In Vitro Study of Calcium Hydroxide Microencapsulation with *Stichopus hermanii* as Pulp Capping Material

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ABSTRACT

Background: *Calcium hydroxide is a material that is widely used in pulp capping treatment. However, the use of this material causes tunnel defects due to the large absorption of calcium. This can be manipulated by creating an encapsulation formula. Stichopus hermanii is a marine biota that contains lots of hyaluronic acid and can be used as an encapsulation material which also plays a role in cell regeneration. Therefore it is necessary to do an in vitro study. The characteristics of the microencapsulated calcium hydroxide preparation with Stichopus hermanii as pulp capping material through an in vitro study.*
Method: *Stichopus hermanii was made freeze dry and dissolved with 1% acetic acid to obtain a gel concentration of 0.2%, 0.4%, and 0.8%. Next, calcium hydroxide powder is added with a ratio of 1: 6 and tripolyphospate (TPP) is added as a cross-linker agent and the mixture is stirred until homogeneous. The test material formed was subjected to SEM, PSA and cytotoxicity tests.*
Result: *The cytotoxicity test showed that all of the test materials (0.2%; 0.4%; 0.8%) were not toxic, especially at a concentration of 0.2%. The PSA test results indicate that this encapsulation shows the micro particle size formed. The SEM test shows a rough and stringy surface shape, where at a concentration of 0.2%, a smoother morphological formation is found.*
Conclusion: *The in vitro study showed that the microencapsulation of calcium hydroxide with Stichopus hermanii as a pulp capping material showed the characteristics of the non-toxic material, the micro size and the rough and fibrous surface shape.*

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INTRODUCTION

Caries is a multifactorial infectious disease of the oral cavity caused by a complex interaction between cariogenic bacteria in the oral cavity and fermented carbohydrates on the tooth surface.[1] Caries lesions begin with the process of demineralization of hard tooth tissue and damage to organic material in hard tooth tissue due to acid which is the result of carbohydrate fermentation by acidogenic bacteria. The classification of pulp disease according to the American Association of Endodontics (AAE) is divided into 3, namely reversible pulpitis, irreversible pulpitis, and pulp necrosis.[2] Reversible pulpitis is an inflammatory condition of the pulp that is not severe. If the cause is removed, the inflammatory symptoms will disappear and the pulp will recover (Tarigan, 2015).[3] One of the treatments in cases of reversible pulpitis is pulp capping. The purpose of pulp capping is to eliminate irritation to the pulp tissue and protect the pulp so that the pulp tissue can maintain its vitality. Pulp capping is divided into two, namely direct pulp capping and indirect pulp capping.[4] The pulp capping material that is often used is calcium hydroxide, which is useful for stimulating odontoblasts to form dentinal bridges.[5] Calcium hydroxide is used as a pulp capping material because it has a high pH (around 12.5-12.8), has good antibacterial ability, can trigger the formation of reparative dentin, can stimulate fibroblasts, can stop internal resorption, is cheap and easy to manipulate.[6] The high pH of calcium hydroxide is the effect of dissociation of calcium hydroxide into calcium ions and hydroxyl ions. The high pH of calcium hydroxide causes superficial irritation of local tissues in direct contact with calcium hydroxide and further stimulates dentin tissue repair through the release of bioactive molecules such as bone morphogenic protein (BMP) and transforming growth factor-beta one (TGF-β1) and followed by mineralization process [7] Calcium hydroxide is known to have several disadvantages, namely having low strength and high solubility.[1] The high solubility of calcium hydroxide can have an impact on the formation of a tunnel effect which can lead to the recurrence of bacterial infections.[8] Tunnel defect is the imperfect morphology of the dentinal bridge that is formed as a result of dissolution of pulp capping material by dentinal fluid which can potentially be an entry point for microorganisms through the formed micro-gaps.[9]

Stichopus hermanii is one of the sea cucumbers which is a marine biota that is used as raw material drugs. Sea cucumbers have been used for hundreds of years as Chinese medicines which are believed to be able to cure various types of diseases. The healing effect is caused by bioactive compounds contained in the body of sea cucumbers.[10] One of the substances that are also found in Stichopus hermanii compared to other sea cucumbers is omega-3. Omega-3 which includes EPA (eicopentaenoic acid) and DHA (Docosahexaenoic acid) is able to repair damaged tissue quickly and regulate the formation of prostaglandins which are key in the wound healing process.[11] Based on observations in previous studies, it was also found that the content of GAGs in gold sea cucumbers was quite large. Hyaluronic acid which is one of the components of GAGs is very abundant in gold sea cucumbers, followed by chondroitin sulfate, dermatan sulfate and heparan sulfate.[12]

