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RESEARCH ARTICLE

Toxicity Test of HA-TCP Variation from Synthesis of Blood Shell with Fluoride Addition as Dental Remineralization Material

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ABSTRACT

Background: *Caries is the most found problem in teeth. Caries begins with fermentation process of carbohydrates. The preventive and curative actions of white spot lesions aim to initiate and improve the process of tooth remineralization. HA TCP from the synthesis of blood shells with fluoride added is expected to be a dental remineralization agent.* ***Objective:*** *To determine the cytotoxicity of HA-TCP variation from the synthesis of blood clam shells (Anadara granosa) with fluoride added as a dental remineralization material.* ***Materials and Methods:*** *This type of research is true experimental with a post test only control design. The data from the research results were then carried out a descriptive test and viability test followed by the Kruskal wallis test and significance test using the Chi Square Test. The number of samples was 15 which were divided into 4 groups, namely the KS (control group of cells, without the addition of scaffolds, P1 (HA-TCP 15% with fluoride addition), P2 (HA TCP 20% with fluoride addition) and P3 (HA-TCP 25% with fluoride addition). The cytotoxicity test was carried out using MTT Assay on BHK-21 cells.* ***Results:*** *The results of cell viability percentages in the KS, P1, P2, and P3 groups were 100%, 61.16%, 79.12% and 88.83% respectively.* ***Conclusion:*** *The cytotoxicity test results of HA-TCP variation from the synthesis of blood clam shells (Anadara granosa) with fluoride added as a dental remineralization material showed non-toxic results.*

Keywords: *Anadara Granosa Shell, Cytotoxicity, HA-TCP, Remineralization*

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INTRODUCTION

Caries is a dental problem, begins with the fermentation process of carbohydrates by bacteria such as *Streptococcus mutans* which produces acid products in the hard tissues of the teeth and decreasing pH of the oral cavity.[1] The condition of continuous pH decrease causes demineralization or loss of mineral structure, especially calcium, phosphate, carbonate and the main mineral hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$) which forms an early lesion of white caries called white spot lesion.[2] Remineralization is the process of reforming lost minerals, especially calcium and phosphate ions that form hydroxyapatite crystals in demineralized enamels.[3] Saliva played a role in affecting the remineralization. Saliva a natural source of calcium and phosphate in the oral cavity.[4] The use of calcium from blood shell powder has been widely studied as remineralization agent. The powder of blood shell can be synthesized into hydroxyapatite (HA) and tricalcium phosphate (TCP) (HA–TCP) which can be used as a tooth remineralization agent

Hydroxyapatite (HA) (Ca10(PO4)6(OH)2) is a type of ceramic known for its excellent biocompatibility due to its chemical and physical composition being identical to that of human bone and tooth minerals. HA powder can be derived from two primary sources: synthetic chemical processes and natural biological materials, such as *Anadara granosa* (blood cockle shells). However, HA has a notable drawback, as it exhibits poor toughness, low wear resistance, and is challenging to degrade.[5] To reduce the degradation process, the combination of hydroxyapatite (HA) and tricalcium phosphate (TCP) is assumed to have higher quality and more stable.[6] This research using HA-TCP 22:78 was due to previous research that hydrothermal method of 18 hours sintering 4 hours will produce HA-TCP 22:78 based on the XRD results.7

The addition of fluor in Tri Calcium Phosphate (fTCP) can controls the delivery of calcium and phosphate ions into tooth. TCP formulations are designed with fluorine to increase remineralization and build acid-resistant minerals without requiring jigh levels of calcium and will produce great benefits when in neutral pH.[8] Topical apllication of fluoride in post eruptive tooth promotes enamel remineralization and inhibit demineralization, reduces glycolisis, and exerts antibacterial effects. The addition of fluoride into restorative materials can reduce the incidence of caries, particularly in high risk individuals.[9,10] In this reasearch, the selection of 900ppm of fluoride because tooth paste with 1000ppm of fluoride can prevent the caries in primary and permanent tooth.[11]

The cytotoxicity test is a component of the biocompatibility assessment aimed at evaluating the biological impact of a material. Its purpose is to ensure that the material does not induce toxic effects that could interfere with biological functions, thereby preventing rejection by the host. Based on this context, this study was carried out to assess the cytotoxicity of cream HA-TCP variations synthesized from blood cockle shells with added fluoride, intended for use as a dental remineralization agent, utilizing the MT-Assay method. The choice of percentages of 15%, 20% and 25% was based on research before which states that a 20% suspension of shell calcium is the optimal concentration for remineralization of enamel that has experienced initial caries.[12]

MATERIALS AND METHODS

This study is a true experimental laboratory study using a post test only control group design. The experimental unit of this research is HA TCP 22-78 cream added with 900ppm fluoride. The subjects in this study were divided into 4 groups, namely cell control (KS), HA TCP 15% (P1) variant, HA TCP 20% (P2) variant and HA TCP 25% (P3) variant.

