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Dengan ini saya kirimkan naskah paper saya dengan judul "The increasing of Bone morphogenetic protein-2 (BMP-2) using chitosan gel with different molecular weight on wound healing process of dental extraction".

Salam hormat,
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(Staf biomaterial FKG UHT, Surabaya)



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Fakultas Kedokteran Gigi Universitas Hang Tuah
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Surabaya

Kami beritahukan bahwa naskah Sejawat dengan judul :
The Increasing of Bone Morphogenetic Protein-2 (BMP-2) using Chitosan Gel with Different Molecular Weight on Wound Healing Process of Dental Extraction

telah kami telaah melalui tinjauan Penyunting Dental Journal (Majalah Kedokteran Gigi). Agar naskah tersebut dapat kami proses lebih lanjut, Sejawat dimohon melakukan perbaikan sesuai dengan catatan penyunting yang tertulis pada naskah.

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Ketua Penyunting Dental Journal (Majalah Kedokteran Gigi)

Ketut Suardita, drg., Ph.D., Sp.KG
NIP. 196811261993031002

3 Attachments

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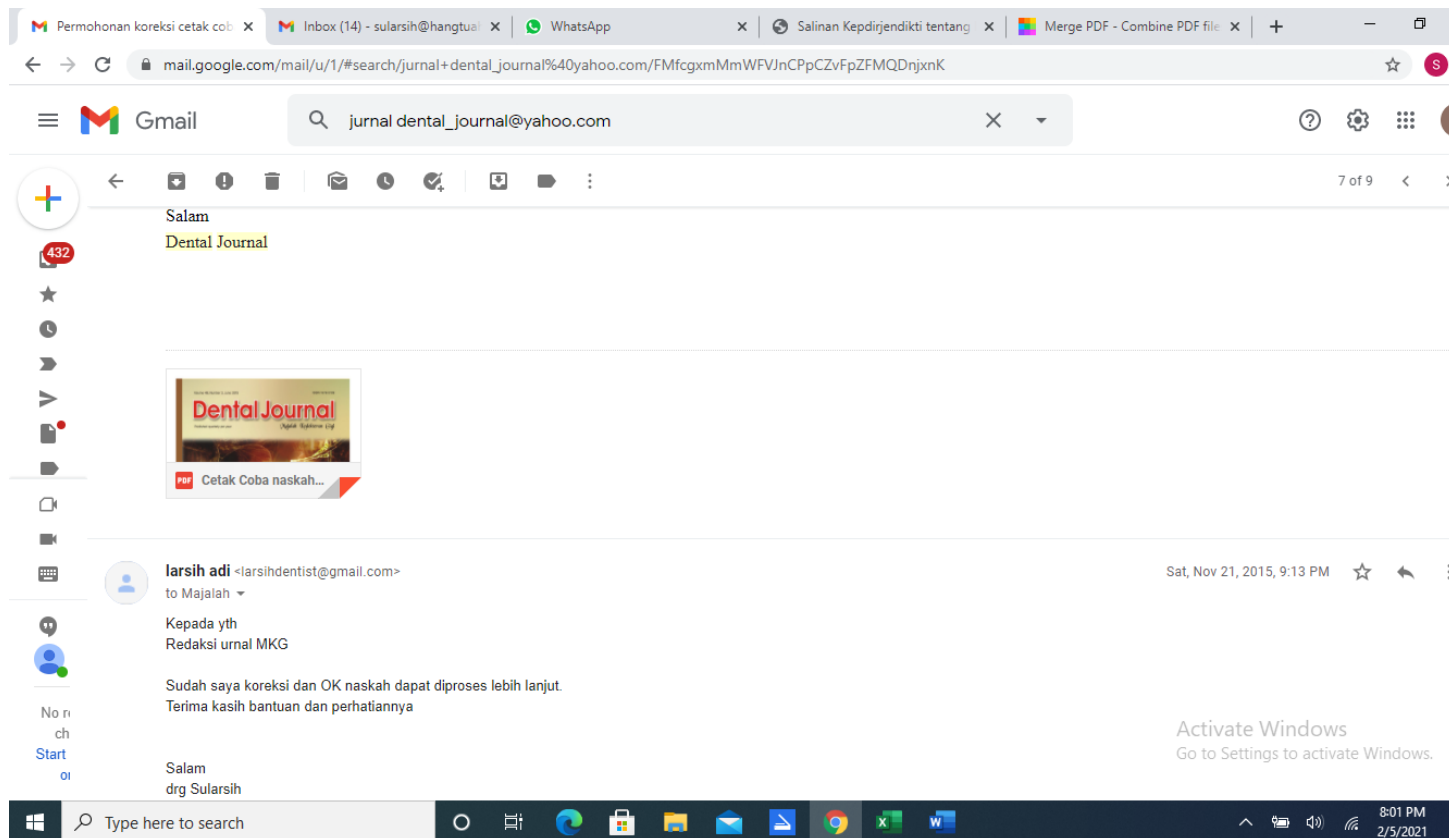
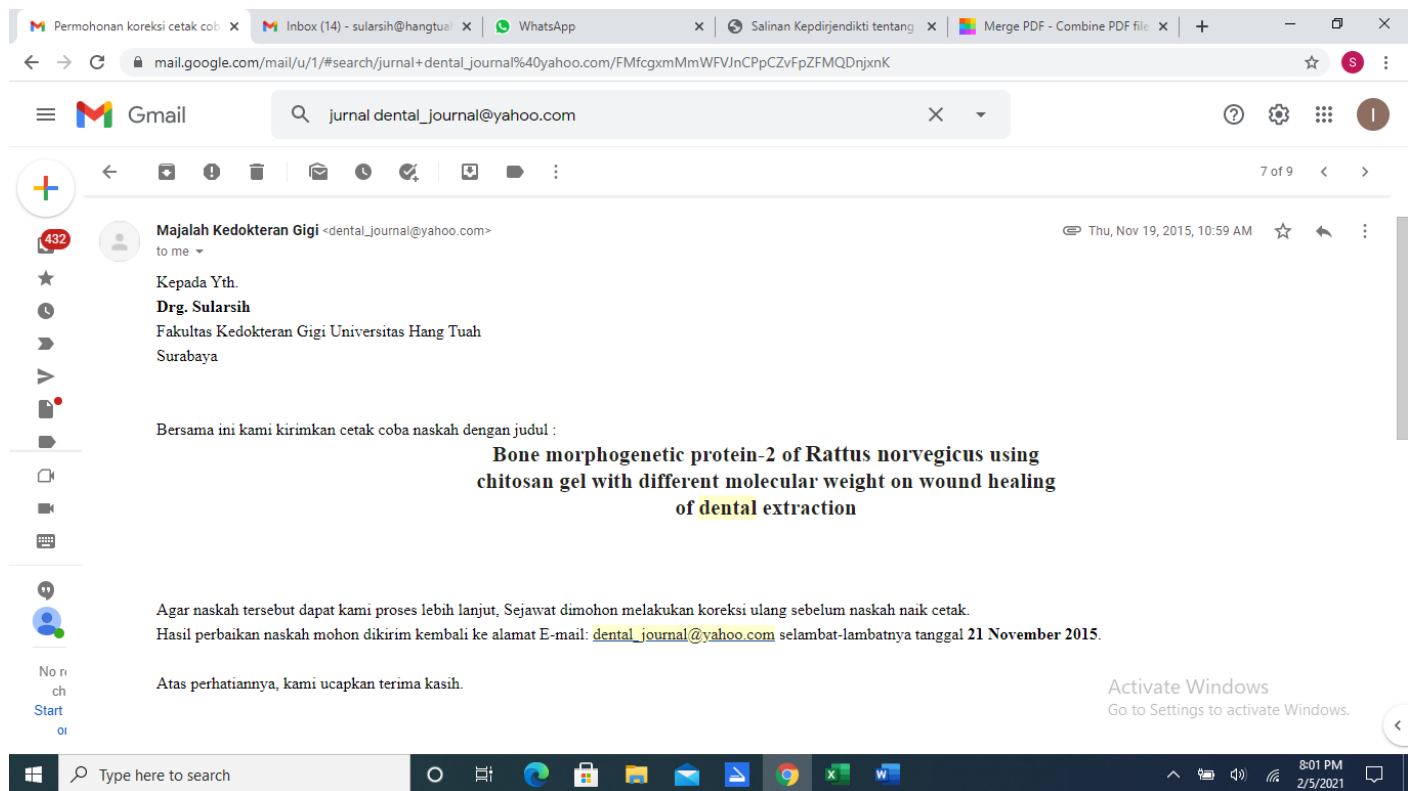
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FORMAT PENILAIAN NASKAH DENTAL JOURNAL HASIL PENELITIAN (untuk Penyunting Ahli)

Judul Naskah : The Increasing of Bone morphogenetic protein-2 (BMP-2) using chitosan gel with different molecular weight on wound healing process of dental extraction

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Tanggal Kembali ke Redaksi : 8/2015

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c)	Metode dan rancangan penelitian sesuai dengan tujuan penelitian ? <u>Keterangan:</u> 		
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e)	Hasil penelitian dapat menjawab <i>research question</i> ? <u>Keterangan:</u> 		
f)	- Pembahasan tidak mengulang hasil ? - Selaras dengan lingkup penelitian dan dibandingkan dengan hasil penelitian sejenis ? - Menerangkan makna hasil penelitian dalam menjawab permasalahan ? <u>Keterangan:</u> 		
g)	Acuan selaras dengan materi penelitian dan menggunakan literatur 10 tahun terakhir? <u>Keterangan:</u> 		

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i) Pustaka perlu ditambahi/ dikurangi**)? <u>Keterangan:</u> 		
5. Apakah ada bagian yang perlu ditambahi/ diringkas**)? <u>Keterangan:</u> -		

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✓ *rattus norvegicus*

The Increasing of Bone morphogenetic protein-2 (BMP-2) using chitosan gel with different molecular weight on wound healing-process of dental extraction

ABSTRACT

Background: Bone morphogenetic protein-2 (BMP-2) is bone stimulator which capable of inducing differentiation of mesenchymal cells into osteoblast, stimulating bone formation in remodeling process. Chitosan is polymer composed N-acetyl-D-glucosamine unit that has been used in various applications in wound healing process and bone tissue engineering. **Purpose:** The objective of this research was to analyzed expressions of BMP-2 for 7,14 and 21 days after using chitosan gel with different molecular weight on wound healing process of dental extraction. **Methods:** The research was an experimental laboratory study. *Rattus norvegicus* strain wistar male, aged 8-16 weeks, divided into 3 treatment groups namely group I and II which given chitosan gel 1 % with high and low molecular weight and group III as control which were not given chitosan gel. Chitosan gel were applied into the socket of dental extraction. Rat was decapitated 7,14 and 21 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination to observe Bone morphogenetic protein-2 (BMP-2). Data were analyzed using ANOVA test. **Results:** The result of this research showed significant differences on BMP-2 for 7,14 and 21 days observation ($p < 0,05$). The increasing of BMP-2 were found in the group which given chitosan gel with high molecular weight. **Conclusion:** It may be concluded that The using of chitosan gel with high molecular weight can enhance the expresion of BMP-2 on wound healing process of dental extraction.