Encapsulation is a technique to protect the core material by a polymer protective material (shell). The protective material is a polymer or resin that functions to coat the core material, generally used in the encapsulation process, including ethyl cellulose, polyethylene, polyamide, gelatin, hydroxyethylcellulose, paraffin, stearic acid, and cellulose acetate. The determination of the appropriate core material and protective polymer in the encapsulation should be based on the physicochemical characteristics of the material, the appropriate method, and its primary purpose.[13] The benefit of the encapsulation method is to protect the core

material from outside influences and can reduce the degree of solubility of the core material. Encapsulation can make the active ingredient components of the core material protected from adverse environmental influences such as damage due to oxidation, hydrolysis, evaporation or degradation by heat. Thus, the core material (active ingredient) will have a longer shelf life and have better process stability.[14] There are various methods used for the encapsulation process. One method that is often used is freeze drying/lyophilization.[15] The freeze drying method is a good technique for heat sensitive materials and the technique is easy to do. Freeze drying is a method of drying process from frozen liquid material which is then lightly heated in a vacuum, so that the ice crystals formed at the freezing stage will sublimate into water vapor without going through the liquid phase and microcapsules are obtained.[16] The freeze drying process is carried out with a Freeze Dryer tool/machine, and is a suitable encapsulation method for heat sensitive materials and is well used in pharmaceutical development.[15] The purpose of this study was to determine the characteristics of calcium hydroxide microencapsulated preparations with Stichopus hermanii as a pulp capping material through an in vitro study.

RESEARCH METHOD

Preparation and Freeze Dry process of Stichopus hermanii

Sample preparation by washing Stichopus hermanii. Next, the center of Stichopus hermanii body is split horizontally but not split into two separate parts. After splitting, the entrails of Stichopus hermanii and the mucus on the surface of Stichopus hermanii stomach are separated. Stichopus hermanii meat which has been separated from the abdominal organs and mucus is washed again and cut into small pieces. After being cut into small pieces, Stichopus hermanii meat was weighed and added with distilled water, and blended until it got a smooth consistency. The refined Stichopus hermanii were put in a plastic container and then stored for the freeze-drying stage. The mashed Stichopus hermanii meat was then freeze-dried to remove the liquid present in Stichopus hermanii to produce powder, then powder produced in the freeze-drying process was then blended to produce a smaller powder size and finally sifted using a 400 mesh sieve.[17]

Preparation of calcium hydroxide microencapsulation with Stichopus hermanii [18]

Stichopus hermanii powder was weighed and dissolved in 1% acetic acid solution to obtain gel concentrations of 0.2%, 0.4%, and 0.8%. Stichopus hermanii gel based on its concentration then added calcium hydroxide powder with a ratio of Stichopus hermanii and calcium hydroxide is 1: 6 based on the ratio of weight. Then, tripolyphosphate (TPP) was added as a cross-linker agent and the mixture was stirred until homogeneous. furthermore, the homogeneous mixture of Stichopus hermanii, calcium hydroxide, and TPP was subjected to a freeze-drying process to remove the water content and form a powder consistency.
The resulting mixed powder is milled to produce a smaller powder size.

PSA (Particle Size Analyzer) Test microencapsulated calcium hydroxide with stichopus hermanii powder

The powder was put into a PSA tube as much as 25 mg and 100 ml of distilled water. Then, it is placed into the PSA (Particle size Analyzer) Cilas 1190. From the PSA test, the particle size of the material being tested is obtained.

Cytotoxicity Test (PUSVETMA Working Standards refer to Freshney, 2010)[19]

BHK-21 cultures and microplates with 96 sterile wells were prepared in laminar flow. Well in the first column, the microplate was filled with Eagle's minimum essential medium (MEM), Kanamycin, Penstrep 1%, Foetal Bovine Serum (FBS) 10% Fungizone 100 units/ml, as much as 100 l as a control medium. Wells in the 2nd to 5th columns of the microplate filled with BHK-21 cells with a density of 3 x 103 in culture medium Eagle's minimum essential medium (MEM), Kanamycin, Penstrep 1%, Foetal Bovine Serum (FBS) 10%, Fungizone 100 units/ml, as much as 100 l in each well.

