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EFFECTIVITY OF WILD PINCTADA MAXIMA-DERIVED CALCIUM CARBONATE ON BONE REGENERATION THROUGH CTX (*C-TERMINAL TELOPEPTIDE*) ANALYSIS

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Running Title : Wild *Pinctada maxima* Calcium for Bone CTX Analysis

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ABSTRACT

Background: Alveolar bone defects are a common consequence of periodontal diseases, leading to tooth mobility, reduced alveolar bone integration, and gingival recession. Pinctada maxima, known for its high calcium carbonate content, shows promise for bone grafting due to its biocompatibility, osteoconductivity, and osteoinductivity. Larger wild Pinctada maxima possess more nacre, providing greater calcium carbonate quantities for bone regeneration. **Methods**: This study processes Pinctada maxima shells into calcium carbonate powder, analyzed using Atomic Absorption Spectroscopy (AAS), X-Ray Fluorescence (XRF), Fourier Transform Infra-Red (FTIR), and X-Ray Diffraction (XRD) to determine content. The powder is then tested on Cavia cobaya with a negative control (no treatment) and a positive control (bovine xenograft) and treatment group Pinctada Maxima-derived calcium carbonate. **Results**: CTX expression were found to be reduced and the greatest number of reduction after 21st day were found to be in the Bonegraft PM (Pinctada Maxima) group in comparison with both control groups. **Conclusion**: Calcium carbonate from wild Pinctada maxima significantly enhances bone regeneration, increasing bone density and integrity of newly formed bone, making it a viable material for treating alveolar bone defects in periodontal disease.

PLAIN LANGUAGE SUMMARY

Alveolar bone loss often occurs due to periodontal disease, leading to tooth instability and gum recession. To help regenerate lost bone, researchers are exploring materials for bone grafts, such as *Pinctada maxima* (a type of pearl oyster) due to its high calcium carbonate content, which supports

bone healing and growth. This study examines the use of *Pinctada maxima* shells, rich in calcium carbonate, as a bone graft material. The shells are processed into a fine powder and tested on guinea pigs to evaluate its effectiveness. Advanced tests confirm the composition of the calcium carbonate in the powder, and it is compared with no treatment and a commonly used bovine bone graft. Findings show that *Pinctada maxima* powder significantly reduces CTX (a marker of bone resorption) and increases new bone formation, particularly after 21 days, compared to both control groups. This study concludes that wild *Pinctada maxima*-derived calcium carbonate is a promising bone graft material that effectively supports bone regeneration and may improve outcomes for patients with bone loss from periodontal disease.

INTRODUCTION

Periodontal disease has a high prevalence in Indonesia, according to RISKESDAS 2018 there are around 74,1% of Indonesians suffering from periodontal diseases and in the adult population in USA, roughly 30%-50% adults are suffering from periodontal diseases. This shows the urgency to treat this disease and prevent the further increase of a very preventable diseases and to increase the quality of life of people suffering from this disease[1,2].

The treatment of choice for periodontal diseases, especially bone losses caused by periodontitis is by using bone grafts in conjunction with periodontal diseases. An ideal bone graft materials needs to be able to have 2 of 3 things, (1) Osteogenesis capabilities, in which it can act as a new bone for the defects, (2) Osteoconductivity, in which it acts as a scaffold for new bone to grow and lastly (3) Osteoinductivity, in which it acts to induce the creation of new bone[3].

One of the materials found to be able to provide both osteoconductivity and osteoinductivity is calcium carbonate. This particular material is amply found in Pinctada Maxima shells, specifically in its nacre or *Mother Of Pearls*. Pinctada Maxima is very commonly found in tropical waters especially in Indonesia and they are usually bred for pearl farming. Distinguishing different found form farm-raised and wild stock of Pinctada Maxima is the size and quality of its nacre, in which the nacre is the source of calcium carbonate. The difference in quality exists because of the lack of genetic diversity from the farm-raised population due in-breeding and thus, in terms of quality of nacre, the wild population were found to be more desirable[4,5,6].

In terms of the ideal marker for bone regeneration, CTX *(C-Terminal Telopeptide*) fits the niche well, this is due to its role in signalling the formation of collagen during the proliferative phase of wound healing, which in turn will be remodelled into new bone in the remodelling phase, as it can be surmised that an increase level of collagen formation is the precursor to new bone formation due to the inherently hereditary nature of healing in which in transitions from one phase to the other with the same materials being reformed and carried over for the purpose of regeneration[7].

METHODS

This research is an *experimental laboratory* with pure experimental design using *pre-post test control* group design. Conducted for 8 months from February-September 2024.

Animals

Animals used for testing are 30 male adult Cavia Cobaya weighing 300 grams that has underwent adaptation for 7 days.

