

# Chitosan

,

. . . .

I.

가  
(Polypeptide Growth Factors,

PGF)

. PGF

3).

가

가

1).

가

가

Zea Mays L.

가 가

가

4).

가

tea

, Egelberg(1987)<sup>2)</sup>

catechin  
Rhizoma)

(Drynariae

2.

(1)

1

1/3

300unit/ml penicillin(Gibco, U.S.A.), 300 µg/ml streptomycin(Gibco, U.S.A.) 1.5 µg/ml amphotericin-B(Gibco, U.S.A.)가 Dulbecco's Modified Eagle's Medium (DMEM, Gibco, U.S.A.) 5

35mm

, 10% fetal bovine serum(FBS, Gibco, U.S.A.) 100unit/ml penicillin, 100 µg/ml streptomycin (Gibco, U.S.A.) 0.5 µg/ml amphotericin-B가 DMEM , 37 °C, 100% , 5% CO<sub>2</sub> (Vision Scientific Co., Korea) 가

0.05% Trypsin/0.02% EDTA

1: 3

4-7

(2)

100g Pentobarbital Sodium (Tokyo Industrial Chem., Japan) 30mg/kg 70%

(3)

1

hyaluronic acid  
chitosan(1-4, 2-amino-2-deoxy- -D-glucan) chitosan  
6, 7)  
8, 9) 가 chitin  
chitosan 10-14)  
15-18) 가 , 5,  
6, 19-22)  
chitosan  
가  
5)  
in vitro chitosan  
chitosan 가  
chitosan

II.

1.

5

4-7

200 µg/ml chitosan chitosan  
0.2% acetic acid

가,

1ml 40 µ

1

g

5 4-7 tometer(Shimatsu Co., Japan) 595nm  
 Bovine serum albumin  
 1.39mg/ml

3.

(1) 1ml 40 µg chitosan (4) 24 well well 5 × 10<sup>4</sup>  
 , chitosan

(2) 24 well (Corning Co., USA) Trypsin/0.02% EDTA 2 3, 7  
 well 2 × 10<sup>4</sup> 1,500 rpm 6 0.05%  
 1ml 40 µg chitosan DMEM 0.5ml 가  
 chitosan (Ultrasonic Dismembrater  
 Model-300, Fisher Co., USA)  
 300 µl (A.I.P.K.kit, )  
 1, 3, 5, 7, 9, 12, 15 UV-VIS spectrophotometer(Shimatsu  
 0.05% Trypsin/0.02% EDTA Co., Japan) 500nm  
 (Gibco, USA) hemocytometer  
 (Olympus Co., Japan)

(3) 24 well well 5 × 10<sup>4</sup> (5) 4 × 10<sup>5</sup>  
 10% Fetal bovine serum, 100unit/ml  
 penicillin, 100 µg/ml streptomycin, 50 µg/ml  
 ascorbic acid, 10mM/ml Na- -glycerophos-  
 phate, 10<sup>-8</sup>M dexamethasone DMEM  
 2  
 0.05% Trypsin/ 0.02% EDTA(Gibco, USA) DMEM ml 40 µg chitosan  
 1,500 rpm 6 11 1.5%  
 0.5ml 가 glutaraldehyde 2 Alizarin  
 (Ultrasonic ,  
 Dismembrater Model-300, Fisher Scientific, red S 0.1M  
 USA) 2% Light Green  
 Bio-rad protein assay dye SF 1.5 ×  
 reagent Vortexing 1.5Cm<sup>2</sup>  
 5 UV-VIS spectropho-

4.

ml 40 µg

chitosan

가

Student t-test(P<0.01)

III.