Microencapsulation powder calcium hydroxide with Stichopus hermanii concentration; 0,2%; 0.4%; 0.8% was sterilized using a UV sterilizer, and 50 l was added to each well in the 3,4,5 columns each. Column 2 was used as a positive control containing only BHK-21 cells and culture media only. Then the microplate was incubated with 5% CO2 at 37oC for 20 hours. The microplate was removed from the incubation apparatus, the culture medium and the microencapsulated powder in the well were taken using a syringe, and BHK-21 cells were left in the well. Each well was refilled with 100 l of culture media. MTT in filtered PBS used 0.20 l millipore for each well, then re-incubated for 3 hours to allow MTT to have metabolic activity. Total incubation time in an incubator at 370 C for 24 hours. After the incubation period is complete, MTT and culture media are taken using a syringe. To dissolve formazan crystals, 50 l . of DMSO was added to each well and then microplate in a shaker for 5 minutes. Formazan optical density value is read with an Elisa reader with a wavelength of 620 nm. To find out the percentage of live BHK-21 cells was done by using the cell viability formula.[20]

$$\% \text{ cell viability} = \frac{\text{OD treatment} - \text{OD media control}}{\text{OD cell control} - \text{OD media control}} \times 100\%$$

SEM (Scanning Electron Microscope) Test Calcium hydroxide powder that has been encapsulated with stichopus hermanii

The sample is placed on a beam, then, the Electron gun generates an electron beam from the filament. The voltage applied to the winding causes heating. The anode will then form a force that can attract electrons to move towards the anode. Magnetic lenses focus electrons toward a point on the sample surface. The focused electron beam scans (scans) the entire sample by being directed by the scanning coil. When electrons hit the sample, there will be scattering of electrons, either Secondary Electron (SE) or Back Scattered Electron (BSE) from the sample surface and will be detected by the detector and displayed in the form of an image on the CRT monitor. From these tests, the morphology, size, and shape of the particles are obtained.

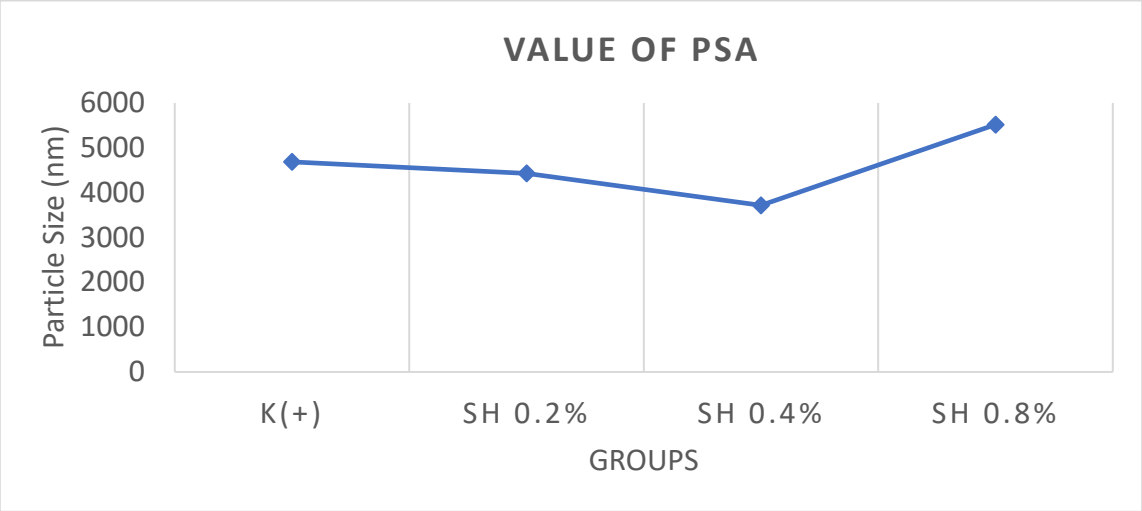
RESULTS

Value of Particle Size Analyze (PSA) microencapsulated calcium hydroxide with Stichopus hermanii