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The produce of HA-TCP cream with fluor addition, started with a clear gel mass was produce with the process of mixing aquabidestilata at a temperature of 80°C with Na CMC and the process of re-adding aquabidestilata at room temperature and then continuing with the process of adding *Anadara granosa* shell material composition of 15%, 20% and 25% and 900 ppm fluoride mixed until homogeneous.

The cytotoxicity test was carried out on BHK 21 cell culture and 96-well microplate. Wells are filled with control media, control cells and research samples with concentrations of 15%, 20% and 25%. Then the microplate is incubated with 5% CO2 at a temperature of 37°C for 20 hours. Then, the microplate is removed from the incubation tool, the culture media and scaffold in the well are taken using a syringe, BHK-21 cells will be left in the well. Then incubated again for 3 hours. Total incubation time in an incubator at 37°C for 24 hours. After the incubation period is complete, MTT and culture media are taken using a syringe The optical density value of formazan was read with an Elisa reader with a wavelength of 620 nm. To determine the percentage of live BHK-21 cells, we use the cell viability formula. Cell viability is defined as the ability of cells to survive. The formula for calculating cell viability is (number of living cells/total number of cells) x 100%.

Data from each examination was analyzed statistically with a significance level (α = 0,05). Normality test in this research was using *Shapiro-Wilk* test, and followed by *Kruskal-Wallis* test.

RESULT

The results of toxicity test showed that the percentage of cell viability is above 50%, which means more and more living cells. The percentage of cell viability from the synthesis of blood clam shells (*Anadara granosa)* measured by ELISA reader can be seen in table 1. The data from the study results then carried out a normality test using *Saphiro-Wilk.*

Table 1. Average and Standard Deviation of live cell and percentage of cell viability in HA-TCP 22-78 with the Addition of 900ppm Fluoride.

Group	Average+Standard Deviation	Percentage of cell viability (%)
P1	0.324 ± 0.219	61.16
P2	0.439 ± 0.308	79.12
P3	22.36 ± 72.514	88.83

Information:
P1 = HA TCP 22-78 with a concentration of 15%
P2 = HA TCP 22-78 with a concentration of 20%
P3 = HA TCP 22-78 with a concentration of 25%

Table 2. Normality Test Results with Shapiro-Wilk

Shapiro-Wilk	
Groups	Sig.
P1	.098
P2	.045
P3	.000

Table 1 shows that the P3 group have the highest result of the average value of living cells, continued with P2 group, and P1 group. Based on table 2, the significance value (sig.) of normality test showed normal undistributed data ($p<0.05$), in the 15%, 25%, and 25% of HA-TCP micro group. These results mean that the normality test is not normally distributed because the significance value (Sig.) is less than ($p<0.05$). The test is continued with a non-parametric test using the *Kruskal Wallis* Test.

Table 3. Hypothesis Test with Kruskal Wallis Test

	Viabilitas
Chi-Square	1.882
Df	2
Asymp. Sig.	.390

The test results using *Kruskal Wallis* test showed that the significance value was 0.390 greater than 0.05 ($p>0.05$), which means that there was no significant difference in cytotoxicity between treatment groups.

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DISCUSSIONS

This study aims to determine the cytotoxicity of the HA-TCP 22-78 from the synthesis of blood clam shells added with fluoride to BHK-21 cells. The selection of fluoride as one of the active ingredients in this study is through the process of forming fluorapatite which plays a role in preventing demineralization and triggering remineralization.[8] The cytotoxicity test on BHK-21 cell culture was chosen because it is stable, easy to grow, easy to culture, and does not show mutations. This cell culture has structural similarities to human fibroblast cells which are often found in the oral cavity.[13,14]