Pinctada Maxima-derived Calcium Carbonate Powder Preparation

Pinctada Maxima-derived powder was formulated using the precipitation method. Firstly the shells were washed and sun-dried thoroughly. The shells are then broken apart and placed in a furnace with 110°C temperature for 2 hours. It were then filtered using -50+60 mesh until a fine powder sample is retrieved. The powder then were tested with *Atomic Absorption Spectrophotometry* (AAS) in order to know the calcium content. Samples were then furnaced with 900°C and calcinated to retrieve to CaO content. It was then synthesized using HNO_3 and mixed using a rotator with 350rpm for 30 minutes and then heated on 60’C heat and NH_4OH we added with a pH of 12 and then filtered. Filtrates received were then flowed using CO_2 for 90 minutes with a flowrate of 120ml/min. Following that, it was filtered again using aquadest until a pH of 7 were obtained, it was then heated in an oven with 110-150’C. The received sample were then characterized with X-Ray *Fluorescence* (XRF), *Fourier Transform Infra Red* (FTIR) and X-Ray Diffraction (XRD) to obtain the necessary compound character needed for a bone graft.

Figure 1. a) Nacre processing to powder b) processed powder was weighed

Figure 2. *Pinctada Maxima* powder was calcinated with 900°C

Figure 3. Porosity test

Experimental Procedure

After adaptation, the cavia cobayas were anesthesized using 500mg/kg of ketamine and Xylazine. Following that the lower front tooth were extracted, after the extraction area has been disinfected with povidone iodine. Randomly, the cavia cobayas are divided to 3 groups each numbering 10. The group were then labeled as Positive Control (KP) in which they are given Bovine Xenograft, Negative Control (KN) in which they are given nothing and lastly Treatment Group (KP) in which they are given the Calcium Carbonate Powder. Suturing were done using absorbable vicryl 5.0 with *interrupted suture* technique and then the cavia cobayas were given prophylaxis antibiotics (Penstrep-400) on the sutured area. For post-surgical medication, the cavia cobayas were given 0,6 mg/kg dexamethasone and 10mg/kg ampycilling. Sacrifice were done on day 14 and 21, afterwards the specimen were taken, fixated and then examined.

RESULTS

In this study, data normality was tested using the Shapiro-Wilk test, as the sample size was fewer than 50 subjects. If the normality test result is $p > 0.05$, the data is normally distributed; otherwise, it is not. Data that is normally distributed will then be tested using ANOVA. If the ANOVA test yields a p-value < 0.05, a posthoc LSD test is conducted. The data used is primary data, and data processing was carried out using IBM SPSS Statistics V.21. Data is presented in tables and graphs.

Table 1 below shows the comparison of CTX expression among the three study groups on days 7, 14, and 21 based on the observation time.

Tabel 1. Comparison of CTX expression between groups

Group	Day-7		Day-14		Day-21	
	Mean	SD	Mean	SD	Mean	SD
Xenograft	11,33	1,53	8,33	1,53	4,33	1,53
Bonegraft PM	12,67	1,53	8,67	1,53	4,00	1,00
Negative Control	4,33	1,53	6,33	1,53	7,67	1,53
p-value	**0.001***		0.210		**0.031***	

**Significant One way anova (P<0,05)*

On day 7, the mean CTX level in the xenograft treatment group was 12.67 ± 1.53, higher than in the positive control group (xenograft) at 11.33 ± 1.54, and in the negative control group at 4.33 ± 1.53. Statistical analysis using a one-way ANOVA test showed a significant difference in CTX levels among the treatment group (Pinctada maxima bone graft), positive control, and negative control groups, with a p-value < 0.05.

On day 14, the mean CTX level in the Pinctada maxima bone graft group decreased to 8.67 ± 1.53, slightly higher in the xenograft group at 8.33 ± 1.53, and in the negative control group at 6.33 ± 1.53. Statistical analysis using a one-way ANOVA test showed no significant difference in CTX levels among the treatment, positive control, and negative control groups, with a p-value > 0.05.

On day 21, the mean CTX level in the Pinctada maxima bone graft group further decreased to 4.00 ± 1.00, lower than in the xenograft group at 4.33 ± 1.53, and higher in the negative control group at 7.67 ± 1.53. Statistical analysis using a one-way ANOVA test indicated a significant difference in CTX levels among the Pinctada maxima bone graft, xenograft, and negative control groups, with a p-value < 0.05.