Key words: Chitosan, Bone morphogenetic protein-2, wound healing

ABSTRAK

Latar belakang: Bone morphogenetic protein-2 (BMP-2) adalah stimulator pada tulang yang memiliki kemampuan menginduksi diferensiasi sel mesenkimal menjadi sel osteoblas dan menstimulasi pembentukan tulang pada proses remodeling. Kitosan adalah polimer yang memiliki komponen unit N-acetyl-D-glucosamine yang dapat digunakan pada proses penyembuhan luka dan rekayasa jaringan pada tulang. **Tujuan:** Penelitian ini bertujuan untuk menganalisa ekspresi BMP-2 pada pengamatan 7,14 dan 21 hari pada proses penyembuhan luka pencabutan gigi dengan menggunakan kitosan gel yang memiliki berat molekul yang berbeda. **Metode:** Penelitian ini merupakan penelitian eksperimental laboratoris. Sampel menggunakan tikus *Rattus norvegicus* strain wistar, jantan, umur 8-16 minggu, dibagi menjadi 3 kelompok perlakuan yaitu kelompok I dan II: kelompok perlakuan dengan pemberian kitosan gel 1 % yang memiliki berat molekul yang berbeda dan kelompok III adalah kelompok kontrol, tanpa pemberian kitosan gel. Kitosan gel diaplikasikan pada soket gigi. Sampel didekapitasi setelah 7,14 dan 21 hari pemberian perlakuan, kemudian dilakukan pemotongan rahang mandibula dan pemeriksaan imunohistokimia untuk menganalisa ekspresi BMP-2. Data dianalisa dengan uji ANOVA. **Hasil:** Terdapat perbedaan

yang signifikan pada ekspresi BMP-2 dengan pengamatan 7,14 dan 21 hari. Ekspresi BMP-2 lebih banyak ditemukan pada kelompok perlakuan dengan kitosan gel yang memiliki berat molekul tinggi. **Kesimpulan:** Penggunaan kitosan gel yang memiliki berat molekul tinggi dapat meningkatkan ekspresi BMP-2 pada penyembuhan luka pencabutan gigi.

Kata kunci: Ktosan, Bone morphogenetic protein-2, penyembuhan luka

INTRODUCTION

Bone morphogenetic protein-2 (BMP-2) has been widely used as an effective growth factor in bone tissue engineering. It is a member of the transforming growth factor- β (TGF- β) superfamily of multifunctional cytokines which has remarkable ability to induce bone formation and bone tissue reconstruction. It plays critical roles in osteogenesis and bone metabolism.¹ The bone matrix is rich in growth factors, among which Bone morphogenetic proteins (BMPs), that are synthesized and secreted by osteoblast and incorporated into the matrix during bone formation. The BMPs, released during osteoclastic bone resorption, are capable of inducing differentiation of mesenchymal cells into osteoblasts (osteinduction), stimulating bone formation in both remodeling and repairing process.^{1,2}

Chitosan is carbohydrate copolymer composed of glucosamine and N-acetyl-D-glucosamine units. Chitosan has a number of properties including biocompatibility, biodegradability, mucoadhesiveness and wound healing capabilities that make it useful as a biomaterial.³ Chitosan is very versatile and can be prepared as films, gels, sponges and other forms and has been used in various applications including wound healing and bone tissue engineering.^{2,3} Biological activity of chitosan depend on their molecular weight and deacetylation degree. The degree of deacetylation and molecular weight are important parameter, which could influences the performance of chitosan in many its application.^{4,5} !

In recent study, chitosan is able to increase transforming growth factor beta 1 (TGF β 1), Platelets release transforming growth factor (PDGF), fibroblasts growth factor 2 (FGFS2), Bone morphogenetic protein expression of mRNA on the seventh day.^{6,7}

Bone morphogenetic protein-2 (BMP-2) has been widely investigated for bone healing due to its properties. BMP-2 recruits stem cells to the bone healing, promotes angiogenesis and causes differentiation of stem cells into osteoblast.^{3,6} The increasing expression of BMP-2 could be achieved by using chitosan. The aim of this study was to

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analyzed expressions of BMP-2 for 7,14 and 21 days after using chitosan gel with different molecular weight on wound healing process of dental extraction.

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MATERIALS AND METHODS

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The material in this experiment were Chitosan powder purchased from Sigma chemical, St. Louis, USA. The degree of deacetylation was more than 75 %. Chitosan with high molecular weight (Product number: 419419, Lot number: MKBH5816V) and chitosan with low molecular weight (Product number= 448869, Lot number= MKBH7256V), asetat acid 2 % p.a (Merck, Germany), buffer formalin 4% and 10% , ketamin (Ketalar,Pfzer), xylazine, alkohol 80%, alkohol 95 %, alkohol 100 % (absolute), xylene, buffer Parafin, EDTA 10 % (JT Baker, USA), NaSO₄ 2 % (Merck, Germany), PBS, Tripsin 0,125 %, H₂O₂ 0,5 %, methanol (Merck, Germany), NaOH 1,25 % and Bone morphogenetic protein-2 monoclonal antibody. The tools used in this experiment were Becker glass, Stirrer, pipette pasteur, Autoclave (Foundry), 5 cc syringe injection (Terumo), 1 cc syringe tuberculin (Terumo), pinset, elevator, Needle holder, non resorbable silk sutures, Bekker glass, Incubator memmert (W Germany), Rotary microtome, Label, slide, cover glass, petri disk Poly-L-lysine, deck glass and mikroskop trinokuler (Olympus CX 31 Japan).

Chitosan gel 1 % (w/v) was made with diluted one gram of chitosan powder in acetic acid 2 %. It added with NaOH 1,25 % solution to get neutral pH. The mixture was stirred until the gel was completely formed. After homogenization, the gels were stored in closed containers at ambient temperature until use. The characteristic of chitosan gel was evaluated includes solubility, pH, viscosity, physical characteristic, homogeneity, consistency, and duration of storage time. The homogeneity test of gel carried out using glass plates after the powder diluted in acetic acid 2 %. It should be observed on optimized homogeneous. Consistency test could be done by using a penetrometer or mechanically sentrifugator. Gel without precipitation will produce a good consistency. Physical characteristic test or Organoleptic analysis during the storage time includes change of colour, form of formulation gel and odorless. Measurement of viscosity using viscometer Ostwald (kapiler method).^{8,9}

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The research was an experimental laboratory study. Rattus norvegicus strain wistar male, aged 8-16 weeks, divided into 3 treatment groups namely group 1 and II which given chitosan gel 1 % with high and low molecular weight and group III as control which were

—bnp vol. 79 amaturkan dlu socket?

not given chitosan gel. Chitosan gel were applied into the socket of dental extraction. Rat was decapitated 7,14 and 21 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination to analyze expression of Bone morphogenetic protein-2 (BMP-2). Fixation was performed using 10 % buffer formalin and decalcification applying EDTA. Further process was dehydration and continued by clearance. The tissue could be cut using microtome in 4-6 μm thickness. Deparaffin and rehydration were subsequently performed. Bone morphogenetic protein-2 monoclonal antibody was diluted by antibody diluents. Next, it was washes by PBS. Streptavidin-biotin was dropped and incubated for 30 minutes, washed by PBS. Counterstained using haematoxyline and washed by flowing water and dried. It was given entelan and covered by cover glass. Light microscope was applied and the evaluation was done. The measuring result were analyzed using ANOVA test. It analyzed the comparison between chitosan treated with high molecular weight group, lower molecular weight group and the control groups ($P < 0,05$).

RESULTS

The expressions of Bone morphogenetic protein-2 (BMP-2) for 7,14 and 21 days observation after using chitosan gel with different molecular weight on wound healing process of dental extraction shown in figure 1, 2 and 3. The region which would be evaluated and observed in third apical socket. The expressions of Bone morphogenetic protein-2 (BMP-2) in third apical socket was done 400 times magnification in all sub groups.

In our study, the expressions of Bone morphogenetic protein-2 (BMP-2) on wound healing process of dental extraction using chitosan was more increasing compared to the control group. The expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days observation after using chitosan gel with high molecular weight was more increasing than the other groups.