In this study, direct contact has been carried out between HA-TCP synthesis of blood clam shells *(Anadara granosa)* in cell culture. The purple color change caused by the addition of formazan crystals was then read the absorption using an ELISA reader. The more intense the color shown, the higher the absorption value and the more cells live. The absorbance value obtained was then included in the cell viability formula to get the percentage of the number of viable cells. Actually, in the cytotoxicity test after obtaining the percentage of cell life/death, the data on the percentage of the number of dead cells can be further calculated by measuring LC_{50} (*lethal concentration*) which shows the concentration value that results in cell death of 50% or IC_{50} (*inhibitory concentration*). It indicates that the concentration value results in the inhibition of cell proliferation by 50% and shows the potential toxicity of a compound to cells.[16.] However, this study was only carried out until the calculation stage of the percentage of cell viability was achieved. The results of the calculation are said to be non-toxic if the percentage of cell viability is more than 50%, and it is said to be toxic if the percentage of viability cell is less than 50%.[15,16]

The sample used in this study is a cream preparation created by initially mixing aquabidestillata at a temperature of 80°C with sodium carboxymethyl cellulose (Na CMC) to form a clear and homogeneous gel mass. After this process, additional aquabidestillata is added at room temperature. Next, we incorporate HA-TCP materials at percentages of 15%, 20%, and 25%, along with 900 ppm of fluoride. This mixture is blended until it becomes homogeneous, resulting in a cream preparation that serves as a candidate for dental remineralization materials. The selection of cream preparations as candidates for remineralization materials is based on several factors. Firstly, they are easy to apply on tooth enamel. Additionally, these preparations effectively deliver important ingredients that can diffuse into the microporosity of the enamel. The cream formulations also adhere well to the enamel layer, facilitating a more efficient absorption process for the medicinal properties of the cream.[17]

Based on the calculation of the data, the results of this study showed that the percentage of cell viability in the P1 group (HA-TCP 15%), has a value of 61.16% while the P2 group (*HA-TCP 20%)* is 79.12% and the P3 group (HA-TCP 25%) is 88.83%. In the KS group (cell control), the percentage of cell viability was 100% because the wells in the microplate only contained media cultures without the administration of HA-TCP. Cell control was used for the normalization of the viability percentage of the other treatment groups. The cell viability percentages in the P1, P2, and P3 groups are all above 50%, indicating that the materials are non-toxic. This conclusion is supported by the toxicity test of the HA-TCP micro scaffold synthesized from blood cockle shells, which yielded non-toxic results.[18]

The addition of fluoride in HA-TCP cream does not affect the results of toxicity tests. This is because the amount of fluoride used is 900 ppm, which is still below the daily dose of topical fluoride (1000-1500ppm).[11,19] Meanwhile, the toxic dose of fluoride in humans is 5,0-10,0 F/kg in weight.[20]

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CONCLUSION

HA-TCP synthesis results of blood clam shells given the addition of 900ppm fluoride showed non-toxic results with cell viability percentages of 61.16%, 79.12% and 88.83%, respectively.

