 8 months beyond its expiration date, as its compressive strength still aligns with ISO 6837:1998 standards, as demonstrated by (Kusumastuti et al., 2017).

4.2 Structural analysis

The crystalline arrangement of calcium sulfate (CS) and calcium sulfate-chitosan (CS-CHT) biomaterials holds significant importance in the context of regenerative medicine. In addition, these random arrangements made the overall surface topography of CS and CS-CHT rough and highly porous. In the context of tissue regeneration, the surface roughness of the materials used in scaffolds and implants plays a critical role in cellular attachment, which is a foundational step for successful tissue growth and regeneration (Boyan et al., 2017).

https://doi.org/10.24191/cos.v12i2.8833

4.3 Surface roughness

In our observation, we found that CS and CS-CHT exhibit rough surface topography which is ideal in periodontal regeneration. Studies show that a 'rough' surface can enhance cell attachment and proliferation (Dalby et al., 2007). Rough surfaces provide more anchoring points for cells, which is crucial for the initial attachment phase. Once cells are securely attached, they can proliferate more effectively, covering the scaffold and beginning the process of tissue formation (Dalby et al., 2007).

Interestingly, in our study, we have observed PDLF attachment to CS by the evidence of cytoplasmic projection on the surface of calcium sulfate. In addition, different levels of roughness can induce stem cells to differentiate into bone, cartilage, or other types of cells, depending on the needs of the regeneration process (Han et al., 2020). This is particularly important in applications where the regeneration of a specific type of tissue is required such as in periodontal regeneration.

The extracellular matrix is a network of proteins and other molecules that provide structural and biochemical support to surrounding cells. Surface roughness can facilitate the formation of a more natural and functional ECM, which is essential for the integration of the new tissue with the surrounding native tissue (Ramirez-San Juan et al., 2017). Furthermore, the surface roughness of CS and CS-CHT is also desired for the angiogenesis process. Surface roughness can affect angiogenesis, with certain surface topographies promoting the formation of blood vessels more effectively than others (Bobbert & Zadpoor, 2017). This is critical for the survival and function of the newly formed tissue.

4.4 Porosity

The success of tissue regeneration is significantly influenced by the porosity of biomaterials, which affects both the structural characteristics of the scaffold and the biological responses it elicits. The concept of porosity refers to the presence and spatial configuration of pores within the biomaterial, a feature essential for promoting the exchange of nutrients and waste. This exchange ensures that cells embedded within the scaffold receive the necessary nutrients and oxygen, while simultaneously facilitating the removal of metabolic waste products, conditions critical for cell viability, growth, and differentiation — key components in the process of tissue regeneration (Abbasi et al., 2020; Gao et al., 2022).

In our investigation, the pore sizes within the CS-CHT scaffold varied widely, with dimensions ranging approximately from 6.0 μm to 90 μm. We postulate that the diversity in pore shapes could extend beyond the parameters observed in our study. This variety is attributed to the heterogeneous mixture and setting process of calcium sulfate and chitosan in the biomaterial. It's important to underscore that the presence of these micro- and macro-pores plays a significant role in facilitating various regenerative processes.

Porosity plays a critical role in enhancing vascularization, or the formation of new blood vessels, by offering the essential spatial framework needed for the growth of these vessels, particularly through macro-pores. Such vascularization is vital for maintaining the supply of nutrients and oxygen across extended areas within the tissue undergoing regeneration. According to research by (Salem et al., 2002), pores smaller than 80 micrometers are necessary to facilitate the migration and infiltration of endothelial cells, which in turn, initiates the formation of blood vessels. Our observations suggest that the pore sizes found within the CS-CHT biomaterials are sufficiently large to support the development of new vascular networks.