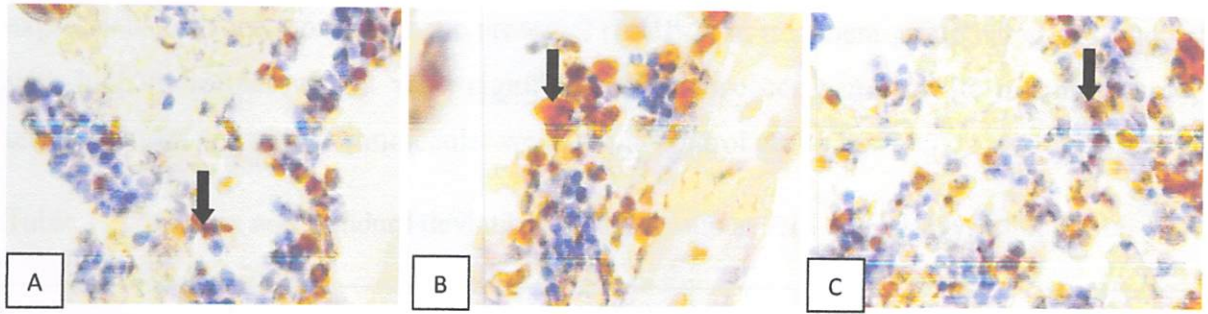


Figure 1. The expressions of Bone morphogenetic protein-2 (BMP-2) at 7 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight. *pembesaran?*

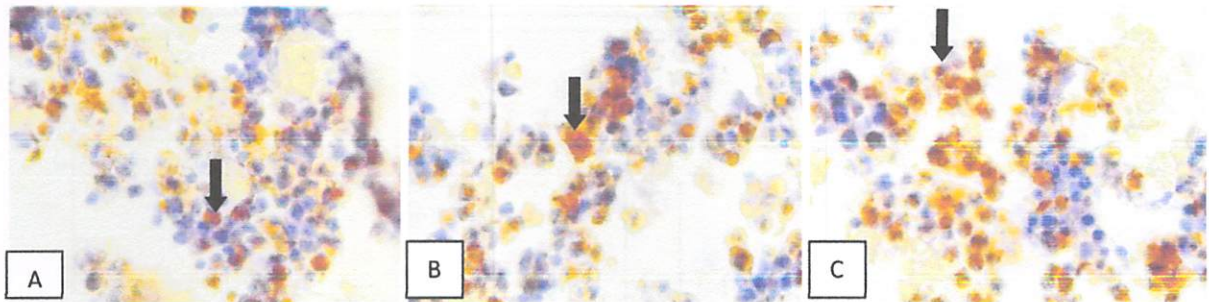


Figure 2. The expressions of Bone morphogenetic protein-2 (BMP-2) at 14 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight. *pembesaran*

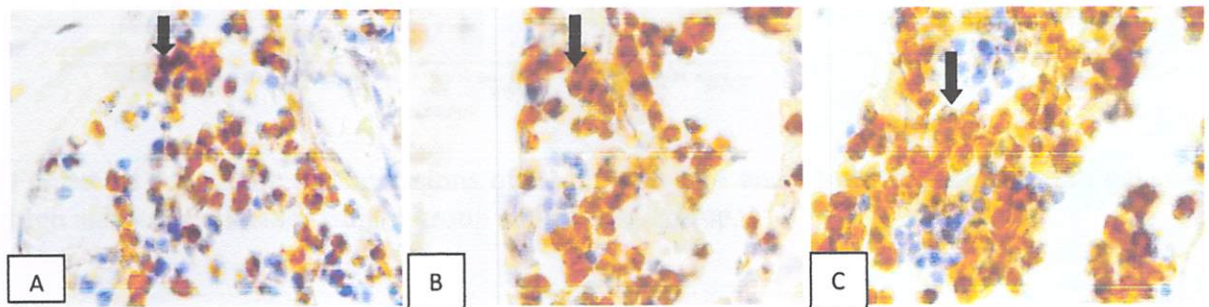


Figure 3. The expressions of Bone morphogenetic protein-2 (BMP-2) at 21 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight. *pembesaran*

Table 1 and figure 4 shown the mean and standard deviation of each group from immunohistochemistry study done in 7,14 and 21 days after treatment. The expressions of Bone morphogenetic protein-2 (BMP-2) for 7,14 and 21 days observation after using chitosan gel with different molecular weight on wound healing process of dental extraction. The data was analyzed using kolmogorov-smirnov statistical test. It showed normal distribution ($p>0.05$) in which fulfilling the requirement of parametric test. The ANOVA test showed,

expressions of Bone morphogenetic protein-2 (BMP-2) in treatment group using chitosan gel with high molecular weight were significant difference compared to the treatment group using chitosan gel with low molecular weight and control group ($p < 0.05$). uji LSD ?

Table 1. The mean and standard deviation of each group at 7, 14 and 21 days after treatment BMP-2

Variable	Treatment	7 days	14 days	21 days
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
The expressions of BMP-2	Control	11,17 $\pm$ 0,75	13,57 $\pm$ 1,81	11,50 $\pm$ 1,22
	Chitosan, High BM	18,25 $\pm$ 4,42	21,33 $\pm$ 2,67	19,29 $\pm$ 4,19
	Chitosan, low BM	11,80 $\pm$ 1,92	14,88 $\pm$ 1,45	14,00 $\pm$ 2,53

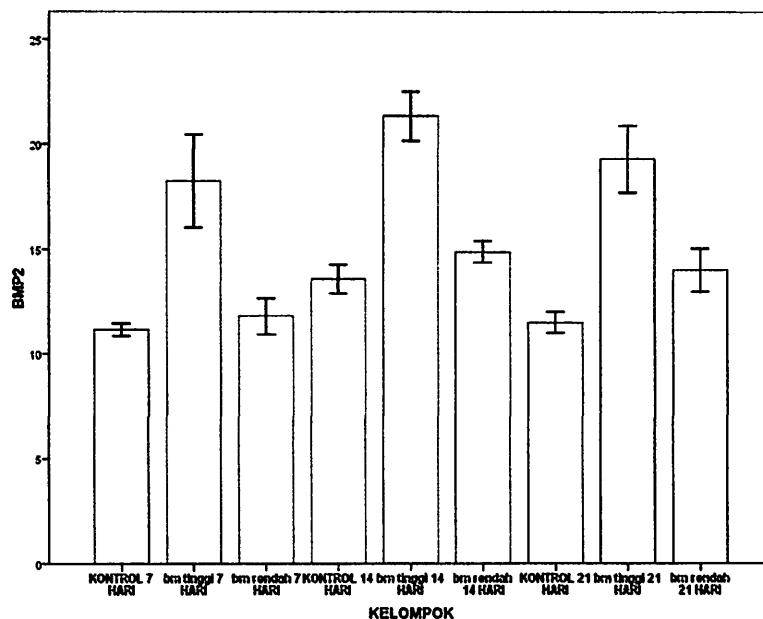


Figure 4. The graphic of expressions of BMP-2 on 7, 14 and 21 days using chitosan gel with high and low molecular weight group and control group.

DISCUSSION

The proliferation and differentiation of sufficient progenitor cells are critical for bone healing. These processes are regulated by growth factors such as TGF- β and BMPs. BMP-2 can efficiently induce bone formation. Many materials, especially the natural polymers, have been researched for controlled release of BMP-2.^{10,11} Chitosan is naturally derived polysaccharide. It has gained much attention as biomaterial in tissue engineering application, due to its antimicrobial activity and biocompatibility.¹⁰

Chitosan is reported to be biocompatible, biodegradable and non toxic, having some biomedical properties such as healing accelerator, antifungal, hemostatic, antimicrobial and analgesic. Several study have shown the activity of chitosan in healing process, where it enhanced the infiltration of inflamamatory cells in the injured area and release growth factor.^{6,12} Chitosan is the partially deacetylated form poly-(1,4)-2-amino-2-deoxy-D-glucose) of chitin, and is a potential biomaterial for bone tissue engineering application.^{1,10,12} In the present study, the chitosan powder was mixed with blood of each person and filled in dental socket, and it found that bone tissue regeneration will be faster in chitosan-filled socket than untreated dental socket.¹³

In our study it was used chitosan gel material with different molecular weight to analyzed expressions of BMP-2 on wound healing process of dental extraction. It showed the expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days ^{mencara} observation ^{pd kel 105} after using chitosan gel was increasing. Chitosan is a polycationic complex carbohydrate with a structure similar to those of glycosaminoglycans specifically hyaluronic acid. Hyaluronic acid is thought to facilitate the migration and proliferation of progenitor cells. Previous studies have demonstared that chitosan might function as a substrate that enhances the migration and differentiation of osteoblast cells and conversely, it might inhibit the function of fibroblast and so indirectly facilitate osteogenesis.^{14,15} Its biological properties of chitosan, including chitosan biodegradation by lysozim that can change chitosan polymer form (N-acetyl-D-glucosamine) to dimer active form. N-acetyl-D-glucosamine dimer active form cross-linked with glycosaminoglycan and glycoprotein that part of matrix macromolecules extracellular as well as stimulate increased Transforming growth factor betha (TGF- β) and Bone morphogenetic protein (BMP) expression of mRNA.^{6,15} In study by Ezzodini ^{nama tidak diketahui} reported that chitosan loading rhBMP-2 can facilitate bone regeneration of periodontal tissue. In the cavities filled with chitosan, not only the bone regeneration was faster, but also the density was similar to the density of the bone of the subject under study.¹³

According to the figure 4, the expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days observation after using chitosan gel with high molecular weight was more increasing than the other groups. Chitosan was the material used to form gels due to its good gel forming property, biodegradability, biocompatibility and non toxic. Gels could be considered as ideal pharmaceutical forms to treat the dental socket. It have the ability to absorb exudates, which moisture on the wound surface. Besides, gels have high water vapor and oxygen permeability, as well as mechanical properties that resemble physiological soft

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tissue. Chitosan gel has a strong tissue-adhesive property.^{12,17,18} The characteristic of chitosan is related with its molecular weight. The absorption mechanism also depend upon the particle size of chitosan powder.^{17,18} The viscosity of chitosan gel with high molecular weight more higher than formulation with low molecular weight. It have high viscosity and better mucoadhesive properties due to strong blood clot is formed in order to eliminate complications of dental extraction such as dry socket. However, it can promote the acceleration of wound healing process on dental socket.^{19,20}

BMP-2 can efficiently induce bone formation. The increasing of expression BMP-2 can increasing Alkaline phosphatase (ALP) activity. ALP is an enzyme regarded to be important in the process of mineralization, since high levels of ALP would promote hydrolysis of phosphate, produce orthophosphate and increase the calcium deposition. ALP activity and calcium deposition are widely used as marker for early and late differentiation of osteoblast cells, respectively.¹⁰ Mature osteoblast produce mineralized bone matrix, and the component of this matrix, such as calcium deposition and osteogenic markers (osteocalcin and osteopontin), are usually used as markers for late stage of osteoblast differentiation. Osteocalcin and osteopontin expression marks the late stage of osteoblast differentiation, and BMP-2 is an important factor for osteoblast maturation. Therefore, these result indicated that the increasing of BMP-2 enhanced the osteoblast activity.^{3,10,15} It may be concluded that The using of chitosan gel which increasing the expression of BMP-2 can accelerate bone healing process of dental extraction.

