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The Healing Effect of Cuttlefish Bone on Fractured Bone in Rat Model

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Background: Fractured bone healing requires three to nine months, which prolongs the patients' morbidity. Long bone fracture is considered to be difficult due to the dependence of bodily mobility and freedom. Bone tissue engineering materials includes hydroxyapatite (HAp), titanium, alumina, and polymers. HAp is found to be heavily abundant in cuttlefish. This study is conducted to prove that the cuttlebone has an effect of accelerating the fractured long bone healing process. **Methods:** This is an experimental study using a total of 32 samples of *Rattus norwegicus*. The Treatment Group received the cuttlebone extract + 0.9% NaCl while the Control Group received only NaCL 0.9%. Both groups were fractured beforehand. On the 14th day the fractured area was harvested and assigned for histopathology and radiographic exam. **Results:** The Treatment Group was found to have thicker callus formation and more osteoblasts. **Conclusions:** The cuttlebone extract application caused thicker callus and higher osteoblast production, proving an accelerated fractured bone healing process.

Keywords: cuttlebone, fracture, long bone, Rattus norwegicus, callus, osteoblast.

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INTRODUCTION

Bone fracture has a considerable long duration of healing process thus the long bone fracture cases is almost always considered to be very dire since these bone represent major bodily function of mobility and freedom. Fracture due to traffic accident is quite high, and often the fracture occurs particularly on the lower extremities, and in this case, that would be the fracture of the tibia bone. The fracture of the tibia shaft is one of the most common long bone fracture, and also quite common open fractures cases seen.[1,2] Marx explained further added that compared to other fractures, tibia fractures are associated closely with a high incidence of infection, delayed union, non-union, and mal-union.

The length of time needed for the fractured bone to heal varies and in the case of tibia fracture, it will need up to 12 – 16 weeks.[3]

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Looking at this fact, people started wondering whether it's possible to shorten the span of bone healing. Classic bone healing process without any exposure from supporting materials might result in permanent deformation. This is due to the fact that bone is the only tissue of the body which is able to heal perfectly without any connective tissue.

The most common biomaterials for bone tissue engineering include hydroxyapatite (HA), titanium, alumina, and polymers.[4] Cuttlebone was composed of an inorganic part of 92% and an organic part of 8%. The former was CaCO3 of aragonite form and the latter was speculated to be mainly chitin.[5] The aim of the present study is to prove that the cuttlebone has an effect of accelerating the fractured long bone healing process.

MATERIALS AND METHODS

The type of research is a Pure Experimental study involving the injection of the mixture of cuttlebone extract and NaCl 0.9% solution after previously being fractured, reposition and fixated. This research couldn't be conducted on human samples since both the tibia bones, of the samples are going to be purposely fractured. This research is a *Randomized Controlled Experiment* using the

design of *Posttest-only Control Group Design.* Samples comes from male *Rattus Norwegicus* (n=32), which is divided into two groups. Qualified samples are the ones who fitted the inclusion criteria: *Rattus Norwegicus*, wistar, Male, 10-12 weeks, 200-300 gram, right legs. The samples are kept in Tropical Disease Center of Airlangga University, Surabaya; (adapted to their cages 2 weeks).

The ratio of saline used with 0.5 cc of HAp is 1:1 in order to dilute the cuttlebone powder into solution. Therefore 0.5 cc of HAp equals 500 mg with the formulae weight of the HAp is 502.31, which after calculation would make the concentration of 0.5 cc of HAp in 0.5 cc of normal saline is 2 x 10^{-3} M.

The right legs of the samples were incised and later broken internally first. After fractured, the Control Group was injected with NaCl 0.9% only (0.5 cc) while the Treatment Group was injected with cuttlebone extract and NaCl 0.9% mixture (0.5 cc). The leg was repositioned and fixated. After 14 days passed, the samples were sacrificed, the tibia bone healing process is observed, from the callus thickness through X-ray photo and measured by the method of callus index (the measurements are obtained using a regular ruler with the accuracy up to millimeters), while the osteoblasts formation by histochemical examination using Hematoxylin and eosin staining, or H&E staining to be counted on one huge field of vision, choosing the one with the highest density of osteoblast population (Hotspot areas).

RESULT

The data collection method is by primary data. Collected data was analyzed by Independent Sample T-Test; the result of the experiment was explained in the form of tables

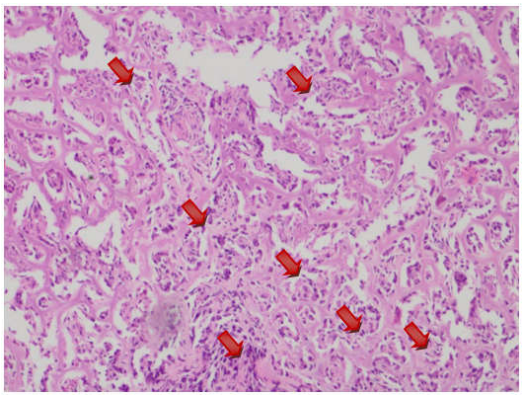

Figure 1
Osteoblast production on the fractured tibia site visible under Microscope 10x with H&E staining from the Treatment Group