The migratory capability of stem cells is a crucial factor in tissue regeneration. Pore size within biomaterials significantly influences this process; too-small pores prevent cell penetration and migration,

https://doi.org/10.24191/cos.v12i2.8833

undermining regenerative potential, while excessively large pores diminish the surface area available for cell attachment, impacting cell adhesion negatively (Murphy et al., 2010). There is a range of perspectives regarding the "ideal" pore size for optimal cellular migration. For example, one study suggests that pore sizes conducive to the growth of fibroblasts, smooth muscle cells, endothelial cells, and nerve cells fall within the 50-160 micrometer range (Bružauskaitė et al., 2016). Furthermore, another research highlights the significance of macropores (greater than 100 micrometers) for facilitating cell infiltration, micropores (1-10 micrometers) for supporting cell adhesion and nutrient exchange, and nanopores (1-50 nanometers) for enhancing interactions between cells and the material (Jeyachandran & Cerruti, 2023). This indicates a nuanced understanding of how different pore scales contribute distinctly to the overall effectiveness of biomaterials in tissue engineering application. Further studies are needed to evaluate cell viability in 3D scaffolds to gain a deeper understanding of cell interactions with the materials.

5. CONCLUSION

The fusion of chitosan with calcium sulfate represents a novel approach in dentistry, making this study the first to explore their combination for periodontal regeneration.

Our findings indicate that the mechanical strength of CS and CS-CHT biomaterials falls within the range of human cancellous bone, between 2.93 MPa and 5.61 MPa. Notably, we observed an overall trend of increasing compressive strength over time, with significant enhancement within a week, especially with the addition of chitosan.

SEM analysis revealed surface roughness and pores of varying sizes in the CS and CS-CHT biomaterials, with PDLFs effectively attaching to these surfaces through cytoplasmic projections.

Further research is needed to compare these findings with commercially available alloplastic materials, such as the EthOss bone graft (β-TCP + CS), to underscore the significance of this study and guide material selection for clinical use. In parallel, antibacterial studies using CS-CHT are essential to refine its properties, contributing to the development of an ideal material for regenerative applications in the near future.

In conclusion, the CS-CHT formulation has shown promising improvements in mechanical strength. Additionally, the observed porosity and surface properties further underscore the regenerative capabilities of these biomaterials, making them promising candidates for future periodontal regeneration strategies.

ACKNOWLEDGEMENTS/FUNDING

The authors would like to acknowledge the support of Universiti Teknologi Mara (UiTM), Cawangan Selangor, Fakulti Pergigian for providing the facilities and financial support on this research.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

https://doi.org/10.24191/cos.v12i2.8833

AUTHORS' CONTRIBUTIONS

Ikhwan Hakimi carried out the research, wrote and revised the article. **Farha Ariffin** conceptualized the central research idea and provided the theoretical framework. **Farha Ariffin and Nik Zarina** designed the research, supervised research progress; **Farha Ariffin** anchored the review, revisions and approved the article submission.

REFERENCES

Abbasi, N., Hamlet, S., Love, R. M. & Nguyen, N.-T. (2020). Porous scaffolds for bone regeneration. *Journal of Science: Advanced Materials and Devices, 5*(1), 1–9. https://doi.org/10.1016/j.jsamd.2020.01.007.

Al Qahtani, M. Q. & Binsufayyan, S. S. (2011). Color change of direct resin-based composites after bleaching: An in vitro study. *King Saud University Journal of Dental Sciences, 2*(1–2), 23–27. https://doi.org/10.1016/J.KSUJDS.2011.03.002.

Aleem, H., Ameen, F. & Rehman, A. (2018). Compressive Strength of Composite Resins at Different Exposure Time Using LED and Halogen Units. *Journal of The Pakistan Dental Association, 27*(1), 22–26. https://doi.org/10.25301/JPDA.271.22.

Bobbert, F. S. L. & Zadpoor, A. A. (2017). Effects of bone substitute architecture and surface properties on cell response, angiogenesis, and structure of new bone. *Journal of Materials Chemistry B, 5*(31), 6175–6192. https://doi.org/10.1039/C7TB00741H.