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FORMAT PENILAIAN NASKAH DENTAL JOURNAL HASIL PENELITIAN (untuk Penyunting Ahli)

Judul Naskah : The Increasing of Bone morphogenetic protein-2 (BMP-2) using chitosan gel with different molecular weight on wound healing process of dental extraction

Tanggal Kirim :

Tanggal Kembali ke Redaksi : 19-6-2015

HAL YANG DISUNTING	YA*	TIDAK*
1. Apakah naskah ini pernah dimuat pada media lain ? <u>Keterangan:</u> Belum pernah & muat, tp mohon penulisan judul & perbaiki, spy lebih fokus 		✓
2. Apakah judul tepat, singkat, jelas, dan menggambarkan kontribusi pengembangan keilmuan ? (maksimal 10 kata, melingkupi variabel yang diteliti) <u>Keterangan:</u> 	✓	
3. Apakah pada naskah hasil penelitian :		
a) Pendahuluan mencakup latar belakang secara jelas ? <u>Keterangan:</u> 	✓	
b) Tujuan cukup jelas ? <u>Keterangan:</u> 	✓	

HAL YANG DISUNTING		
c) Metode dan rancangan penelitian sesuai dengan tujuan penelitian ? <u>Keterangan:</u> 	✓	
d) Prosedur penelitian diuraikan secara tepat dan rinci, sehingga menjamin validitas internal/ eksternal <u>Keterangan:</u> 	✓	
e) Hasil penelitian dapat menjawab <i>research question</i> ? <u>Keterangan:</u> 	✓	
f) - Pembahasan tidak mengulang hasil ? - Selaras dengan lingkup penelitian dan dibandingkan dengan hasil penelitian sejenis ? - Menerangkan makna hasil penelitian dalam menjawab permasalahan ? <u>Keterangan:</u> 	✓	
g) Acuan selaras dengan materi penelitian dan menggunakan literatur 10 tahun terakhir? <u>Keterangan:</u> 	✓	

HAL YANG DISUNTING	YA*	TIDAK*
h) Kesimpulan sesuai dengan judul, permasalahan? - Hasil penelitian memberi kontribusi untuk pengembangan Ilmu kedokteran gigi ? - Melakukan sintesis berdasar hasil penelitian sejenis yang mendahului <u>Keterangan:</u> 	✓	
i) Pustaka perlu ditambahi/ dikurangi**)? <u>Keterangan:</u> 		
5. Apakah ada bagian yang perlu ditambahi/ diringkas**)? <u>Keterangan:</u> Hanya keterangan gambar dan tabel → ukuran huruf diperkecil. Penulisan judul spy lebih fokus. -		

Catatan:

1. *) Bubuhkan tanda tilik (✓), **) Coret yang tidak perlu
2. Koreksi dapat dilakukan langsung pada naskah
3. Apabila form keterangan kurang, mohon ditulis pada lembar tambahan

REKOMENDASI untuk KETUA PENYUNTING

- [.....] 1. Naskah dapat dimuat tanpa perubahan.
- [.✓] 2. Naskah dapat dimuat dengan perbaikan sesuai dengan arahan Penyunting Ahli (saran perbaikan mohon ditulis langsung pada naskah)

Keterangan:

- [.....] 3. Naskah tidak dapat dimuat

Alasan:

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Sby, 28 Mei 2015

Penyunting Ahli,

(.....
Wisnu Setyari.....)

The Increasing of Bone morphogenetic protein-2 (BMP-2) using chitosan gel with different molecular weight on wound healing process of dental extraction

ABSTRACT

Background: Bone morphogenetic protein-2 (BMP-2) is bone stimulator which capable of inducing differentiation of mesenchymal cells into osteoblast, stimulating bone formation in remodeling process. Chitosan is polymer composed N-acetyl-D-glucosamine unit that has been used in various applications in wound healing process and bone tissue engineering. **Purpose:** The objective of this research was to analyzed expressions of BMP-2 for 7,14 and 21 days after using chitosan gel with different molecular weight on wound healing process of dental extraction. **Methods:** The research was an experimental laboratory study. *Rattus norvegicus* strain wistar male, aged 8-16 weeks, divided into 3 treatment groups namely group I and II which given chitosan gel 1 % with high and low molecular weight and group III as control which were not given chitosan gel. Chitosan gel were applied into the socket of dental extraction. Rat was decapitated 7,14 and 21 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination to observe Bone morphogenetic protein-2 (BMP-2). Data were analyzed using ANOVA test. **Results:** The result of this research showed significant differences on BMP-2 for 7,14 and 21 days observation ($p < 0,05$). The increasing of BMP-2 were found in the group which given chitosan gel with high molecular weight. **Conclusion:** It may be concluded that The using of chitosan gel with high molecular weight can enhance the expresion of BMP-2 on wound healing process of dental extraction.

Key words: Chitosan, Bone morphogenetic protein-2, wound healing

ABSTRAK

Latar belakang: Bone morphogenetic protein-2 (BMP-2) adalah stimulator pada tulang yang memiliki kemampuan menginduksi diferensiasi sel mesenkimal menjadi sel osteoblas dan menstimulasi pembentukan tulang pada proses remodeling. Kitosan adalah polimer yang memiliki komponen unit N-acetyl-D-glucosamine yang dapat digunakan pada proses penyembuhan luka dan rekayasa jaringan pada tulang. **Tujuan:** Penelitian ini bertujuan untuk menganalisa ekspresi BMP-2 pada pengamatan 7,14 dan 21 hari pada proses penyembuhan luka pencabutan gigi dengan menggunakan kitosan gel yang memiliki berat molekul yang berbeda. **Metode:** Penelitian ini merupakan penelitian eksperimental laboratoris. Sampel menggunakan tikus *Rattus norvegicus* strain wistar, jantan, umur 8-16 minggu, dibagi menjadi 3 kelompok perlakuan yaitu kelompok I dan II: kelompok perlakuan dengan pemberian kitosan gel 1 % yang memiliki berat molekul yang berbeda dan kelompok III adalah kelompok kontrol, tanpa pemberian kitosan gel. Kitosan gel diaplikasikan pada soket gigi. Sampel didekapitasi setelah 7,14 dan 21 hari pemberian perlakuan, kemudian dilakukan pemotongan rahang mandibula dan pemeriksaan imunohistokimia untuk menganalisa ekspresi BMP-2. Data dianalisa dengan uji ANOVA. **Hasil:** Terdapat perbedaan

yang signifikan pada ekspresi BMP-2 dengan pengamatan 7,14 dan 21 hari. Ekspresi BMP-2 lebih banyak ditemukan pada kelompok perlakuan dengan kitosan gel yang memiliki berat molekul tinggi. Kesimpulan: Penggunaan kitosan gel yang memiliki berat molekul tinggi dapat meningkatkan ekspresi BMP-2 pada penyembuhan luka pencabutan gigi.

Kata kunci: *Kitosan, Bone morphogenetic protein-2, penyembuhan luka*

INTRODUCTION

Bone morphogenetic protein-2 (BMP-2) has been widely used as an effective growth factor in bone tissue engineering. It is a member of the transforming growth factor- β (TGF- β) superfamily of multifunctional cytokines which has remarkable ability to induce bone formation and bone tissue reconstruction. It plays critical roles in osteogenesis and bone metabolism.¹ The bone matrix is rich in growth factors, among which Bone morphogenetic proteins (BMPs), that are synthesized and secreted by osteoblast and incorporated into the matrix during bone formation. The BMPs, released during osteoclastic bone resorption, are capable of inducing differentiation of mesenchymal cells into osteoblasts (osteinduction), stimulating bone formation in both remodeling and repairing process.^{1,2}

Chitosan is carbohydrate copolymer composed of glucosamine and N-acetyl-D-glucosamine units. Chitosan has a number of properties including biocompatibility, biodegradability, mucoadhesiveness and wound healing capabilities that make it useful as a biomaterial.³ Chitosan is very versatile and can be prepared as films, gels, sponges and other forms and has been used in various applications including wound healing and bone tissue engineering.^{2,3} Biological activity of chitosan depend on their molecular weight and deacetylation degree. The degree of deacetylation and molecular weight are important parameter, which could influences the performance of chitosan in many its application.^{4,5} In recent study, chitosan is able to increase transforming growth factor beta 1 (TGF β 1), Platelets release transforming growth factor (PDGF), fibroblasts growth factor 2 (FGFS2), Bone morphogenetic protein expression of mRNA on the seventh day.^{6,7}

Bone morphogenetic protein-2 (BMP-2) has been widely investigated for bone healing due to its properties. BMP-2 recruits stem cells to the bone healing, promotes angiogenesis and causes differentiation of stem cells into osteoblast.^{3,6} The increasing expression of BMP-2 could be achieved by using chitosan. The aim of this study was to

analyzed expressions of BMP-2 for 7,14 and 21 days after using chitosan gel with different molecular weight on wound healing process of dental extraction.

MATERIALS AND METHODS

The material in this experiment were Chitosan powder purchased from Sigma chemical, St. Louis, USA. The degree of deacetylation was more than 75 %. Chitosan with high molecular weight (Product number: 419419, Lot number: MKBH5816V) and chitosan with low molecular weight (Product number= 448869, Lot number= MKBH7256V), asetat ^{asetat} acid 2 % p.a (Merck, Germany), buffer formalin 4% and 10% , ketamin (Ketalar,Pfzer), xylazine, alkohol 80%, alkohol 95 %, alkohol 100 % (absolute), xylene, buffer Parafin, EDTA 10 % (JT Baker, USA), NaSO₄ 2 % (Merck, Germany), PBS,Tripsin 0,125 %, H₂O₂ 0,5 %, methanol (Merck, Germany), NaOH 1,25 % and Bone morphogenetic protein-2 monoclonal antibody. The tools used in this experiment were Becker glass, Stirrer, pipette pasteur, Autoclave (Foundry), 5 cc syringe injection (Terumo), 1 cc syringe tuberculin (Terumo), pinset, elevator, Needle holder, non resorbable silk sutures, Bekker glass, Incubator memmert W Germany, Rotary microtome, Label, slide, cover glass, petri disk Poly-L-lysine, deck glass and mikroskop trinokuler Olympus CX 31 Japan).

