

# The density of collagen fiber in alveolus mandibular bone of rabbit after augmentation with powder demineralized bone matrix post incisivus extraction

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## ABSTRACT

*The bone defect due to tooth extraction contributes the most cases reported in the aspects of oral surgery. The defect can be preventively managed by adding powder bone matrix intended for augmentation which eventually induces the formation of new bones. This hard tissue wound healing is preceded by the presence of collagen fibers. The aim of this study was to determine the density of collagen fiber in the alveolus mandibular bone of rabbit which was augmented using powder demineralized bone matrix (DBM) post incisivus extraction. Twenty four male rabbits aged 2.5–3 months weighed 900–1,100 grams were randomly divided into two groups. The treated rabbits were augmented with DBM after the incisivus extraction on mandible. The mucosa was then sutured. On the other hand, the controlled rabbits received similar treatments with those of the treated rabbits except there was no augmentation of DBM. Decapitation of treated and controlled rabbits was made on day 5, 7, 10, and 14 days post surgery, each with three rabbits. Mandibles were cut, decalcified, and imbedded in paraffin block. The staining was done using Mallory. Significant differences in the density of collagen were noted on day 10 and 14 post surgery, indicating that powder demineralized bone matrix successfully induced the stimulation of collagen.*

**Key words:** demineralized bone matrix, augmentation, collagen

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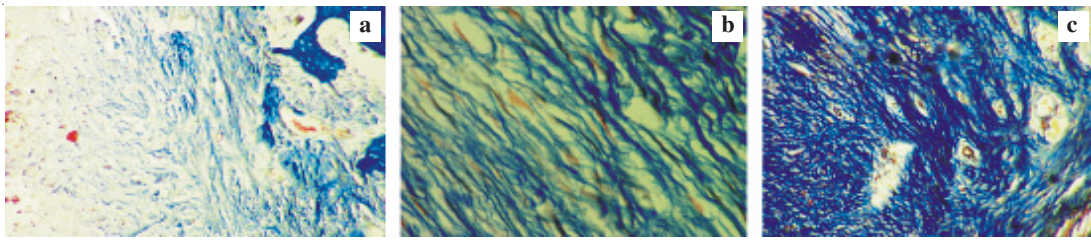
## INTRODUCTION

The advancement of science in developing tissue transplantation has triggered an increase in the need for tissue replacement, so-called graft. The graft is required for either reconstruction or preventing further tissue deterioration on damaged body tissues. It has been reported that the abnormality of bone in the aspect of oral surgery is bone defect, in most cases engendered by tooth extraction.<sup>1</sup> Bone grafting is often essential in providing the best possible set of conditions for the anchorage of implants or augmentation in areas lacking in quality or quantity of bone to induce bone formation.<sup>2</sup> There are heaps of different materials available for the augmentation of the alveolar bone. Freeze dried powder demineralized bone graft or demineralized bone matrix (DBM) is a bone that has been treated with acid, then washed and followed by sterilization using  $\gamma$  (gamma) radiation to help extend shelf life.

The utilisation of that type of allograft was reported effective with less risk of complication.<sup>3</sup> Previous studies show that DBM is able to induce ectopic bone formation in subcutaneous and intramuscular pockets in rodents.<sup>4,5</sup> This bone induction process has been studied extensively.<sup>6,7</sup> Histological and biochemical analyses show that cartilage

appears 5–10 days after implantation of active DBM.<sup>8</sup> Study of Wang<sup>6</sup> reports that this cartilage is mineralized by day 7–14 and subsequently replaced by bone. As DBM-related bone formation can be observed afterwards to occur at ectopic sites, it is assumed that pluripotent mesenchymal are attracted to the site of implantation. The use of allograft powder DBM leads bone tissue to be fully incorporated into patient's tissue by a well established biological mechanism, and in treating bone defects has been proven beneficial for bone regeneration of both animals and humans.<sup>9,10</sup> Demineralized bone matrix (DBM) powder form comprises collagenous and non-collagenous. Urist determined that the osteoinductive fraction could be extracted from the organic component of bone, which he termed bone morphogenic protein (BMP). The BMPs are embedded in bone matrix.<sup>11</sup> In addition to BMPs, other growth factor such as platelet derived growth factor (PDGF) and transforming growth factor (TGF- $\beta$ ) were found.<sup>12</sup>