1.

chitosan

$2 \times 10^4$ cells/ml

2

$6.63 \times 10^4$ cells/ml,  $8.42 \times$

$10^4$ cells/ml, 5

$11.75 \times 10^4$ cells/ml,

$12.80 \times 10^4$ cells/ml

9

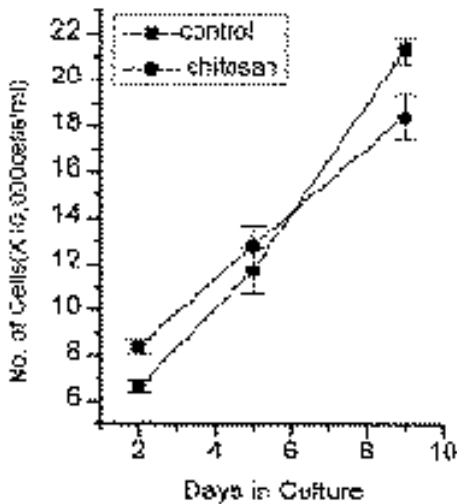
$21.32 \times 10^4$ cells/ml,

$18.43 \times 10^4$ cells/ml

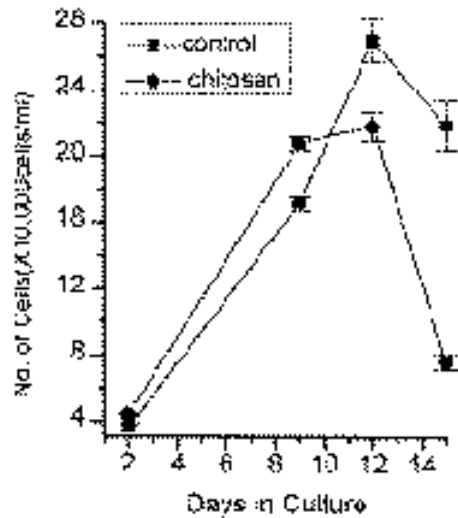
( 1, 1).

2 (3.6 ×

$10^4$ cells/ml,  $4.4 \times 10^4$ cells/ml) 9 (17.2 ×



1 The effect of chitosan on the proliferation of human periodontal ligament cell



2 The effect of chitosan on the proliferation of rat calvaria cell

1 Effect of Chitosen on the Proliferation of Human Periodontal Ligament Cell

Group	Number of Cells (×10,000cells/ml)		
	2day	5day	9day
control	6.63 ± 0.252	11.75 ± 0.988	21.32 ± 0.548
chitosan	8.42 ± 0.305*	12.80 ± 0.905	18.43 ± 1.948*

Values are mean ± S. E., n=3

Number of intial inoculated cell :  $2 \times 10^4$ cells/ml

\* : Statistically significant difference from control group (P<0.01).

## 2 Effect of Chitosan on the Proliferation of Rat Calvaria Cell

Group	Number of Cells( ×10,000cells/ml)			
	2day	9day	12day	15day
control	3.6 ± 0.212	17.2 ± 0.448	27.0 ± 1.310	21.9 ± 1.491
chitosan	4.4 ± 0.035*	20.8 ± 0.395*	21.8 ± 0.901*	7.6 ± 0.473*

Values are mean ± S. E., n=3

\* : Statistically significant difference from control group (P<0.01).

## 3 Effect of Chitosan on the Proliferation of Human Gingival Fibroblast

Group	Number of Cells( ×10,000cells/ml)		
	1 day	3 day	7 day
control	3.31 ± 0.188	9.75 ± 0.125	11.25 ± 0.562
chitosan	1.94 ± 0.188	8.69 ± 0.563	9.69 ± 0.469

