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RESEARCH ARTICLE

The Expression Of Macrophage Cell On Wound Healing Process In Rattus Norvegicus Using Chitosan Gel With Different Molecular Weight

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ABSTRACT

Objectives: *The infiltration of macrophage cell on wound healing process has important role to release a number of cytokines and synthesize extracellular matrix. The aim of this study was to account the the expression of macrophage cell on wound healing process of dental extraction in Rattus norvegicus for 3 and 4 days using chitosan gel with different molecular weight.* ***Methods:*** *Rattus nornegicus strain wistar male, aged 8-16 weeks, divided into 3 groups, namely group I which given chitosan gel 1% with high molecular weight, group II which given chitosan gel 1% with low molecular weight and group III as control which were not given chitosan gel. Chitosan gel 1% were applied into the socket of dental extraction. Rat was decaputated 3 and 4 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination using macrophage cell monoclonal antibody to observethe expression of macrophage cell. Data were analyzed using ANOVA test.* ***Results:*** *The expression of macrophage cells were found higher in the group which given chitosan gel 1% with high molecular weight. The result showed significant differences in expression of macrophage cell for 3 and 4 days observation compared to control group ($p<0,05$).* ***Conclusion:*** *The application chitosan gel 1 % with high molecular weight stimulates macrophages cells on wound healing process of dental extraction.*

Keywords: *Chtosan gel 1 %, molecular weight, macrophage cell*

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BACKGROUND

The macrophage is important inflammatory cell in wounds healing process. The macrophage cells have role to many functions in wound healing, including host defense, promotion of inflammation and support of cell proliferation on wound healing process.[1] It include the following growth factors that promote cellular proliferation and protein synthesis, proteases and extra-cellular matrix molecules. It produce a large number of mediators and cytokines including interleukin-1, interleukin-6, interleukin-12, TNFα, and inducible nitric oxide synthase (iNOS). The macrophage cell stimulate the production of growth factors such as TGF-beta1, vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF)-1. These growth factor promote the proliferation cells in wound healing process.[1,2,3]

Chitosan is naturally derived polysaccharide that have many application in tissue engineering due to its antimicrobial activity ,biocompatibility and having some properties to accelerate wound healing process.[4] In recent study the application of chitosan gel on wound healing process of dental extraction can increase the number of type 1 collagen on remodeling process of dental extraction.[5] Kojima *et al.* reported that chitosan is able to stimulates Platelets derived growth factor (PDGF). It can stimulates the migration and proliferation of macrophages and fibroblast cell on wound healing. Futhermore, PDGF activates the synthesis of Tranforming growth factor beta (TGF β) in macrofag, which also activates the synthesis of collagen in fibroblast.[5,6,7] The application of chitosan depends on the characteristic of chitosan include the molecular weight and deacetylation degree.[8,9]

The infiltration of macrophage cell on wound healing process has important role to release cytokines, some mediator and synthesize extracellular matrix. Chitosan gel is property to accelerate wound healing process of dental extraction. The aim of this study was to account the the expression of macrophage cell on wound healing process of dental extraction in Rattus norvegicus for 3 and 4 days using chitosan gel with different molecular weight.

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 %, H_2O_2 0,5 %, methanol (Merck, Germany), NaOH 1,25 % and Macrophage monoclonal antibody. The tools used in this experiment were Becker glass, Stirer, 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.[10,11]

The research was an experimental laboratory study. Rattus nornegicus strain wistar male, aged 8-16 weeks, divided into 3 treatment groups namely group 1 which given chitosan gel 1 % with high molecular weight dan high viscosity. Group 2 given chitosan gel 1 % with low molecular weight and low viscosity, and group III as control which were not given chitosan gel. Chitosan gel were applied into the socket of dental extraction. Rat was decaputated 3 and 4 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination to analyze expression of Macrophage cell. 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. Deparafin 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 mean and standard deviation of each group at 3 and 4 days after treatment. The expression of macrophage cell in 3 and 4 days after treatment using chitosan with high molecular weight and high viscosity more higher compared to group using chitosan with low molecular weight and low viscosity. The data was analysized using kolmogorov-smirnov statatistical test. It showed normal distribution ($p>0.05$) in which fulfiiling the requirement of parametric test. ANOVA test showed there were

significant difference ($p<0.05$) in all group.