Wound healing is basically a complex process in which a variety of cellular and matrix components act in concert to re-establish the integrity of injured tissues. The complexity of this process may be simplified into temporal sequence of normal healing: haemostasis, inflammation, cell proliferation (repair), and tissue remodelling. During



**Figure 1.** (a) Score of the collagen fiber density showing the range of low collagen density, (b) Medium collagen density, and (c) High collagen density.

the evolution process, a group of structural protein develops, variously modified in terms of rigidity, elasticity, and strength levels, depending on the environmental influence and functional needs known as collagen. For instance, collagen on skin, bone, cartilage, and basal membrane. Collagen is protein mostly found in humans and reaches 30% of the dry weight. The collagen consists of glycine amino acid (33.5%), prolin (12%), hidroxy prolin (10%), and the remaining substances such as other amino acids. Type I collagen is the most spread one, found in the tissue as a classical structure renowned as collagen fiber. These fibers support bones, dentin, tendon dermis, etc. Type II collagen is mostly found in hyaline and elastic cartilage, whose shape is fibrous and very smooth.<sup>13</sup> On day 7 in the process of wound healing on bone, the synthesis of type II collagen appears whilst type I collagen generally prevails on day 10–12.<sup>7</sup> When observed by light microscope, the collagen fiber is acidophilic, appears to be pink by Van Geyson and blue by Mallory trycom. Collagen fibers comprise thick fibers solidly suppress each other with diameters approximately 75nm in mammals. In many parts of body, collagen fibers are conceived in parallel series forming collagen bundles.<sup>13</sup> The objective of this study was to determine the density of collagen fibers on alveolus mandibular bone of rabbit which was augmented using powder DBM post-incisivus extraction.

#### MATERIALS AND METHODS

This study was done pure experimentally using post-test only group control design. Twenty-four male rabbits aged 2.5–3 months with weights of 900–1,100 grams were randomly divided into two groups. The first treated group I was augmented by powder DBM (370–710  $\mu$ m) post-incisivus extraction, whilst the other group was not augmented, hence used as a controlled group. Demineralized bone matrix (DBM) in the form of allograft made from cortical bone of rabbit was prepared by the Bank of Tissue Dr Soetomo RSUD Dr. Soetomo, Surabaya. The rabbits were injected with intra muscular with penobarbital sodium 100 mg/kg body weight; then after they were calm and sleepy, they were injected with 0,2ml/kg body weight of 2% Lidocaine HCl (Phapros, Semarang) on incivus tooth

labial. After the extraction ended, the wounds were cleaned by sterilized cotton, and gingival were pressed to control bleeding problem. Subsequently, they were immediately augmented by DBM that has been mixed with the solution of lactate ringer. DBM was pressed to get in and fill the tooth sockets on mandible. Mucosa crease was then sutured by non-absorbable suture No. 5 (Ethyon silk suture). Analgesic (250 mg) was also included into the liquid of ad libitum 250 ml only for two days after surgery. The food given was pellet (Japfa Comfeed, Indonesia) and aquadest.

On days 5, 7, 10 and 14 post-surgery, three rabbits from each group randomly took to be decapitated. Mandible as wide as incisivus tooth was cut and submerged into the solution of Lylis as the fixative solution and decalcification for 3–5 days. Then the process was followed by 70% of alcohol as bone submersion solution for three days. Subsequently, the bone was vertically cut in line with tooth axis using microtome and was imbedded in paraffin block. Furthermore, the tissue was again cut as wide as 6 mm and put on object glass and processed for Mallory staining. The observation on collagen fiber density was conducted on ten view fields under light microscope with the 4x 100 objective. Criteria for valuation are: Score 1 shows that the density of collagen fiber is low (Figure 1-a); score 2 depicts that collagen fiber is medium (Figure 1-b); and score 3 shows very high density (Figure 1-c). In order to analyse the density data of the collagen fibers, Mann-Whitney Test was utilized.