REFERENCES

1. Marwah N. 2019. Textbook of : Introduction to Pediatric Dentistry. 3rd Ed. 1–1004. Jaypee Pub. P: 1-18.
2. Jeanny KH, Lunardhi CGJ, Subiyanto A. Kemampuan Bioaktif Glass (Novamin) dan Casein Peptide Amorphous Calcium Phosphate (CPP-ACP) terhadap Demineralisasi Enamel The Potential of Bioactive Glass (Novamin) and Casein Peptide Amorphous Calcium Phosphate (CPP-ACP) on Enamel Demineralization. 2017; 7. Conservative Dentistry Journal 7(2); 2017: 111-119.
3. Vo Truong Nhu N, Pham Thi Hong T, Le AQ, Minh ST, Nguyen Thi Thu P. The Effect of Casein Phosphopeptide-amorphous Calcium Fluoride Phosphate on the Remineralization of Artificial Caries Lesions: An In Vitro Study. Journal of Dentistry Indonesia. 2017;24(2): 45-49. doi:10.14693/jdi.v24i2.1044
4. Talaat DM, Abdelrahman AAM, Abdelaziz RH, Nagy D. Effect of Two Remineralizing Agents On Initial Caries-Like Lesions in Young Permanent Teeth: An in vitro study. Journal of Contemporary Dental Practice. 2018; 19 (10): 1181–8.
5. Neel EAA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, et al. Demineralization – Remineralization Dynamics in Teeth And Bone. International Journal of Nanomedicine. 11(1); 2016: 4743-4763.
6. Ferdynanto RA, Dharmayanti PES, Dewi PTK, Prananingrum W. The effect of various concentrations of HA-TCP derived from cockle shell synthesis on scaffold porosity. Dent J. 2018; 51 (3): 114–8.
7. Sari RP, Kurniawan H. Effectiveness of Anadara granosa shell-Stichopus hermanni granules at accelerating woven bone formation fourteen days after tooth extraction. Dent J. 2019; 52 (4): 177–82.
8. Tiffany AS, Wahyuni S. Pengaruh Pengolesan Bahan Remineralisasi FTCP (Clinpro®) Terhadap pH Saliva. Jurnal Kesehatan Gigi dan Mulut (JGKM). 2020; 2(1): 14.
9. Mankar N, Kumbhare S, Nikhade P, Mahapatra J, Agrawal P. Role of Fluoride in Dentistry: A Narrative Review. J Cureus. 2023; 15 (12): 12. DOI: 10.7759/cureus.50884
10. Zirk M, Schievelkamp S, Kemnitz E, Lausch J, Wierichs RJ, Esteves-Oliveira M, et al. Evaluation of Novel Nanoscaled Metal Fluorides On Their Ability to Remineralize Enamel Caries Lesions. Sci Rep. 2019; 9(1): 1-8.
11. Walsh T, Worthington HV, Glenny AM et al. Fluoride Toothpastes of Different Concentrations For Preventing Dental Caries. Cochrane Database Syst Rev. 2019; 3(3): 1-13.
12. Abdelnabi A, Hamza NK, El-Borady OM, Hamdy TM. Effect of Different Formulations and Application Methods of Coral Calcium on Its Remineralization Ability on Carious Enamel. Open Access Maced J Med Sci. 2020; 8: 94–9.
13. Adnyana VC. Uji Sitotoksisitas Periodontal Dressing Yang Mengandung Ekstrak Kulit Buah Kakao 15% Terhadap Kultur Sel Fibroblas Baby Hamster Kidney-21 (BHK-21). Skripsi. Jember: Fakultas Kedokteran Gigi, Universitas Jember; 2017. P:19.
14. Emilda Y, Budipramana E, Kuntari DS. Uji Toksisitas Ekstrak Bawang Putih (Allium Sativum) Terhadap Kultur Sel Fibroblast (Garlic Allium Sativum) Extract Toxicity Test On Fibroblast Cell Culture). Dent. J. (Maj. Ked. Gigi) 2014; 47(4) : 215–219.
15. Michael Josef Kridanto Kamadjaja, Bambang Agustono, Innocencio Kresna Pratama. Biocompatibility of Crab Shell-Derived Graft Sterilized by Ultraviolet Light Towards Human Gingival Fibroblast Cell Culture. World Journal of Advanced Research and Reviews. 2023; 17 (3): 728–35.
16. Arquitectura EY, Introducci TI, 赫晓霞, Iv T, Teatinas LAS, Conclusiones TVII, et al. No 主観的健康感を中心とした在宅高齢者における 健康関連指標に関する共分散構造分析Title. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis [Internet]. 2015; 53 (9): 1689–99. Available from: http://publications.lib.chalmers.se/records/fulltext/245180/245180.pdf%0Ahttps://hdl.handle.net

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/20.500.12380/245180%0Ahttp://dx.doi.org/10.1016/j.jsames.2011.03.003%0Ahttps://doi.org/10.1016/j.gr.2017.08.001%0Ahttp://dx.doi.org/10.1016/j.precamres.2014.12
17. Effendi MD, Suhendra N. Pengaruh Rasio HA/TCP terhadap Karakteristik Komposit BCP-Gelatin-CMC sebagai Bahan Injectable Bone Substitute. Jurnal Inovasi dan Teknologi Material. 2020; 1 (2): 15–20.
18. Subagiyo S, Margino S, Triyanto T, Ari Setyati WA. Effects of pH, Temperature and Salinity In Growth and Organic Acid Production of Lactic Acid Bacteria Isolated From Penaeid Shrimp Intestine. Ilmu Kelaut. 2015; 20 (4) :187.
19. Clark MB, Keels MA, Slayton RL. Fluoride Use in Caries Prevention in the Primary Care Setting. Pediatrics. 2020; 146 (6): 1-11. DOI: https://doi.org/10.1542/peds.2020-034637
20. Susanto A, Padjadjaran U. The Effect of the Application of HA-TCP Bone Graft towards Gingival Crevicular Fluid Osteocalsin in the Treatment of Periodontal Infrabony Defects. Journal of International Dental and Medical Research. 2020; 13 (4): 12–7.

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