Table 1. Average PSA of calcium hydroxide microencapsulated with Sichopus hermanii (nano)

Group	N	Average ± Standard deviation
K(+)	3	4689±511
1	3	4427±527
2	3	3717±328
3	3	5517±111

Note :
K (+) : Positive control group 1 (calcium hydroxide powder)
P1 : Treatment group 1 microencapsulated calcium hydroxide with Stichopus hermanii 0.2%
P2 : Treatment group 2 microencapsulated calcium hydroxide with Stichopus hermanii 0.4%
P3 : Treatment group 3 microencapsulated calcium hydroxide with Stichopus hermanii 0.8%



Based on table 4.13, it is known that the smallest PSA average value is in group P2, namely the calcium hydroxide microencapsulation group with Stichopus hermanii concentration of 0.4% with an average PSA value of 3717 nano, while the largest particle size average value is in the group. P3 is a calcium hydroxide microencapsulated group with a concentration of 0.8% Stichopus hermanii with an average PSA value of 5517 nano. The calcium hydroxide microencapsulated group with 0.2% concentration of Stichopus hermanii had an average PSA value of 4427 nano, which was smaller than the P3 group, but larger than the P2 group and had a PSA value close to the PSA value in the K(+) group.

Cytotoxicity of microencapsulated calcium hydroxide with Stichopus hermanii

Cytotoxicity of calcium hydroxide encapsulation with gold sea cucumber was tested using MTT Assay by measuring the optical density of the resulting solution. The results of optical density were read with the help of an Elisa reader at a wavelength of 620 nm. The calculation results are declared non-toxic if the percentage of living cells is more than 50%, while toxic if the percentage of living cells is less than 50%.[19]

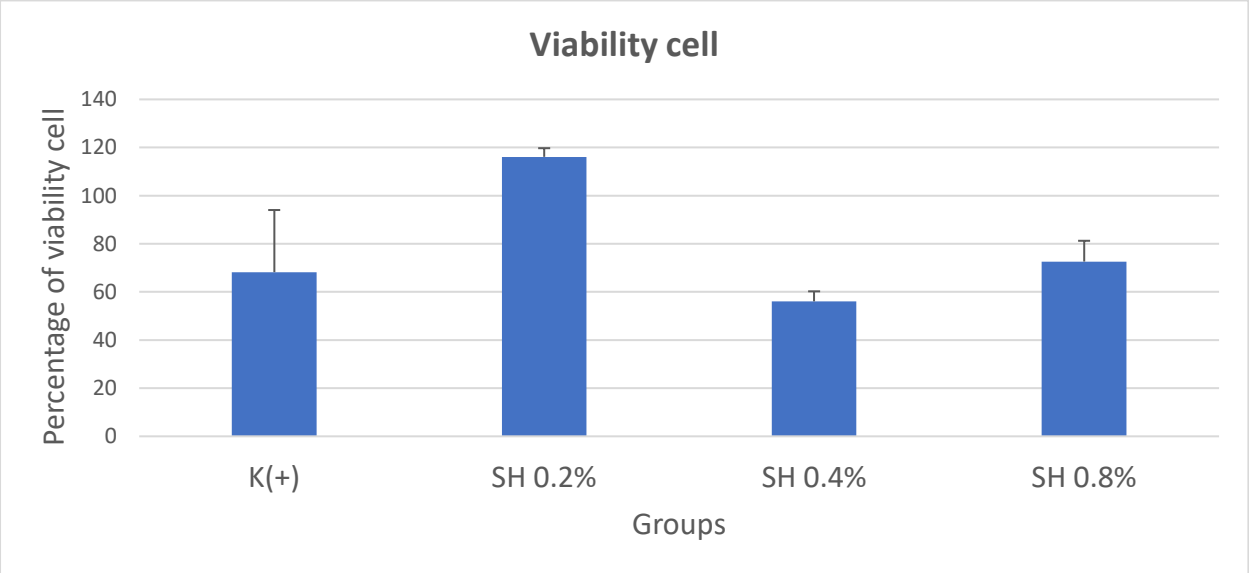
Table 2 Cytotoxicity test of calcium hydroxide microencapsulated with Sichopus hermanii by measuring Optical Density