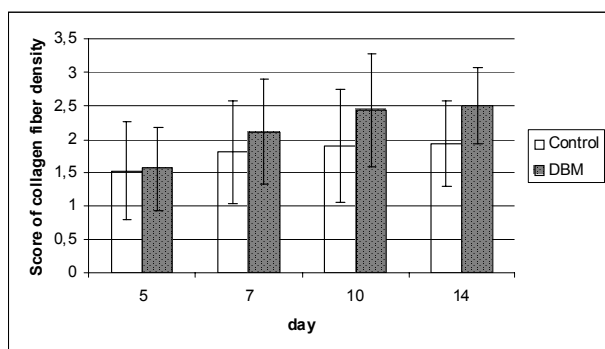
#### RESULTS

Table 1 below shows the mean and standard deviation of the collagen fiber density of mandibular bone for each group. The pattern of collagen fiber density was summarized in Figure 2.

General description shown in Figure 2 indicates that on day 7, collagen fiber density of DBM group was higher (denser) than that control group (without augmentation). Meanwhile, histological description depicts that the formation of collagen fiber in DBM-augmented group is getting higher on day 7 after being wounded although histologically there has yet to show the prevalence of collagen bundle. This phenomenon is of difference from

**Table 1.** Mean and standard deviation of the density of mandibular fiber collagen on control and DBM groups

Time after augmentation	N	Control	DBM
		$\bar{X} \pm SD$	$\bar{X} \pm SD$
Day 05	30	1.53 ± 0.73	1.56 ± 0.62
Day 07	30	1.80 ± 0.76	2.11 ± 0.79
Day 10	30	1.90 ± 0.84	2.43 ± 0.67
Day 14	30	1.93 ± 0.63	2.50 ± 0.57

**Figure 2.** The increase in density of collagen fiber on the mandibular bone post surgery based on observation day of control and DBM groups.

that on day 5, showing that collagen fibers of the two groups are relatively few with similar mean values and without twist of collagen fiber. On day 10 after being wounded, DBM group shows the existence of collagen fiber bundle which appears to be denser after 14 days of treatment. On the other hand, the controlled group has also shown collagen bundle but the distance is not as dense as that of DBM group. Subsequently, the mean rank of collagen fiber density in each group predicated upon treatment days is described in Table 2.

The difference significance of collagen fiber density of mandibular bone is graphed in Table 3. As seen, there was no significant difference of collagen fiber density on day 5 and 7 after the extraction wounds. The difference was significant on day 10 and 14 in the wake of DBM

**Table 2.** Mean rank and sum of ranks of the density of the mandibular collagen fiber on control and DBM groups

Group	Time after augmentation	N	Mean Rank	Sum of Ranks
Control	Day 5	30	29.60	888.00
DBM		30	31.40	942.00
Control	Day 7	30	26.70	801.00
DBM		30	34.30	102.90
Control	Day 10	30	25.25	757.50
DBM		30	35.75	1072.50
Control	Day 14	30	23.78	713.50
DBM		30	37.22	1116.50

Note: DBM = powder demineralized bone matrix.

**Table 3.** Mann-Whitney test of the density of the mandibular collagen fiber on control and DBM groups

Time after Augmentation	Assym. Sig (2-tailed)
Day 05	0.653
Day 07	0.73
Day 10	0.013**
Day 14	0.010**

augmentation. The results indicate that powder demineralized bone matrix induces the collagen fiber stimulation after the augmentation on rabbit alveolus mandibular post- incisivus extraction.

## DISCUSSION

Results of this study tend to indicate that DBM powder of allograft can induce bone collagen fiber stimulation on mandible in post-extraction period. This fact substantiates previous studies that also show the capability of DBM of inducing collagen stimulation.<sup>7,14</sup> Since the initial studies conducted by Urist<sup>4</sup> the osteoinductive capacity of DBM has been well established.<sup>15</sup> DBM is produced by the extraction of human cortical bone and the components of the bone that remain behind including the non-collagenous proteins; bone osteoinductive growth factors, the most significant of which is BMP, and the type I collagen.<sup>14</sup> DBM in this study is allograft in the form of freeze dried powder which could be stored at room temperature and is sterilized using  $\gamma$  (gamma) ray. These processes yield a dry granular material that consists of collagen matrix in which BMPs and other insoluble growth factors are embedded. This condition is also in line with the use reported as effective grafting materials.<sup>3</sup>