Chitosan gel 1 % (w/v) was made with diluted one gram of chitosan powder in acetic acid 2 %. It added with NaOH 1,25 % solution to get neutral pH. The mixture was stirred until the gel was completely formed. After homogenization, the gels were stored in closed containers at ambient temperature until use. The characteristic of chitosan gel was evaluated includes solubility, pH, viscosity, physical characteristic, homogeneity, consistency, and duration of storage time. The homogeneity test of gel carried out using glass plates after the powder diluted in acetic acid 2 %. It should be observed on optimized homogeneous. Consistency test could be done by using a penetrometer or mechanically sentrifugator. Gel without precipitation will produce a good consistency. Physical characteristic test or Organoleptic analysis during the storage time includes change of colour, form of formulation gel and odorless. Measurement of viscosity using viscometer Ostwald (kapiler method).^{8,9}

The research was an experimental laboratory study. Rattus norvegicus strain wistar male, aged 8-16 weeks, divided into 3 treatment groups namely group I and II which given chitosan gel 1 % with high and low molecular weight and group III as control which were

not given chitosan gel. Chitosan gel were applied into the socket of dental extraction. Rat was decapitated 7,14 and 21 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination to analyze expression of Bone morphogenetic protein-2 (BMP-2). Fixation was performed using 10 % buffer formalin and decalcification applying EDTA. Further process was dehydration and continued by clearance. The tissue could be cut using microtome in 4-6 μm thickness. Deparaffin and rehydration were subsequently performed. Bone morphogenetic protein-2 monoclonal antibody was diluted by antibody diluents. Next, it was washes by PBS. Streptavidin-biotin was dropped and incubated for 30 minutes, washed by PBS. Counterstained using haematoxyline and washed by flowing water and dried. It was given entelan and covered by cover glass. Light microscope was applied and the evaluation was done. The measuring result were analyzed using ANOVA test. It analyzed the comparison between chitosan treated with high molecular weight group, lower molecular weight group and the control groups ($P<0,05$).

RESULTS

The expressions of Bone morphogenetic protein-2 (BMP-2) for 7,14 and 21 days observation after using chitosan gel with different molecular weight on wound healing process of dental extraction shown in figure 1, 2 and 3. The region which would be evaluated and observed in third apical socket. The expressions of Bone morphogenetic protein-2 (BMP-2) in third apical socket was done 400 times magnification in all sub groups.

In our study, the expressions of Bone morphogenetic protein-2 (BMP-2) on wound healing process of dental extraction using chitosan was more increasing compared to the control group. The expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days observation after using chitosan gel with high molecular weight was more increasing than the other groups.

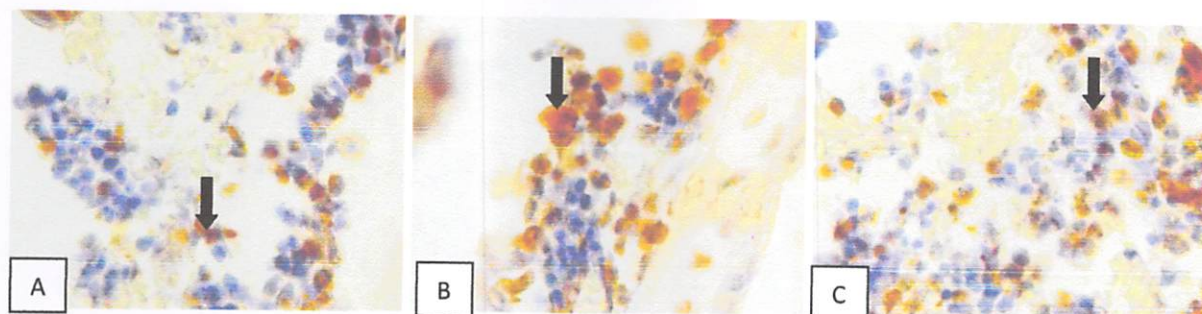


Figure 1. The expressions of Bone morphogenetic protein-2 (BMP-2) at 7 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight.

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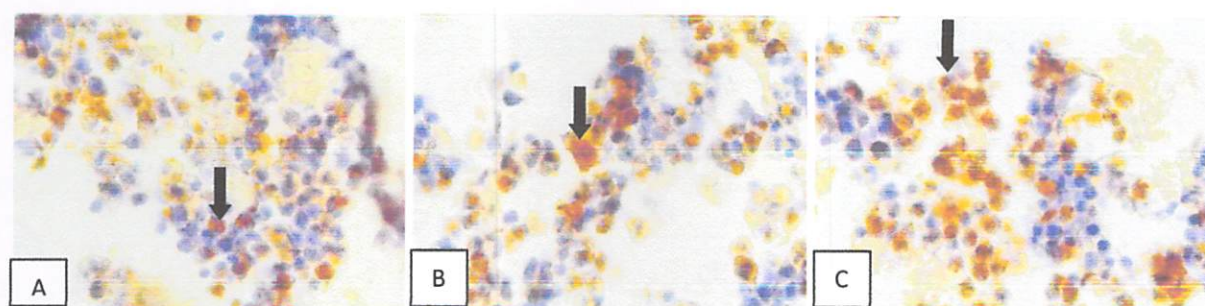


Figure 2. The expressions of Bone morphogenetic protein-2 (BMP-2) at 14 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight.

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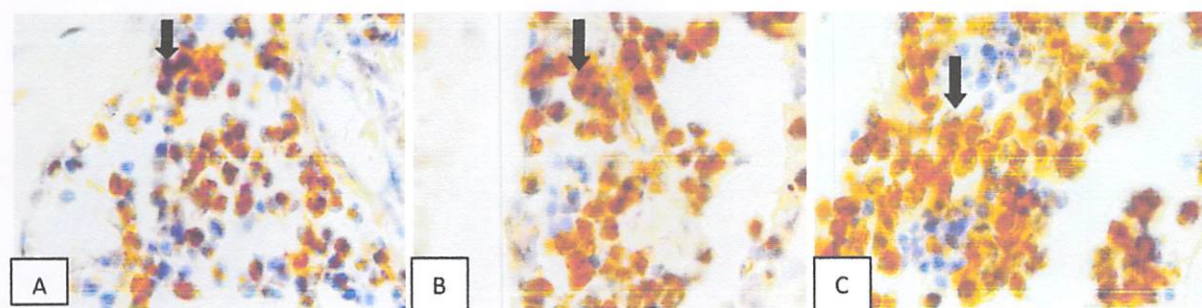


Figure 3. The expressions of Bone morphogenetic protein-2 (BMP-2) at 21 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight.

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Table 1 and figure 4 shown the mean and standard deviation of each group from immunohistochemistry study done in 7,14 and 21 days after treatment. The expressions of Bone morphogenetic protein-2 (BMP-2) for 7,14 and 21 days observation after using chitosan gel with different molecular weight on wound healing process of dental extraction. The data was analyzed using kolmogorov-smirnov statistical test. It showed normal distribution ($p>0.05$) in which fulfilling the requirement of parametric test. The ANOVA test showed,

expressions of Bone morphogenetic protein-2 (BMP-2) in treatment group using chitosan gel with high molecular weight were significant difference compared to the treatment group using chitosan gel with low molecular weight and control group ($p < 0.05$).

Table 1. The mean and standard deviation of each group at 7,14 and 21 days after treatment

Variable	Treatment	7 days	14 days	21 days
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
The expressions of BMP-2	Control	11,17 $\pm$ 0,75	13,57 $\pm$ 1,81	11,50 $\pm$ 1,22
	Chitosan,High BM	18,25 $\pm$ 4,42	21,33 $\pm$ 2,67	19,29 $\pm$ 4,19
	Chitosan, low BM	11,80 $\pm$ 1,92	14,88 $\pm$ 1,45	14,00 $\pm$ 2,53

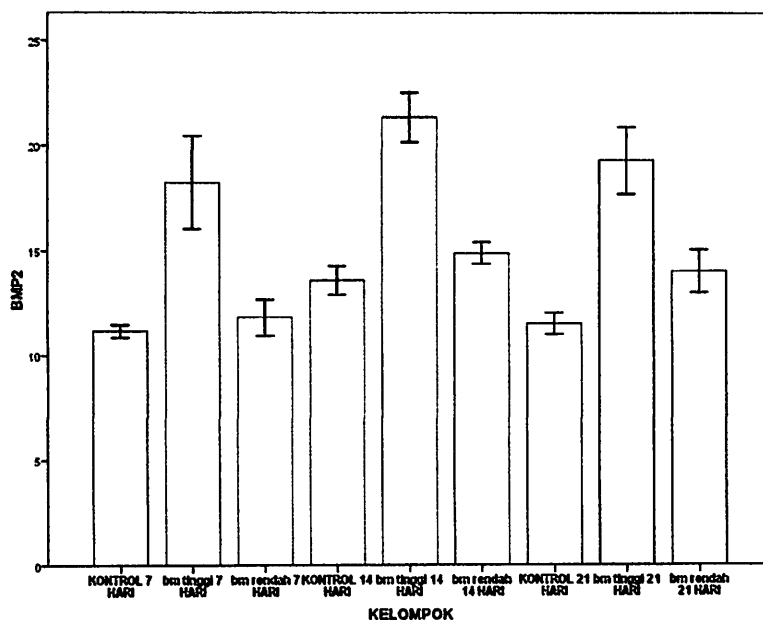


Figure 4. The graphic of expressions of BMP-2 on 7,14 and 21 days using chitosan gel with high and low molecular weight group and control group. } font L

DISCUSSION

The proliferation and differentiation of sufficient progenitor cells are critical for bone healing. These processes are regulated by growth factors such as TGF- β and BMPs. BMP-2 can efficiently induce bone formation. Many materials, especially the natural polymers, have been researched for controlled release of BMP-2.^{10,11} Chitosan is naturally derived polysaccharide. It has gained much attention as biomaterial in tissue engineering application, due to its antimicrobial activity and biocompatibility.¹⁰

Chitosan is reported to be biocompatible, biodegradable and non toxic, having some biomedical properties such as healing accelerator, antifungal, hemostatic, antimicrobial and analgesic. Several study have shown the activity of chitosan in healing process, where it enhanced the infiltration of inflamamatory cells in the injured area and release growth factor.^{6,12} Chitosan is the partially deacetylated form poly-(1,4)-2-amino-2-deoxy-D-glucose) of chitin, and is a potential biomaterial for bone tissue engineering application.^{1,10,12} In the present study, the chitosan powder was mixed with blood of each person and filled in dental socket, and it found that bone tissue regeneration will be faster in chitosan-filled socket than untreated dental socket.¹³