N	K(+)	P1	P2	P3
Media control	0,088	0,088	0,108	0,108
1	0,155	0,381	0,123	0,173
2	0,382	0,359	0,153	0,196
3	0,150	0,353	0,157	0,196
4	0,194	0,385	0,125	0,185
5	0,093	0,386	0,117	0,187
6	0,127	0,381	0,136	0,269
Cell control	0,31	0,313	0,312	0,313

Table 3. Cytotoxicity test by calculating the percentage of the number of living BHK-21 cells (% cell viability)

N	K(+)	P1	P2	P3
Media control	0,088	0,088	0,108	0,108
1	61%	100%	55%	67%
2	100%	100%	62%	72%
3	60%	100%	63%	72%
4	71%	100%	55%	70%
5	45%	100%	54%	70%
6	55%	100%	58%	90%
Cell control	0,31	0,313	0,312	0,313

From the calculation of the percentage of cell viability in group K (+), namely calcium hydroxide powder, group P1 (microencapsulated calcium hydroxide with Stichopus hermanii concentration 0.2 %), group P2 (microencapsulated calcium hydroxide with Stichopus hermanii concentration 0.4%) and group P3 (microencapsulated calcium hydroxide with Stichopus hermanii concentration of 0.8 %), all of them are not toxic because the value of the percentage of cell viability (number of living cells) is greater than 50%. In group P1 (microencapsulated calcium hydroxide with Stichopus hermanii concentration 0.2%) the percentage of cell viability (number of living cells) was 100%.



Scanning Electron Microscope (SEM) microencapsulated calcium hydroxide with Stichopus hermanii

The results of the Scanning Electron Microscope (SEM) of a particle will be displayed in the form of an image on a CRT monitor and from the test obtained images of morphology, size and particle shape. The following is a picture of the SEM results for each group in this study.

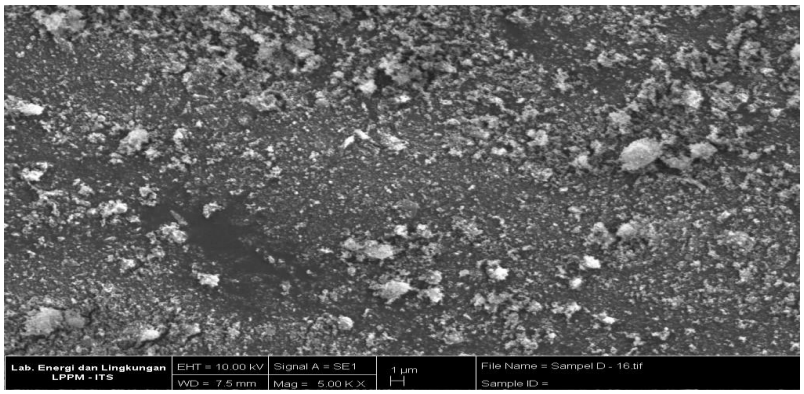


Figure 1. Results of examination of the shape and surface morphology of particles calcium hydroxide microencapsulated with Stichopus hermanii at 0.2% concentration using a Scanning Electron Microscope (SEM) at 1500Kx magnification

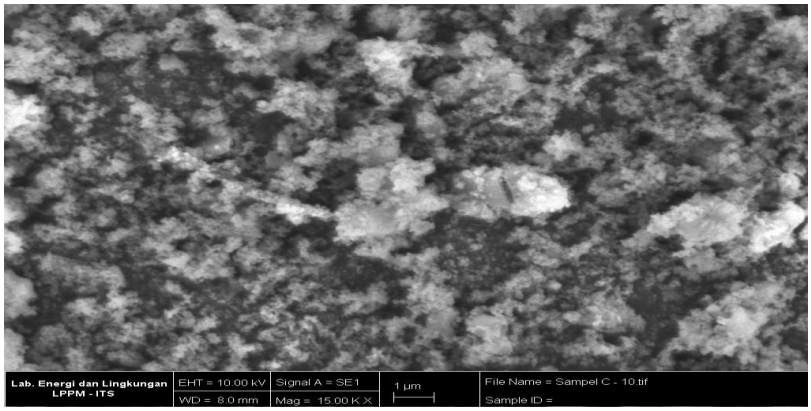


Figure 2. Results of examination of the shape and surface morphology of particles calcium hydroxide microencapsulated with Stichopus hermanii at a concentration of 0.4% using a Scanning Electron Microscope (SEM) at 1500Kx magnification