The major phases of the osteoinduction are chemotaxis, mitosis, and differentiation.<sup>16</sup> Chemotaxis may be defined as the directed migration of cells in response to a chemical gradient. Implantation of allograft DBM powder promotes chemotaxis of cells to vicinity. Plasma fibronectin binds avidly to the implanted bone matrix.<sup>17,18</sup> Fibronectin is a protein that has affinity for collagen, fibrin, and heparin, the major components in the site of any skeletal trauma. It is well known that peptides of fibronectin are chemotactic and perhaps mitogenic.<sup>7</sup>

The major constituents of the demineralized bone matrix (powder) are collagen and other matrix proteins. The mineralization process that occurs in hard tissues of the body relies upon this extracellular matrix. It has generally been agreed that fibrillar collagen, the primary component of this matrix, has the capacity to serve as the structural framework in tissue that is undergo mineralization.<sup>19</sup>

One of the bone healing parameters is the growing collagen fibers. This study's results indicate a significant difference of collagen fiber density on day 10 and 14 after the tooth extraction. It is apparent that collagen fiber

density, supposed to be type I and II on DBM-augmented alveolus, is denser. Collagen fibers on mandibular alveolus are mostly type I and II. This condition is in line with the study of Reddi *et al.*<sup>7</sup> reporting that the synthesis of type I collagen prevails between day 10 and 12 in the process of bone induction of DBM implantation. Bone matrix consists mainly of collagen fibers (approximately 90%) and non-collagenous proteins. Type I collagen, which plays structural as well as morphogenic roles and provides scaffolding for mineral crystals, is the major species roles and within lamellar bone, the fibers are forming arches for optimal bone strength.<sup>20</sup> Accordingly, allograft powder of DBM as the augmentation materials is capable of hastening bone reconstruction by inducing biosynthesis of collagen fibers stimulation for bone strength.

The effect of bone healing augmented by powder allograft of DBM is possibly caused by several growth factors that it contains, such as BMP, PDGF, GDF and TGF- $\beta$ 1, that synergically cooperating with local recipient cells that influence the proliferation of cells involved in bone healing. The main action of BMPs is to commit undifferentiated pluripotential cells to differentiate into cartilage and bone-forming cells.<sup>21,22</sup> BMPs are basically abundant in bone and belong to the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily, which consists of a group of related peptide growth factors.<sup>23</sup> The mechanisms of polypeptide growth and differentiation factors (GDFs) on the repair and regeneration of tissues have been shown to have pleiotropic effects on wound repair in nearly all tissues.<sup>22,24</sup> The expression of various GDFs following bone and soft tissue injury may regulate the repair and or regenerative process.

Previous studies have shown that acidic and basic FGF are found in bone matrix<sup>25</sup> and *in vitro*; both forms stimulate DNA synthesis and cell replication.<sup>26</sup> Of significance, the bFGF potently stimulates angiogenesis that is critical for the vascular invasions of bone.<sup>27</sup> TGF- $\beta$ 1 has itself shown to be a strong promoter of extracellular matrix production in many cell types. The effects of TGF- $\beta$ 1 appear to be highly dependent upon bone cell source and local environment.<sup>28</sup> This growth factor stimulates type I collagen, fibronectin, and osteonectin biosynthesis as well as bone matrix deposition and chemotaxis.<sup>29</sup> This study's results substantiate that of Conran *et al.*,<sup>30</sup> finding that the collagen synthesis in the healing process is influenced by some factors such as PDGF, FGF, TGF- $\beta$ , and cytokins IL-1 and IL-4 secreted by leukocyte and fibroblast.

The conclusion of the stimulation of collagen synthesis on alveolus of rabbit mandibular bone post-incisus extraction has proven significantly induced by demineralized bone matrix augmentation. The density of collagen fiber per se is of significant difference on day 10 and 14 after the augmentation of demineralized bone matrix post incisus extraction.

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