In our study it was used chitosan gel material with different molecular weight to analyzed expressions of BMP-2 on wound healing process of dental extraction. It showed the expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days observation after using chitosan gel was increasing. Chitosan is a polycationic complex carbohydrate with a structure similar to those of glycosaminoglycans specifically hyaluronic acid. Hyaluronic acid is thought to facilitate the migration and proliferation of progenitor cells. Previous studies have demonstared that chitosan might function as a substrate that enhances the migration and differentiation of osteoblast cells and conversely, it might inhibit the function of fibroblast and so indirectly facilitate osteogenesis.^{14,15} Its biological properties of chitosan, including chitosan biodegradation by lysozim that can change chitosan polymer form (N-acetyl-D-glucosamine) to dimer active form. N-acetyl-D-glucosamine dimer active form cross-linked with glycosaminoglycan and glycoprotein that part of matrix macromolecules extracellular as well as stimulate increased Transforming growth factor betha (TGF- β) and Bone morphogenetic protein (BMP) expression of mRNA.^{6,15} In study by Ezzodini reported that chitosan loading rhBMP-2 can facilitate bone regeneration of periodontal tissue. In the cavities filled with chitosan, not only the bone regeneration was faster, but also the density was similar to the density of the bone of the subject under study.¹³

According to the figure 4, the expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days observation after using chitosan gel with high molecular weight was more increasing than the other groups. Chitosan was the material used to form gels due to its good gel forming property, biodegradability, biocompatibility and non toxic. Gels could be considered as ideal pharmaceutical forms to treat the dental socket. It have the ability to absorb exudates, which moisture on the wound surface. Besides, gels have high water vapor and oxygen permeability, as well as mechanical properties that resemble physiological soft

tissue. Chitosan gel has a strong tissue-adhesive property.^{12,17,18} The characteristic of chitosan is related with its molecular weight. The absorption mechanism also depends upon the particle size of chitosan powder.^{17,18} The viscosity of chitosan gel with high molecular weight is more higher than formulation with low molecular weight. It has high viscosity and better mucoadhesive properties due to strong blood clot is formed in order to eliminate complications of dental extraction such as dry socket. However, it can promote the acceleration of wound healing process on dental socket.^{19,20}

BMP-2 can efficiently induce bone formation. The increasing of expression BMP-2 can increase Alkaline phosphatase (ALP) activity. ALP is an enzyme regarded to be important in the process of mineralization, since high levels of ALP would promote hydrolysis of phosphate, produce orthophosphate and increase the calcium deposition. ALP activity and calcium deposition are widely used as marker for early and late differentiation of osteoblast cells, respectively.¹⁰ Mature osteoblasts produce mineralized bone matrix, and the component of this matrix, such as calcium deposition and osteogenic markers (osteocalcin and osteopontin), are usually used as markers for late stage of osteoblast differentiation. Osteocalcin and osteopontin expression marks the late stage of osteoblast differentiation, and BMP-2 is an important factor for osteoblast maturation. Therefore, these results indicated that the increasing of BMP-2 enhanced the osteoblast activity.^{2,10,15} It may be concluded that the using of chitosan gel which increases the expression of BMP-2 can accelerate bone healing process of dental extraction.

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DAFTAR TILIK PENYUNTING PELAKSANA (Penyuntingan Bahasa dan Format)

Judul Naskah : The Increasing of Bone morphogenetic protein-2 (BMP-2) using chitosan gel with different molecular weight on wound healing process of dental extraction

Tanggal Kirim :

Tanggal Kembali ke Redaksi : 1/7/2015

HAL YANG DISUNTING	KETERANGAN
FORMAT	
Apakah panjang naskah cukup memadai? ▪ (10-12 halaman, 1,5 spasi, dengan ukuran kertas HVS A4, times new roman ukuran font 12)	Ya/Tidak
▪ Bagian-bagian isi naskah proporsional (Pembahasan lebih panjang dari Pendahuluan)	Ya/Tidak
Judul	
▪ Sesuai dengan masalah, tujuan dan memuat variabel utama	Ya/Tidak
▪ Tidak terlalu panjang (maksimal 10 kata) dengan huruf kecil diawali huruf kapital	Ya/Tidak
Abstrak (dalam bahasa Inggris dan bahasa Indonesia)	
▪ Panjang < 250 kata, 1 spasi, terstruktur dalam 1 paragraf	Ya/Tidak
▪ Kata kunci sesuai dengan variabel/konsep utama dalam bahasa Indonesia dan bahasa Inggris	Ya/Tidak
▪ Kata kunci maksimal 5 kata/frase	Ya/Tidak
▪ Abstrak terstruktur, satu paragraph, terdiri atas: latar belakang (background), tujuan (purpose), metode (method), hasil (result), kesimpulan (conclusion)	Ya/Tidak
Acuan	
▪ Cara mengacu: sistem Vancouver	Ya/Tidak
Gambar dan tabel	
▪ Sesuai dengan gaya selingkung jurnal (kebenaran, kelengkapan judul dan keterangan/legend) dan dicantumkan acuannya	Ya/Tidak
▪ Jumlah gambar/tabel pada research report dan literature review maksimal 4	Ya/Tidak
▪ Jumlah gambar/tabel pada case report maksimal 8	Ya/Tidak
▪ Gambar/tabel ditulis terpisah dengan teks	Ya/Tidak

lebih dari 4 tapi mungkin
kelebihan 8 images
kelompok.

HAL YANG DISUNTING	KETERANGAN ¹⁾
Daftar Pustaka (Sistem Vancouver)	
▪ Sesuai dengan daftar pustaka dan menurut gaya selingkung jurnal (sistem Vancouver)	Ya/ Tidak
▪ 10 tahun terakhir	Ya/ Tidak
▪ Acuan primer $\pm$ 70% (jurnal, thesis, disertasi, dokumen paten)	Ya/ Tidak
▪ Nomor / volume dan halaman jurnal sudah tercantum	Ya/ Tidak
▪ Edisi, penerbit, kota dan halaman buku sudah tercantum	Ya/ Tidak
▪ Urut pemunculan pada teks	Ya/ Tidak
▪ Nama pengarang ditulis semua	Ya/ Tidak
▪ Konsistensi penyingkatan nama penulis	Ya/ Tidak
▪ Download dari internet cantumkan waktu dan alamat website	Ya/ Tidak
▪ Cara menyingkat judul jurnal sesuai dengan indeks dental dan indeks medicus	Ya/ Tidak
BAHASA	
▪ Tidak enumeratif	Ya/ Tidak
▪ Tidak terjadi kesalahan ketik	Ya/ Tidak
▪ Ejaan baku	Ya/ Tidak
▪ Kalimat baku (subyek, predikat, obyek)	Ya/ Tidak
▪ Satu paragraf, satu pokok pikiran (>2 kalimat)	Ya/ Tidak
HASIL PENELITIAN	
FORMAT	
▪ Sistematika naskah hasil penelitian terdiri dari pendahuluan, bahan dan metode, hasil, pembahasan diakhiri kesimpulan, daftar pustaka.	Ya/ Tidak
Pendahuluan	
▪ Latar belakang empirik/teoritik	Ada/ Tidak
▪ Masalah/tujuan	Ada/ Tidak
Bahan dan Metode	
▪ Rancangan (jenis, masa (waktu), tempat penelitian)	Ada/ Tidak
▪ Teknik pengambilan sampel	Ada/ Tidak
▪ Cara kerja penelitian	Ada/ Tidak

Format Penyuntingan Bahasa dan Format Naskah Dental Journal

HAL YANG DISUNTING	KETERANGAN*)
▪ Analisa data	Ada/Tidak
Hasil	
▪ Paparan data	Ada/Tidak
▪ Analisa hasil	Ada/Tidak
Pembahasan	
▪ Pembahasan tidak mengulang hasil	Ya/Tidak
▪ Selaras dengan lingkup penelitian dan dibandingkan dengan hasil penelitian sejenis?	Ya/Tidak
▪ Menerangkan makna hasil penelitian dan menjawab permasalahan	Ya/Tidak
▪ Kesimpulan	Ada/Tidak
▪ Saran	Ada/Tidak
LITERATURE REVIEW	
FORMAT	
▪ Sistematika naskah <i>Literature Review</i> terdiri dari pendahuluan, bagian isi (terdiri dari kasus subjudul), dan pembahasan diakhiri kesimpulan, daftar pustaka.	Ya/Tidak
▪ Abstrak terstruktur satu paragraf terdiri atas: latar belakang (<i>background</i>), tujuan (<i>purpose</i>), review, kesimpulan (<i>conclusion</i>)	
▪ Daftar pustaka (lebih dari 30 pustaka)	Ya/Tidak
LAPORAN KASUS	
FORMAT	
▪ Sistematika naskah <i>Case Report</i> terdiri dari pendahuluan, tatalaksana kasus, pembahasan diakhiri kesimpulan, dan daftar pustaka.	Ya/ Tidak
▪ Abstrak terstruktur satu paragraf terdiri atas: latar belakang (<i>background</i>), tujuan (<i>purpose</i>), kasus (<i>case</i>), tatalaksana kasus (<i>case management</i>), kesimpulan (<i>conclusion</i>)	

Catatan:

1. *) Coret yang tidak perlu
2. Apabila tidak ada kesesuaian antara penulis dan penyunting seyogyanya dipertemukan untuk mendapatkan solusi.

Format Penyuntingan Bahasa dan Format Naskah Dental Journal

Rekomendasi Penyunting Pelaksana (pilih salah satu)

1. Naskah dapat diproses tanpa perbaikan oleh penulis

2. Naskah dapat diproses dengan perbaikan oleh penulis, yaitu pada bagian :

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.....*gigit & naskah*.....
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3. Naskah ditolak, karena

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Penyunting Pelaksana

Julia

(.....)