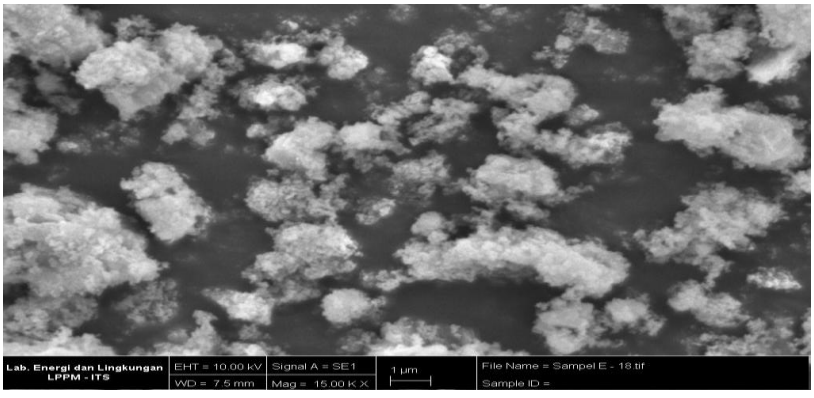


Figure 3. Results of examination of the shape and surface morphology of particles calcium hydroxide microencapsulated with Stichopus hermanii at a concentration of 0.8% using a Scanning Electron Microscope (SEM) at 1500Kx magnification

DISCUSSION

This study aims to determine the effect of microencapsulation of gold sea cucumber (Stichopus hermanii) powder in three different concentrations on calcium hydroxide powder. Calcium hydroxide has been the "material of choice" sub-base for many years globally because it has the advantage of being able to form reparative dentin, has strong antibacterial properties, and can act as a good insulating layer.[21] This material, however, has a drawback, namely high solubility, which causes "tunnel defects" in the formed barrier layer and has the potential to fail pulp capping treatment.[22] The encapsulation of gold sea cucumber powder in calcium hydroxide is expected to reduce the solubility of calcium hydroxide ingredients. Golden sea cucumber (Stichopus hermanii) has a high content of hyaluronic acid, where hyaluronic acid is a bioactive polymer that can be used as a protective layer for calcium hydroxide particles through the microencapsulation method.[23] The process of encapsulation of gold sea cucumber powder and calcium hydroxide was carried out using the freeze-drying method.[15]

This study used 3 kinds of concentrations of gold sea cucumbers in mixed powder preparations, namely concentrations of 0.2%, 0.4%, and 0.8%. The determination of the three concentrations was based on the results of a study by Casale et al.[24] regarding the effect of hyaluronic acid in various concentrations on soft tissues and hard tissues of the oral cavity, which stated that 0.2% hyaluronic acid helps the process of soft tissue regeneration and at a concentration of 0.8% helps hard tissue regeneration process.[22] Preparation of microencapsulated powder at each concentration was carried out by mixing gold sea cucumber powder and calcium hydroxide powder with a weight ratio of 1:6. This comparison figure is determined based on preliminary research that has been previously carried out. After the microencapsulation process was completed, the material characteristics were tested, among others PSA test, toxicity test, and SEM test.

In the PSA test, the smallest particle size was calcium hydroxide microencapsulated powder with a concentration of 0.4% Stichopus hermanii with an average PSA value of 3717 nano. In comparison, the largest particle size average value was calcium hydroxide microencapsulated powder with Stichopus hermanii at a 0.8% concentration, with an average PSA value of 5517 nano. The calcium hydroxide microencapsulated powder group with 0.2% concentration of Stichopus hermanii had an average PSA value of 4427 nano, which was smaller than the P3 group, but larger than the P2 group and had a PSA value close to the PSA value for the calcium hydroxide powder particle size. pure. At a concentration of 0.2%, the size of Stichopus hermanii powder that encapsulates calcium hydroxide is also thinner, this causes the PSA value to be smaller than Stichopus hermanii with a concentration of 0.4% and 0.8%, respectively. It has been repeatedly shown that treatment with nanomaterials stimulates the development of a dentinal bridge with many dentinal tubules.[25] One of the most important and commonly applied criteria for assessing the biomaterials in question is the production of tertiary dentin. Reactionary and reparative dentin are two further classifications for tertiary dentin. Reactionary dentin is morphologically distinguished by tubular dentin resembling primary and secondary dentin, while reparative dentin is typified by amorphous calcified tissue.[26]