Table 1. The mean and standard deviation of each group at 3 and 4 days after treatment

Variable	Treatment	3 days	4 days
		Mean± SD	Mean± SD
The expression of macrophage cell	Chitosan high MW,visco	16.00±2.37	22.00±2.00
	Chitosan low MW,visco	12.40±2.30	13.33±2.25
	Control	2.83±0.98	3.40±1.14

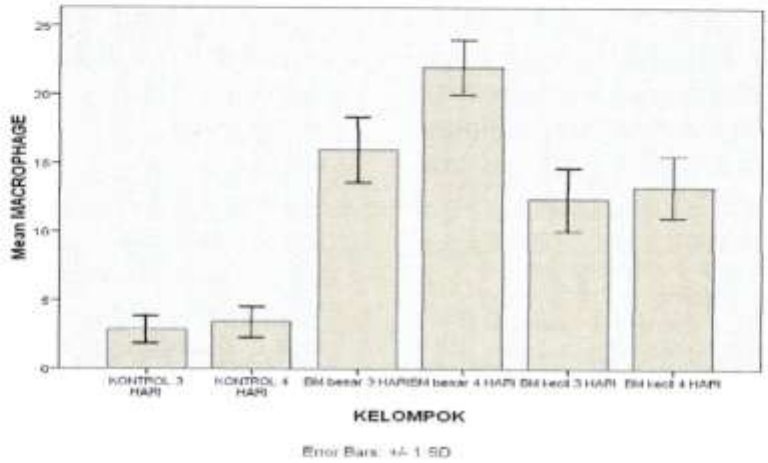

Figure 1. The graphic of expression macrophage cell on 3 and 4 days using chitosan with high molecular weight, lower molecular weight and control group

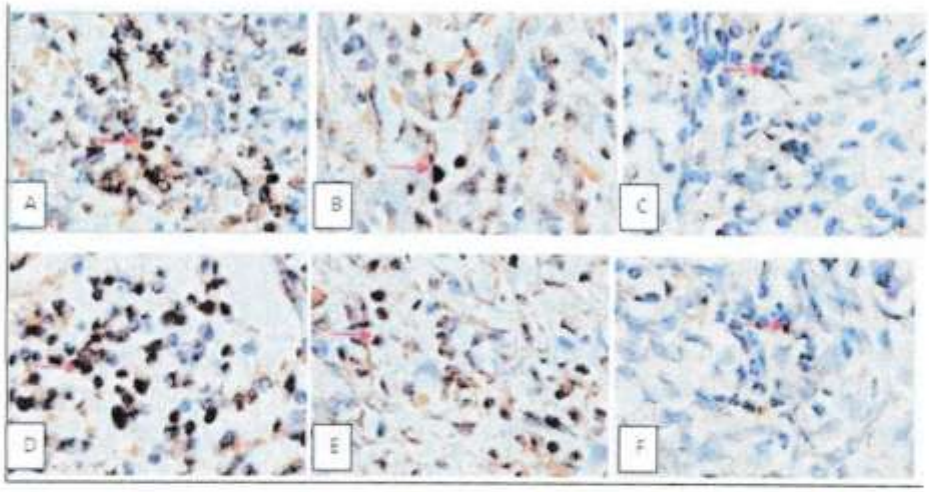

Figure 2. The expression of macrophage cell at 3 days observation: (A) Chitosan with high molecular weight and high viscosity, (B) Chitosan with low molecular weight and low viscosity, (C) Control group, without using chitosan; The expression of macrophage cell at 4days observation: (D) Chitosan with high molecular weight and high viscosity, (E) Chitosan with low molecular weight and low viscosity, (F) Control group, without using chitosan

The expression of macrophage cell on wound healing process of dental extraction using chitosan gel shown in figure 2. Figure 2 showing the expression of macrophage cell in 3 and 4 days after dental extraction. In our study, the expression of macrophage cell on wound healing process of dental extraction using chitosan was more higher compared to control group. The expression of macrophage cell in 3 and 4 days using chitosan gel with high molecular weight and high viscosity was more higher than using chitosan with low molecular weight and low viscosity.