The Increasing of Bone morphogenetic protein-2 (BMP-2) using chitosan gel with different molecular weight on wound healing process of dental extraction

ABSTRACT

Background: Bone morphogenetic protein-2 (BMP-2) is bone stimulator which capable of inducing differentiation of mesenchymal cells into osteoblast, stimulating bone formation in remodeling process. Chitosan is polymer composed N-acetyl-D-glucosamine unit that has been used in various applications in wound healing process and bone tissue engineering. **Purpose:** The objective of this research was to analyzed expressions of BMP-2 for 7,14 and 21 days after using chitosan gel with different molecular weight on wound healing process of dental extraction. **Methods:** The research was an experimental laboratory study. *Rattus norvegicus* strain wistar male, aged 8-16 weeks, divided into 3 treatment groups namely group I and II which given chitosan gel 1 % with high and low molecular weight and group III as control which were not given chitosan gel. Chitosan gel were applied into the socket of dental extraction. Rat was decapitated 7,14 and 21 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination to observe Bone morphogenetic protein-2 (BMP-2). Data were analyzed using ANOVA test. **Results:** The result of this research showed significant differences on BMP-2 for 7,14 and 21 days observation ($p < 0,05$). The increasing of BMP-2 were found in the group which given chitosan gel with high molecular weight. **Conclusion:** It may be concluded that The using of chitosan gel with high molecular weight can enhance the expresion of BMP-2 on wound healing process of dental extraction.

Key words: Chitosan, Bone morphogenetic protein-2, wound healing

ABSTRAK

Latar belakang: Bone morphogenetic protein-2 (BMP-2) adalah stimulator pada tulang yang memiliki kemampuan menginduksi diferensiasi sel mesenkimal menjadi sel osteoblas dan menstimulasi pembentukan tulang pada proses remodeling. Kitosan adalah polimer yang memiliki komponen unit N-acetyl-D-glucosamine yang dapat digunakan pada proses penyembuhan luka dan rekayasa jaringan pada tulang. **Tujuan:** Penelitian ini bertujuan untuk menganalisa ekspresi BMP-2 pada pengamatan 7,14 dan 21 hari pada proses penyembuhan luka pencabutan gigi dengan menggunakan kitosan gel yang memiliki berat molekul yang berbeda. **Metode:** Penelitian ini merupakan penelitian eksperimental laboratoris. Sampel menggunakan tikus *Rattus norvegicus* strain wistar, jantan, umur 8-16 minggu, dibagi menjadi 3 kelompok perlakuan yaitu kelompok I dan II: kelompok perlakuan dengan pemberian kitosan gel 1 % yang memiliki berat molekul yang berbeda dan kelompok III adalah kelompok kontrol, tanpa pemberian kitosan gel. Kitosan gel diaplikasikan pada soket gigi. Sampel didekapitasi setelah 7,14 dan 21 hari pemberian perlakuan, kemudian dilakukan pembedahan rahang mandibula dan pemeriksaan imunohistokimia untuk menganalisa ekspresi BMP-2. Data dianalisa dengan uji ANOVA. **Hasil:** Terdapat perbedaan

yang signifikan pada ekspresi BMP-2 dengan pengamatan 7,14 dan 21 hari. Ekspresi BMP-2 lebih banyak ditemukan pada kelompok perlakuan dengan kitosan gel yang memiliki berat molekul tinggi. **Kesimpulan:** Penggunaan kitosan gel yang memiliki berat molekul tinggi dapat meningkatkan ekspresi BMP-2 pada penyembuhan luka pencabutan gigi.

Kata kunci: Kitosan, Bone morphogenetic protein-2, penyembuhan luka

INTRODUCTION

→ penelitian di kelas ad. sbknnya

Bone morphogenetic protein-2 (BMP-2) has been widely used as an effective growth factor in bone tissue engineering. It is a member of the transforming growth factor- β (TGF- β) superfamily of multifunctional cytokines which has remarkable ability to induce bone formation and bone tissue reconstruction. It plays critical roles in osteogenesis and bone metabolism.¹ The bone matrix is rich in growth factors, among which Bone morphogenetic proteins (BMPs), that are synthesized and secreted by osteoblast and incorporated into the matrix during bone formation. The BMPs, released during osteoclastic bone resorption, are capable of inducing differentiation of mesenchymal cells into osteoblasts (osteinduction), stimulating bone formation in both remodeling and repairing process.^{1,2}

Chitosan is carbohydrate copolymer composed of glucosamine and N-acetyl-D-glucosamine units. Chitosan has a number of properties including biocompatibility, biodegradability, mucoadhesiveness and wound healing capabilities that make it useful as a biomaterial.³ Chitosan is very versatile and can be prepared as films, gels, sponges and other forms and has been used in various applications including wound healing and bone tissue engineering.^{2,3} Biological activity of chitosan depend on their molecular weight and deacetylation degree. The degree of deacetylation and molecular weight are important parameter, which could influences the performance of chitosan in many its application.^{4,5} In recent study, chitosan is able to increase transforming growth factor beta 1 (TGF β 1), Platelets release transforming growth factor (PDGF), fibroblasts growth factor 2 (FGFS2), Bone morphogenetic protein expression of mRNA on the seventh day.^{6,7}

Bone morphogenetic protein-2 (BMP-2) has been widely investigated for bone healing due to its properties. BMP-2 recruits stem cells to the bone healing, promotes angiogenesis and causes differentiation of stem cells into osteoblast.^{3,6} The increasing expression of BMP-2 could be achieved by using chitosan. The aim of this study was to

analyzed expressions of BMP-2 for 7,14 and 21 days after using chitosan gel with different molecular weight on wound healing process of dental extraction.

MATERIALS AND METHODS

The material in this experiment were Chitosan powder purchased from Sigma chemical, St. Louis, USA. The degree of deacetylation was more than 75 %. Chitosan with high molecular weight (Product number: 419419, Lot number: MKBH5816V) and chitosan with low molecular weight (Product number= 448869, Lot number= MKBH7256V), asetat acid 2 % p.a (Merck, Germany), buffer formalin 4% and 10%, ketamin (Ketalar,Pfzer), xylazine, alkohol 80%, alkohol 95 %, alkohol 100 % (absolute), xylene, buffer Parafin, EDTA 10 % (JT Baker, USA), NaSO₄ 2 % (Merck, Germany), PBS, Tripsin 0,125 %, H₂O₂ 0,5 %, methanol (Merck, Germany), NaOH 1,25 % and Bone morphogenetic protein-2 monoclonal antibody. The tools used in this experiment were Becker glass, Stirrer, pipette pasteur, Autoclave (Foundry), 5 cc syringe injection (Terumo), 1 cc syringe tuberculin (Terumo), pinset, elevator, Needle holder, non resorbable silk sutures, Bekker glass, Incubator memmert W Germany, Rotary microtome, Label, slide, cover glass, petri disk Poly-L-lysine, deck glass and mikroskop trinokuler Olympus CX 31 Japan).

Chitosan gel 1 % (w/v) was made with diluted one gram of chitosan powder in acetic acid 2 %. It added with NaOH 1,25 % solution to get neutral pH. The mixture was stirred until the gel was completely formed. After homogenization, the gels were stored in closed containers at ambient temperature until use. The characteristic of chitosan gel was evaluated includes solubility, pH, viscosity, physical characteristic, homogeneity, consistency, and duration of storage time. The homogeneity test of gel carried out using glass plates after the powder diluted in acetic acid 2 %. It should be observed on optimized homogeneous. Consistency test could be done by using a penetrometer or mechanically sentrifugator. Gel without precipitation will produce a good consistency. Physical characteristic test or Organoleptic analysis during the storage time includes change of colour, form of formulation gel and odorless. Measurement of viscosity using viscometer Ostwald (kapiler method).^{8,9}

The research was an experimental laboratory study. *Rattus norvegicus* strain wistar male, aged 8-16 weeks, divided into 3 treatment groups namely group I and II which given chitosan gel 1 % with high and low molecular weight and group III as control which were

not given chitosan gel. Chitosan gel were applied into the socket of dental extraction. Rat was decapitated 7,14 and 21 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination to analyze expression of Bone morphogenetic protein-2 (BMP-2). Fixation was performed using 10 % buffer formalin and decalcification applying EDTA. Further process was dehydration and continued by clearance. The tissue could be cut using microtome in 4-6 μm thickness. Deparaffin and rehydration were subsequently performed. Bone morphogenetic protein-2 monoclonal antibody was diluted by antibody diluents. Next, it was washes by PBS. Streptavidin-biotin was dropped and incubated for 30 minutes, washed by PBS. Counterstained using haematoxyline and washed by flowing water and dried. It was given entelan and covered by cover glass. Light microscope was applied and the evaluation was done. The measuring result were analyzed using ANOVA test. It analyzed the comparison between chitosan treated with high molecular weight group, lower molecular weight group and the control groups ($P<0,05$).

RESULTS

The expressions of Bone morphogenetic protein-2 (BMP-2) for 7,14 and 21 days observation after using chitosan gel with different molecular weight on wound healing process of dental extraction shown in figure 1, 2 and 3. The region which would be evaluated and observed in third apical socket. The expressions of Bone morphogenetic protein-2 (BMP-2) in third apical socket was done 400 times magnification in all sub groups.

In our study, the expressions of Bone morphogenetic protein-2 (BMP-2) on wound healing process of dental extraction using chitosan was more increasing compared to the control group. The expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days observation after using chitosan gel with high molecular weight was more increasing than the other groups.

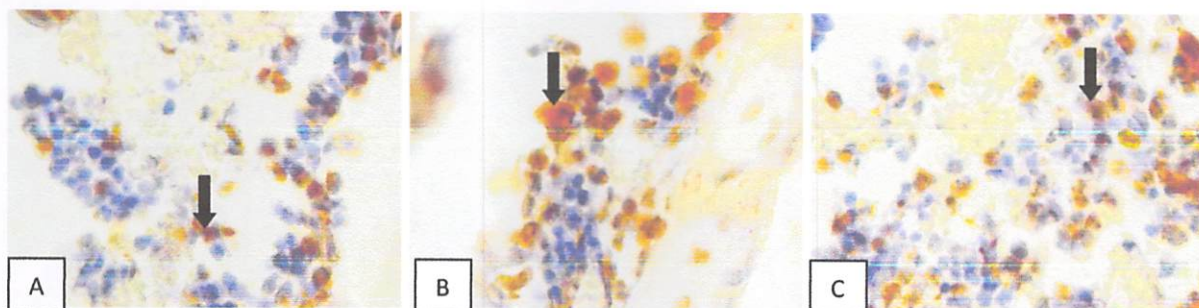


Figure 1. The expressions of Bone morphogenetic protein-2 (BMP-2) at 7 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight.