Toxicity tests on all groups, both the control group (calcium hydroxide) and the calcium hydroxide microencapsulated treatment group with Stichopus hermanii concentrations of 0.2%, 0.4%, and 0.8% showed that the percentage value of cell viability (the number of BHK21 fibroblast cells) was more than 50 %, this means that all materials are non-toxic. In microencapsulation of calcium hydroxide with 0.2% concentration of Stichopus hermanii, the percentage value of cell viability (number of living cells) was 100%, this indicates that there was

no fibroblast cell death tested. Scanning Electron Microscope (SEM) test on calcium hydroxide microencapsulated powder with Stichopus hermanii concentrations of 0.2%, 0.4%, and 0.8% obtained a rough and fibrous surface shape, where at 0.2% concentration a smoother morphology was found. The reactions of tissues to calcium hydroxide are not always foreseeable, though. After pulp capping, a restoration needs to be used. As a result, it is necessary to take into account both the toxicity of the pulp-capping material and the restoration material. It is important to highlight that the cytotoxicity test only measures the early toxicity of the microparticles used in this investigation.[27]

CONCLUSION

Calcium hydroxide microencapsulated with Stichopus hermanii concentrations of 0.2%, 0.4%, and 0.8% as pulp capping materials proved to be non-toxic. Particle size (PSA) of calcium hydroxide encapsulated preparations with Stichopus hermanii concentration of 0.4% showed a smaller size than 0.2% and 0.8%. The morphology of the calcium hydroxide encapsulated dosage form with Stichopus hermanii concentration is 0.2% smoother than the 0.4% and 0.8% that appear stringy.

REFERENCES

1. Garg, N., & Garg, A. 2015. *Textbook of Operative Dentistry 3rd Edition*. New Delhi: Jaypee Brothers Medical Publishers.
2. Hargreaves KM, Cohen S. 2016. Cohen's pathways of the pulp. 17th ed. St Louis: Mosby Elsevier.
3. Tarigan, R. 2015. Karies Gigi. Edisi 2. EGC: Jakarta
4. Torabinejad, M., Walton, R. E., & Fouad, A. F. 2015. *Endodontics Principles and Practice 5th Edition.pdf* (Fifth Edit). St. Louis Mossouri: Elsevier Saunders.
5. Nurhayati, I. S., Kajian Pengendalian Mastitis Subklinis Melalui Pemberian Anbiotik pada Saat Kering Kandang di KPSBU Lembang Jawa Barat, Thesis, IPB University, 2014.
6. Mustafa, M., Jain, D., Taluka, S., Lecturer, S., Dentistry, C., & Capping, P. 2012. Role of Calcium Hydroxide in Endodontics : A Review. *Global Journal of Medicine and Public Health*, *1*(1)
7. Baranwal, R., Singh, B., Dubey, A., & Avinash, A. 2016. Review Article Calcium Hydroxide in Dentistry. *Chettinad Health City Medical Journal*, *5*(January), 30–33.
8. Mostafa, N., & Moussa, S. A. 2018. Mineral Trioxide Aggregate (MTA) vs Calcium Hydroxide in Direct Pulp Capping – Literature Review. *Online Journal of Dentistru & Oral Health*, *1*(2), 1–6. https://doi.org/10.20431/2456-0030.0302004
9. Morotomi, T., Washio, A., & Kitamura, C. 2019. Current and Future Options for Dental Pulp Therapy. *Japanese Dental Science Review*, *55*(1), 5–11. https://doi.org/10.1016/j.jdsr.2018.09.001
10. Albuntana, A., Yasman., dan Wardhana, W., 2011. Uji Toksisitas Ekstrak Empat Jenis Teripang Suku Holothuriidae dari Pulau Penjaliran Timur, Kepulauan seribu, Jakarta menggunakan Brine Shrimp Lethality Test (BSLT), Jurnal Ilmu Teknologi Kelautan Tropis, 3:65-72.
11. Collins, N., and Sulewski, C., 2011. Omega-3 fatty acids and wound healing. Nutrition 411, Ostomy Wound Management.
12. Sari RP, Budijono SCS. Characterization And Potency Of Stichopus Hermanii Ethanol Extract On Oral Wound Healing. Odonto Dental Journal, 2020; 7 (1) : 9-17
13. Gupta, A., and Dey, B. 2013. Microencapsulation for controlled drug delivery: a comprehensive review. Sunsari Technical College Journal, 1(1):48-54.
14. Hidayah N. 2016. Perbandingan Berbagai Teknik Mikroenkapsulasi Pakan dalam Menghasilkan Daging Sapi Sehat. Malang: SenasPro 2016 Universitas Muhammadiyah Malang, pp. 143- 151.
15. Astuti W.M., Dewi E.N., dan Kurniasih R. A., 2019. Pengaruh Perbedaan Jenis pelarut dan Suhu Pemanasan se;ama Ekstraksi terhadap Stabilitas Mikrokapsul Fikosianin dari Spirulina platensis. Jurnal Ilmu dan Teknologi Perikanan 1(1):8-14.
16. Nireesha, G., L. Divya, C. Sowmya, N. Venkateshan, M. Babu, and V. Lavakumar. 2013. Lyophilization/freeze drying: An review. World Journal of Pharmaceutical Research 4 (8):516–43.
17. Sari RP, Kusumawardani RP, Damaiyanti DW, Rahayu ARP, Sudjarwo SA. The Effectivity Of Scaffold From Anadara Granosa Shell-Stichopus Hermanii On Blood Vessel Counts After Tooth Extraction**.** Odonto Dental Journal, 2021; 8 (2) : 11-17.