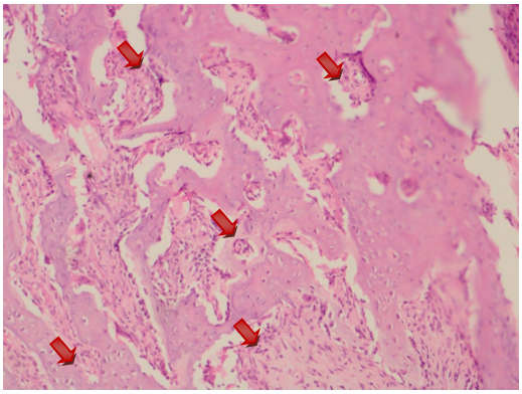

Figure 2
Osteoblast production on the fractured tibia site visible under Microscope 10x with H&E staining from the Control Group

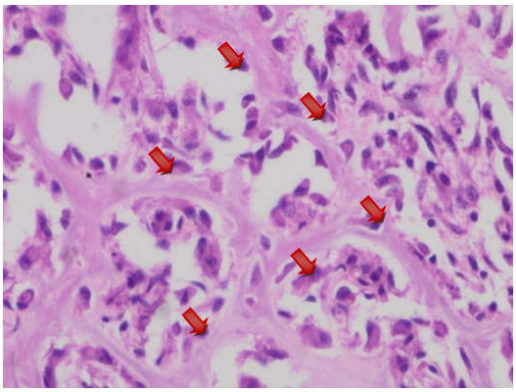

Figure 3
Osteoblast production on the fractured tibia site visible under Microscope 40x with H&E staining from the Treatment Group

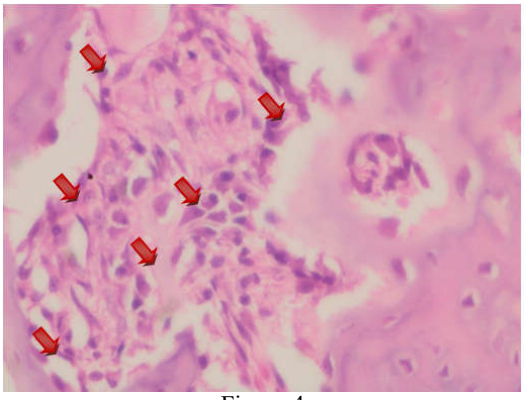

Figure 4
Osteoblast production on the fractured tibia site visible under Microscope 40x with H&E staining from the Control Group

Figure 5. Callus formation from the control (above) and treatment (below) group

Tab 1. Description of both treatment and Control Group of Rattus norwegicus

Variable	NaCL 0,9%	Cuttlefih Bone + NaCl 0.9%	p-value
Callus Index	2.9±0.5	4.6±1.3	p<0.0001
Osteoblast	100.1±46.2	164.6±60.2	0.002

This research used 32 samples of Rattus norwegicus, utilizing the right tibia bone as the research unit. The histopathology examination is conducted to examine the number of osteoblasts on the bone healing process, while the radiologic examination is conducted to obtain the callus index of the callus on the bone healing process.

The group sample which received the cuttlefish bone extract + NaCl 0.9% were statistically higher (4.6±1.3mm) compared to the callus index of the Control Group sample which only received NaCl 0.9% (2.9±0.5mm) with a significance number of p <0.0001. While for the osteoblast result compared between the two groups there's a significance number of p <0.05. This indicates a significant difference between the sample groups which received the cuttlefish bone extract compared to the controlled group.

DISCUSSIONS

The osteoblast produced from the Treatment Group was significantly higher in quantity than the ones from the Control Group. The process of osteoblast generation indicated that the proliferative phase was currently underway, signaling the end of the inflammatory phase.[4-7] This is as accordance to previous research conducted by Hench and Wilson.[8,9] The higher production of osteoblast signified that there was higher proliferative activity of osteoblast. This result showed that the application of the cuttlebone extract (Hydroxyapatite) affected the fractured bone healing process. In the case of higher production of osteoblast, the healing process would be accelerated.

The fibroblasts within the granulation tissue develop into chondroblasts which also form hyaline cartilage.[8] These processes culminate in a new mass of heterogeneous tissue which is known as the fracture callus.[5] To observe the callus thickness, simple Lateral X-Ray was conducted and later in order to measure its thickness, the method of callus index is used, which is a method where callus thickness is defined as the ratio of the maximum callus diameter to bone diameter at the same level as the callus.[11-13] The result of callus thickness in the Treatment Group almost reaches one and half times of difference compared to the Control Group but since observation period took up 2-3 weeks, the observable callus which formed on both groups of samples was limited to only the soft callus, which is correct in the process of bone healing process.[5] The thicker callus formation was the result of higher osteoblast production (the increased proliferation rate) from the application of the cuttlebone extract With this result, it can be said that a high number of osteoblast production means a thicker callus production. The callus that starts as being cartilaginous and fibrocartilaginous acts as a stabilizer the fracture site, an action which then favors bone formation.[14-16]

The fact that the source came from cuttlebone was also advantageous, since cuttlebone from common cuttlefish was considered an organic waste the cost was very affordable and abundantly ready especially in country such as Indonesia which is practically surrounded by the ocean.

CONCLUSIONS

The cuttlebone extract application caused thicker callus and higher osteoblast production, proving an accelerated fractured bone healing process.

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