Boyan, B. D., Lotz, E. M. & Schwartz, Z. (2017). Roughness and Hydrophilicity as Osteogenic Biomimetic Surface Properties. *Tissue Engineering Part A, 23*(23–24), 1479–1489. https://doi.org/10.1089/ten.tea.2017.0048.

Bružauskaitė, I., Bironaitė, D., Bagdonas, E. & Bernotienė, E. (2016). Scaffolds and cells for tissue regeneration: different scaffold pore sizes—different cell effects. *Cytotechnology, 68*(3), 355–369. https://doi.org/10.1007/s10616-015-9895-4.

Dalby, M., Gadegaard, N., G. Curtis, A. & C. Oreffo, R. (2007). Nanotopographical Control of Human Osteoprogenitor Differentiation. *Current Stem Cell Research & Therapy, 2*(2), 129–138. https://doi.org/10.2174/157488807780599220.

Dawood, S. H., Kandil, M. M. & El-Korashy, D. I. (2019). Effect of Aging on Compressive Strength, Fluoride Release, Water Sorption, and Solubility of Ceramic-reinforced Glass Ionomers: An In Vitro Study. *Journal of Contemporary Dentistry, 9*(2), 78–84. https://doi.org/10.5005/jp-journals-10031-1260.

Gao, J., Yu, X., Wang, X., He, Y. & Ding, J. (2022). Biomaterial–Related Cell Microenvironment in Tissue Engineering and Regenerative Medicine. *Engineering, 13*, 31–45. https://doi.org/10.1016/j.eng.2021.11.025.

Ginebra, M.-P. & Montufar, E. B. (2019). Cements as bone repair materials. *In Bone Repair Biomaterials* (pp. 233–271). Elsevier. https://doi.org/10.1016/B978-0-08-102451-5.00009-3.

Halawany, H. S. (2012). A review on miswak (Salvadora persica) and its effect on various aspects of oral health. *The Saudi Dental Journal, 24*(2), 63–69. https://doi.org/10.1016/j.sdentj.2011.12.004.

Han, S., Kim, J., Lee, G. & Kim, D. (2020). Mechanical Properties of Materials for Stem Cell Differentiation. *Advanced Biosystems, 4*(11). https://doi.org/10.1002/adbi.202000247.

Jeyachandran, D. & Cerruti, M. (2023). Glass, Ceramic, Polymeric, and Composite Scaffolds with Multiscale Porosity for Bone Tissue Engineering. *Advanced Engineering Materials, 25*(17). https://doi.org/10.1002/adem.202201743.

Kusumastuti, K. S., Irawan, B. & Damiyanti, M. (2017). Effect of shelf life on compressive strength of type iv gypsum. *Journal of Physics: Conference Series, 884,* 012092. https://doi.org/10.1088/1742-6596/884/1/012092.

Landis, F. A., Stephens, J. S., Cooper, J. A., Cicerone, M. T. & Lin-Gibson, S. (2006). Tissue engineering scaffolds based on photocured dimethacrylate polymers for in vitro optical imaging. *Biomacromolecules, 7*(6), 1751–1757. https://doi.org/10.1021/BM0600466.

Low, A., Mohd Yusof, H., Reza, F., Abdullah Nurul, A., Sritharan, S., Haania Zain Ali, N., Subhi Azeez, H. & Husein, A. (2015). Gypsum-based biomaterials: Evaluation of physical and mechanical properties, cellular effects and its potential as a pulp liner. *Dental Materials Journal, 34*(4), 522–528. https://doi.org/10.4012/dmj.2015-029.

Murphy, C. M., Haugh, M. G. & O'Brien, F. J. (2010). The effect of mean pore size on cell attachment, proliferation and migration in collagen–glycosaminoglycan scaffolds for bone tissue engineering. *Biomaterials, 31*(3), 461–466. https://doi.org/10.1016/j.biomaterials.2009.09.063.

Prashant, H. (2011). Preserving Natural Tooth Structure with Composite Resin. *Journal of Cosmetic Dentistry, 3,* 26–34.