DISCUSSION

Macrophage cell appear In inflammatory phase of wound healing process, 48 until 72 hours after injury and continue the process phagocytosis. These cells Attracted to the wound site by chemoattractive agents, including clotting factors, complement components, cytokines such as PDGF, TGF-β and platelet factor IV, as well as elastin and collagen. Macrophages cells have a longer lifespan than neutrophils. It has important role as regulatory cells and providing an abundant reservoir of potent tissue growth factors, TGF-β, as well as other mediators (TGF-α, heparin binding epidermal growth factor, fibroblast growth factor [FGF], collagenase), activating keratinocytes, fibroblasts and endothelial cells. If there no macrophage cell would cause delayed fibroblast proliferation, angiogenesis and maturation[2,12].

In our study the expression of macrophage cell in 3 and 4 days after treatment using chitosan gel have more higher than the treatment of group control. Chitosan exhibits several valuable properties such as antibacterial, antifungal, nontoxic, hemostatic, biodegradable as well as hydrogel formation properties. Which these properties, chitosan applications has important role in many fields for tissue engineering.[13] Chitosan gel also acts as an ideal wound dressing and more importantly chitosan gel accelerates wound healing.[14] Chitosan is metabolized by certain human enzymes, such as lysozyme. Thus, chitosan is biodegradable. It has structural similarities to glycosaminoglycans and is hydrophilic. Chitosan's monomeric unit, *N*-acetylglucosamine is an extracellular macromolecule that is important in wound healing.[14,15] When chitosan is applied to the wound, it biodegraded by lysozymes, Chitosan modulates macrophage function and the secretion of numerous enzymes collagenase and cytokines include interleukins and tumor necrosis factor during the wound healing process. Chitosan structurally glycosaminoglycans (GAG), which have long-chain, unbranched, repeating disaccharide units maintaining cell morphology, differentiation and function. Glycosaminoglycans and proteoglycans are widely distributed throughout modulate cytokines and growth factors, including heparin and heparan sulfate. Hence, the cell-binding and cell-activating properties of chitosan are important for wound healing. Moreover, *N*-acetylglucosamine is an anti-inflammatory drug and is synthesized in the human body from

glucose.[15] It is incorporated into glycosaminoglycans and glycoproteins. Chitosan exerts anti-inflammatory effects by inhibiting prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2) protein expression. The application of chitosan increases the expression of the anti-inflammatory cytokine. The degradation of chitosan into monomers and oligomers at a wound site significantly accelerates the wound healing process. [13,15]

The characteristic of chitosan is related with its molecular weight. The expression of macrophage cell after treatment using chitosan gel with high molecular weight and high viscosity shown more higher than treatment using chitosan gel with low molecular weight and low viscosity. Chitosan gel has a strong tissue-adhesive property. When chitosan dissolved in acidic solution gives viscous solutions.The viscosity of chitosan is influenced by its molecular weight. The monomers of chitosan powder with high molecular weight and high viscosity were directly effective because it monomers more quickly absorbed and biodegraded by some enzymes. N-acetyl-D-glucosamine dimer active of chitosan cross-linked with glycosaminoglycan and glycoprotein that part of matrix macromolecules extracellular as well as stimulate increased.[16,17,18] The macrophage cell is key of inflmatory process in wound healing process. It produces some mediators, sitokin and growth factor which crucial role in wound healing process of dental extraction.[2] Chitosan gel were found to stimulate the expression of macrophage cell, significantly it could promote the the wound healing process of dental extraction.

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