18. Diana Soesilo, Aprilia, Moh.Basroni Rizal. InvitroMicro Encapsulation of Beta Tri Calcium Phosphate from Anadara granosa Shell Synthesis. Denta Jurnal Kedokteran Gigi, 2020; 14(2) : 71-76.
19. Freshney, R.I. 2010. Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications. 6th Edition, John Wiley & Sons Ltd., Hoboken. https://doi.org/10.1002/9780470649367
20. Ratna Putri*, Twi Agnita Cevanti**, Henu Sumekar. Sitotoksisitas Ekstrak Daun Mangrove Daruju(Acanthus Ilicifolius) Sebagai Bahan Irigasi Saluran Akar. Denta Jurnal Kedokteran Gigi, 2018; 12(1) : 44-50
21. Pannu R dan Berwal V. 2017. Calcium Hydroxide in Dentistry: A Review. Haryana: *Journal of Applied Dental and Medical Sciences*, 3(3), pp. 24-31.
22. Ziad NA. 2017. Calcium Hydroxide Liners – A Literature Review. Zababdeh: *Clinical, Cosmetic, and Investigational Dentistry*, 9, pp. 67-72.
23. Ahmadzadeh S, Herasaki Z, dan Zamanian A. 2011. Preparation and Characterisation of Calcium Phosphate – Hyaluronic Acid Nanocomposite Bone Cement. Tehran: *Advances in Applied Ceramics* Institute of Materials, Minerals, and Mining, 110(6), pp. 340-345.
24. Casale, M., Moffa, A., Vella, P., Sabatino, L., Capuano, F., Salvinelli, B., Salvinelli, F. 2016. Hyaluronic acid: Perspectives in dentistry. A systematic review. *International Journal of Immunopathology and Pharmacology*, *29*(4), 572–582.
25. Imura, K., Hashimoto, Y., Okada, M., Yoshikawa, K., & Yamamoto, K. (2019). Application of hydroxyapatite nanoparticle-assembled powder using basic fibroblast growth factor as a pulp-capping agent. *Dental Materials Journal*, *38*(5), 713–720. https://doi.org/10.4012/dmj.2018-198.
26. Lin, H. P., Tu, H. P., Hsieh, Y. P., & Lee, B. S. (2017). Controlled release of lovastatin from poly(lactic-co-glycolic acid) nanoparticles for direct pulp capping in rat teeth. *International Journal of Nanomedicine*, *12*, 5473–5485. https://doi.org/10.2147/IJN.S138410.
27. Priyadarshini, B. M., Selvan, S. T., Narayanan, K., & Fawzy, A. S. (2017). Characterization of chlorhexidine-loaded calcium-hydroxide microparticles as a potential dental pulp-capping material. *Bioengineering*, *4*(3). https://doi.org/10.3390/bioengineering4030059.