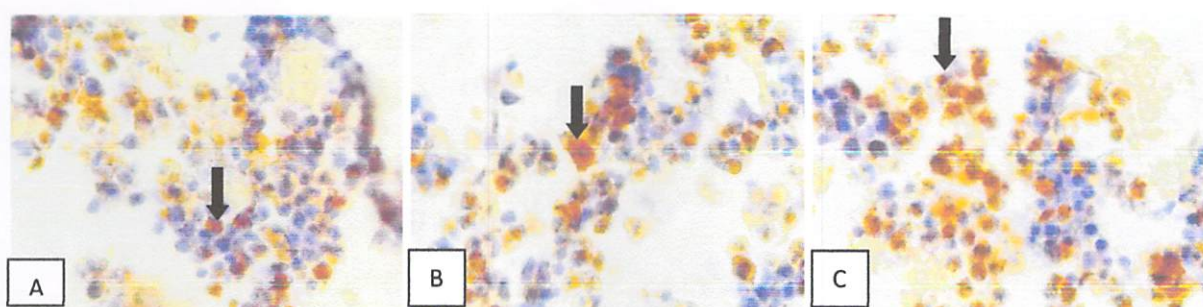


Figure 2. The expressions of Bone morphogenetic protein-2 (BMP-2) at 14 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight.

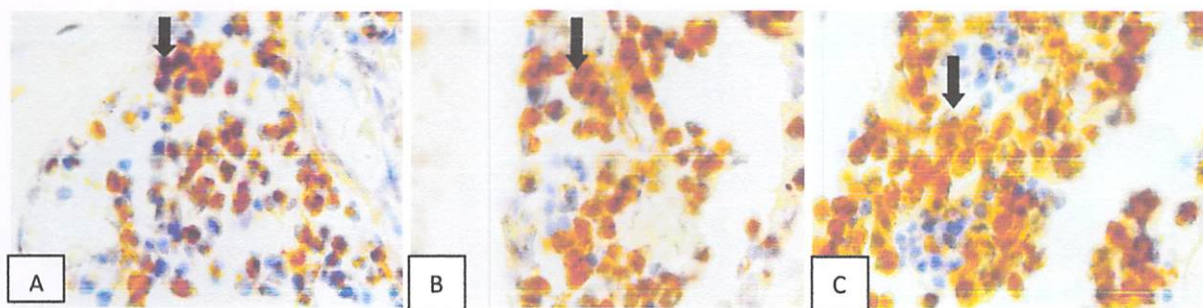


Figure 3. The expressions of Bone morphogenetic protein-2 (BMP-2) at 21 days observation, (A) Control group, without using chitosan, (B) Treatment group using chitosan gel with high molecular weight, (C) Treatment group using chitosan gel with low molecular weight.

Table 1 and figure 4 shown the mean and standard deviation of each group from immunohistochemistry study done in 7,14 and 21 days after treatment. The expressions of Bone morphogenetic protein-2 (BMP-2) for 7,14 and 21 days observation after using chitosan gel with different molecular weight on wound healing process of dental extraction. The data was analyzed using kolmogorov-smirnov statistical test. It showed normal distribution ($p>0.05$) in which fulfilling the requirement of parametric test. The ANOVA test showed,

expressions of Bone morphogenetic protein-2 (BMP-2) in treatment group using chitosan gel with high molecular weight were significant difference compared to the treatment group using chitosan gel with low molecular weight and control group ($p < 0.05$).

Table 1. The mean and standard deviation of each group at 7,14 and 21 days after treatment

Variable	Treatment	7 days	14 days	21 days
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
The expressions of BMP-2	Control	11,17 $\pm$ 0,75	13,57 $\pm$ 1,81	11,50 $\pm$ 1,22
	Chitosan,High BM	18,25 $\pm$ 4,42	21,33 $\pm$ 2,67	19,29 $\pm$ 4,19
	Chitosan, low BM	11,80 $\pm$ 1,92	14,88 $\pm$ 1,45	14,00 $\pm$ 2,53

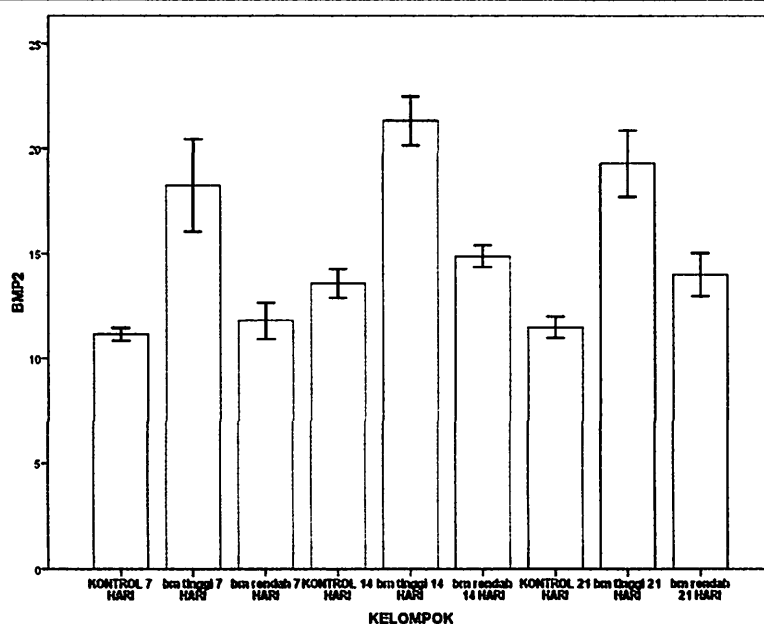


Figure 4. The graphic of expressions of BMP-2 on 7,14 and 21 days using chitosan gel with high and low molecular weight group and control group.

DISCUSSION

The proliferation and differentiation of sufficient progenitor cells are critical for bone healing. These processes are regulated by growth factors such as TGF- β and BMPs. BMP-2 can efficiently induce bone formation. Many materials, especially the natural polymers, have been researched for controlled release of BMP-2.^{10,11} Chitosan is a naturally derived polysaccharide. It has gained much attention as a biomaterial in tissue engineering application, due to its antimicrobial activity and biocompatibility.¹⁰

Chitosan is reported to be biocompatible, biodegradable and non toxic, having some biomedical properties such as healing accelerator, antifungal, hemostatic, antimicrobial and analgesic. Several study have shown the activity of chitosan in healing process, where it enhanced the infiltration of inflamamatory cells in the injured area and release growth factor.^{6,12} Chitosan is the partially deacetylated form poly-(1,4)-2-amino-2-deoxy-D-glucose) of chitin, and is a potential biomaterial for bone tissue engineering application.^{1,10,12} In the present study, the chitosan powder was mixed with blood of each person and filled in dental socket, and it found that bone tissue regeneration will be faster in chitosan-filled socket than untreated dental socket.¹³

In our study it was used chitosan gel material with different molecular weight to analyzed expressions of BMP-2 on wound healing process of dental extraction. It showed the expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days observation after using chitosan gel was increasing. Chitosan is a polycationic complex carbohydrate with a structure similar to those of glycosaminoglycans specifically hyaluronic acid. Hyaluronic acid is thought to facilitate the migration and proliferation of progenitor cells. Previous studies have demonstared that chitosan might function as a substrate that enhances the migration and differentiation of osteoblast cells and conversely, it might inhibit the function of fibroblast and so indirectly facilitate osteogenesis.^{14,15} Its biological properties of chitosan, including chitosan biodegradation by lysozim that can change chitosan polymer form (N-acetyl-D-glucosamine) to dimer active form. N-acetyl-D-glucosamine dimer active form cross-linked with glycosaminoglycan and glycoprotein that part of matrix macromolecules extracellular as well as stimulate increased Transforming growth factor betha (TGF- β) and Bone morphogenetic protein (BMP) expression of mRNA.^{6,15} In study by Ezzodini reported that chitosan loading rhBMP-2 can facilitate bone regeneration of periodontal tissue. In the cavities filled with chitosan, not only the bone regeneration was faster, but also the density was similar to the density of the bone of the subject under study.¹³

According to the figure 4, the expressions of Bone morphogenetic protein-2 (BMP-2) in 7,14 and 21 days observation after using chitosan gel with high molecular weight was more increasing than the other groups. Chitosan was the material used to form gels due to its good gel forming property, biodegradability, biocompatibility and non toxic. Gels could be considered as ideal pharmaceutical forms to treat the dental socket. It have the ability to absorb exudates, which moisture on the wound surface. Besides, gels have high water vapor and oxygen permeability, as well as mechanical properties that resemble physiological soft

tissue. Chitosan gel has a strong tissue-adhesive property.^{12,17,18} The characteristic of chitosan is related with its molecular weight. The absorption mechanism also depends upon the particle size of chitosan powder.^{17,18} The viscosity of chitosan gel with high molecular weight is more higher than formulation with low molecular weight. It has high viscosity and better mucoadhesive properties due to strong blood clot is formed in order to eliminate complications of dental extraction such as dry socket. However, it can promote the acceleration of wound healing process on dental socket.^{19,20}

BMP-2 can efficiently induce bone formation. The increasing of expression BMP-2 can increase Alkaline phosphatase (ALP) activity. ALP is an enzyme regarded to be important in the process of mineralization, since high levels of ALP would promote hydrolysis of phosphate, produce orthophosphate and increase the calcium deposition. ALP activity and calcium deposition are widely used as marker for early and late differentiation of osteoblast cells, respectively.¹⁰ Mature osteoblasts produce mineralized bone matrix, and the component of this matrix, such as calcium deposition and osteogenic markers (osteocalcin and osteopontin), are usually used as markers for late stage of osteoblast differentiation. Osteocalcin and osteopontin expression marks the late stage of osteoblast differentiation, and BMP-2 is an important factor for osteoblast maturation. Therefore, these results indicated that the increasing of BMP-2 enhanced the osteoblast activity.^{2,10,15} It may be concluded that the using of chitosan gel which increases the expression of BMP-2 can accelerate bone healing process of dental extraction.

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