Ramirez-San Juan, G. R., Oakes, P. W. & Gardel, M. L. (2017). Contact guidance requires spatial control of leading-edge protrusion. *Molecular Biology of the Cell, 28*(8), 1043–1053. https://doi.org/10.1091/mbc.e16-11-0769.

Salem, A. K., Stevens, R., Pearson, R. G., Davies, M. C., Tendler, S. J. B., Roberts, C. J., Williams, P. M. & Shakesheff, K. M. (2002). Interactions of 3T3 fibroblasts and endothelial cells with defined pore features. *Journal of Biomedical Materials Research, 61*(2), 212–217. https://doi.org/10.1002/jbm.10195.

Saunders, S. K., Cole, S. Y., Sierra, V. A., Bracamonte, J. H., Toldo, S. & Soares, J. S. (2022). Evaluation of perfusion-driven cell seeding of small diameter engineered tissue vascular grafts with a custom-designed seed-and-culture bioreactor. *PLoS ONE, 17*(6). https://doi.org/10.1371/JOURNAL.PONE.0269499.

Sharma, A. & Mishra, P. (2016). Time-dependent Variation in Compressive Strengths of Three Posterior Esthetic Restorative Materials: An in vitro Study. *International Journal of Prosthodontics and Restorative Dentistry, 6*(3), 63–65. https://doi.org/10.5005/jp-journals-10019-1157.

Silva, M. A. B. da, Vitti, R. P., Consani, S., Sinhoreti, M. A. C., Mesquita, M. F. & Consani, R. L. X. (2012). Linear dimensional change, compressive strength and detail reproduction in type IV dental stone dried at room temperature and in a microwave oven. *Journal of Applied Oral Science, 20*(5), 588–593. https://doi.org/10.1590/S1678-77572012000500016.

Subhi, H., Reza, F., Husein, A., Al Shehadat, S. A. & Nurul, A.-A. (2018). Gypsum-Based Material for Dental Pulp Capping: Effect of Chitosan and BMP-2 on Physical, Mechanical, and Cellular Properties. *International Journal of Biomaterials, 2018,* 1–7. https://doi.org/10.1155/2018/3804293.

Vyas, R., Vaddamanu, S. K., Gurumurthy, V., Kuruniyan, M. S., Quadri, S. A. & Kanji, M. A. (2019). Variations in the Compressive Strength of a Die Stone with Three Different Sulfates at Eight Different Concentrations: An In Vitro Study. *The Journal of Contemporary Dental Practice, 20*(11), 1297–1306.

Wang, W., Caetano, G., Ambler, W. S., Blaker, J. J., Frade, M. A., Mandal, P., Diver, C. & Bártolo, P. (2016). Enhancing the Hydrophilicity and Cell Attachment of 3D Printed PCL/Graphene Scaffolds for Bone Tissue Engineering. *Materials, 9*(12). https://doi.org/10.3390/MA9120992.

6. APPENDIX

A. About the authors

Ikhwan Hakimi Mohamad serves as a lecturer in the Periodontic Unit at the School of Dental Sciences, Universiti Sains Malaysia. His primary research focuses on periodontal regeneration, as well as both non-surgical and surgical periodontal therapies. He can be contacted via email at drikhwanhakimi@usm.my.

Farha Ariffin is an Associate Professor in the Centre of Periodontology Studies, Faculty of Dentistry, Universiti Teknologi MARA. Her main research activity is in the area of periodontal regeneration, biomaterials and dental education. She can be reached through her email at drfarha@uitm.edu.my.

Nik Zarina Nik Mahmood is an Associate Professor at SEGi University, Kota Damansara, with over 31 years of clinical experience. Her primary areas of interest include aesthetic dentistry, fixed prosthodontics, endodontic immunology and microbiology, as well as restorative dental materials, particularly composite resin and adhesive materials. She can be contacted via email at nikzarinanikmahmood@segi.edu.my.