Table 2. Post-Hoc LSD test for CTX expression between groups

Group	Day-7			Day-14			Day-21		
	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value
Xenograft Bonegraft PM	11,33 12,67	1,53 1,53	0.326	8,33 8,67	1,53 1,53	0.798	4,33 4,00	1,53 1,00	0.776
Xenograft Negative Control	11,33 7,33	1,53 1,53	**0.001***	8,33 6,33	1,53 1,53	0.160	4,33 7,67	1,53 1,53	**0.025***
Bonegraft PM Negative Control	12,67 4,33	1,53 1,53	**0.001***	8,67 6,33	1,53 1,53	0.111	4,00 7,67	1,00 1,53	**0.017***

*Significant posthoc LSD (P<0,05)

Table 2 shows the comparison of CTX expression between the Pinctada maxima bone graft group and the negative control group on days 7, 14, and 21. There is a significant difference in CTX expression between the Pinctada maxima bone graft group and the negative control group on days 7, 14, and 21 (p < 0.05). However, when comparing CTX expression between the Pinctada maxima bone graft group and the xenograft group on days 7, 14, and 21, there is no significant difference (p > 0.05). A significant difference in CTX expression is observed between the Pinctada maxima bone graft group and the negative control group on days 7 and 21 (p < 0.05). The CTX expression in each group is also displayed in the bar chart below.

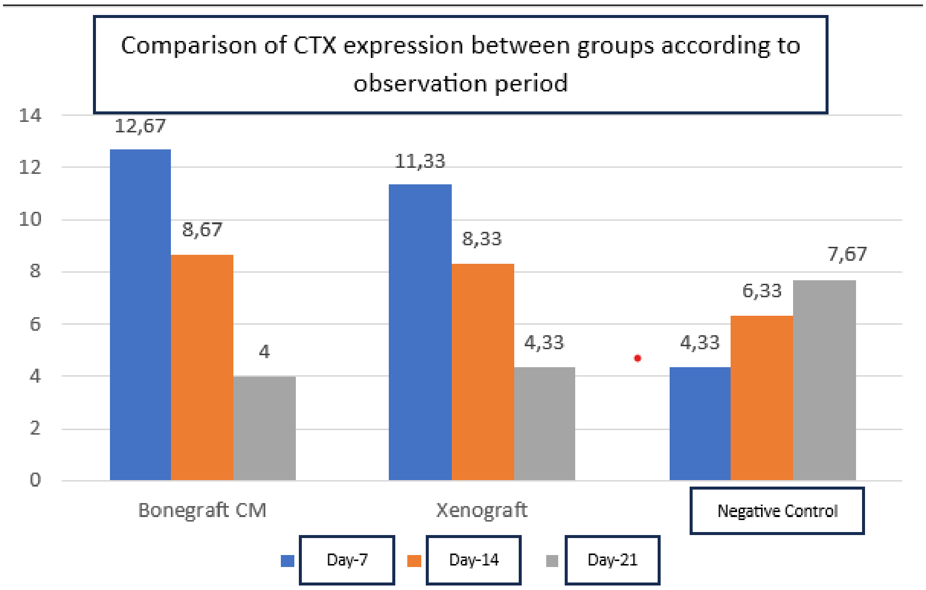

Figure 4 CTX Expression on the 7th, 14th and 21st day

DISCUSSION

Treatment of periodontal condition requires a thorough understanding of its aetiology and pathogenesis, meaning that an understanding in both microorganisms involved and the right treatment are of paramount importance[8,9].

One of the main treatment of periodontitis is regenerative surgery with the purpose of reducing tooth mobility, increasing Clinical Attachment Level (CAL), reducing Probing Depth (PD), reducing bleeding on probing (BOP) and bone level gain. However the true purpose of regenerative treatment is to accelerate bone and periodontal tissue healing rate using the technique of *Guided Tissue Regeneration* (GTR) and *Guided Bone Regeneration* (GBR)[10,11]. These 2 techniques focuses on tissue and bone regeneration respectively, however in terms of bone level gain, GBR is combined with the use of bone grafting materials[12,13].

Calcium carbonate has proven its potential in bone regeneration and thus it can be surmised that it will have a profound effect on bone regeneration. One of the sources of calcium carbonate is the nacre of Pinctada Maxima[14]. The nacre of Wild Pinctada maxima has the advantage in quality in comparison with farm-raised ones, due to the increased of genetic diversity due to its wild nature and thus having more amount of nacre[6]. Another consideration is that the formation of nacre from farm-raised ones, due to its commercial nature in pearl farming, leaves a lot to be desired in terms of calcium carbonate content[16,17].

The quality of the materials used will be helpful in increasing the prognosis of the treatment[18]. Compared to non-regenerative periodontal surgery such as *Open Flap Debridement* (OFD), the usage of regenerative materials effectively increases the end-result of the treatment, namely the formation of new bone or even the closure of the defects entirely. To put it simply, regenerative materials not only increase the prognosis, but also increases the overall quality of the treatment, as demonstrated by reduced PD and increased CAL on OFD combined with regenerative materials[19].

CONCLUSION

Calcium carbonate shows a positive effects in bone regeneration, showed by an decreased CTX activity on the grafted area from the test subjects, furthermore from Micro-CT analysis showed an increase bone density which indicates an increased quality of the newly formed bone.

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