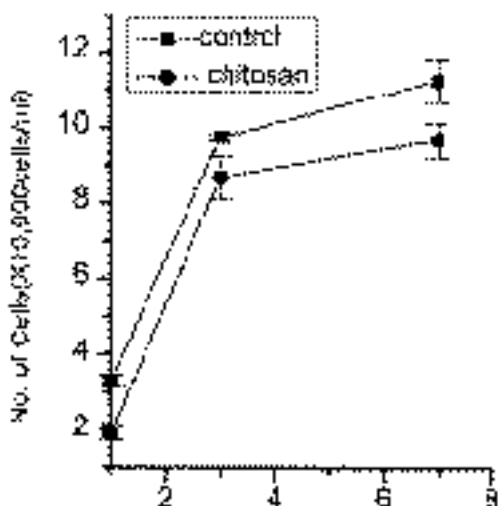
Values are mean ± S. E., n=3

## 4 Effect of Chitosan on the Protein Level in Human Periodontal Ligament Cell

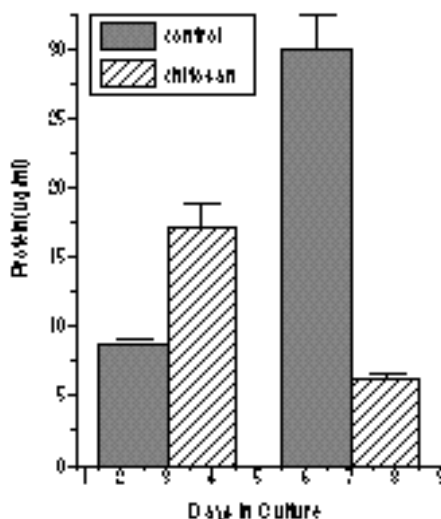
Group	protein( µg/ml)	
	3 day	7 day
control	8.876 ± 0.257	30.513 ± 3.578
chitosan	16.729 ± 1.453*	6.101 ± 0.332*

Values are mean ± S. E., n=3

\* : Statistically significant difference from control group (P<0.01).



3 The Effect of Chitosan on the Proliferation of Human Gingival Fibroblast.



4 Effect of chitosan on the protein level in human periodontal ligament cell

$10^4$ cells/ml,  $20.8 \times 10^4$ cells/ml) 3  
 , 12 (27.0  $\times$  19.89  $\mu$ g/ml, 20.60  $\mu$ g/ml  
 $10^4$ cells/ml,  $21.8 \times 10^4$ cells/ml), 15 (21.9  $\times$  7  
 $10^4$ cells/ml,  $7.6 \times 10^4$ cells/ml) 20.81  $\mu$ g/ml, 14.05  $\mu$ g/ml  
 ( 5, 5)  
 ( 2, 2) 3 (23.16  
 1 (3.31  $\mu$ g/ml, 24.12  $\mu$ g/ml), 7 (25.35  $\mu$ g/ml,  
 $\times 10^4$ cells/ml,  $1.94 \times 10^4$ cells/ml), 3 (9.75  $\times$  23.93  $\mu$ g/ml)  
 $10^4$ cells /ml,  $8.69 \times 10^4$ cells/ml), 7 (11.25  $\times$   
 $10^4$ cells/ ml,  $9.69 \times 10^4$ cells/ml) (P< 0.01)( 6, 6)

3.

(P< 0.01)( 3, 3)

chitosan

1ml 40  $\mu$ g

2.

, 3 1.41IU, 2.11IU, 3  
 8.88  $\mu$ g/ml, 16.73  $\mu$ g/ml 1.98IU, 5.55IU 7  
 , 7 가 (P<0.01)( 7,  
 30.51  $\mu$ g/ml, 6.10  $\mu$ g/ml 7).  
 ( 4, 4). 3

#### 5 Effect of Chitosan on the Protein Level in Rat Calvaria Cell

Group	protein( $\mu$ g/ml)	
	3 day	7 day
control	19.891 $\pm$ 0.985	20.808 $\pm$ 1.816
chitosan	20.597 $\pm$ 1.984	14.054 $\pm$ 0.236*

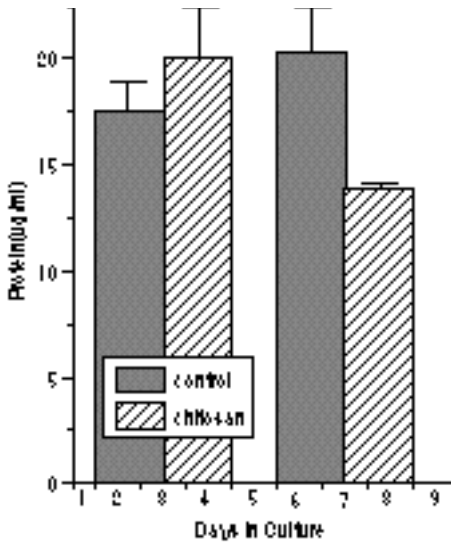
Values are mean  $\pm$  S. E., n=3

\* : Statistically significant difference from control group (P<0.01).

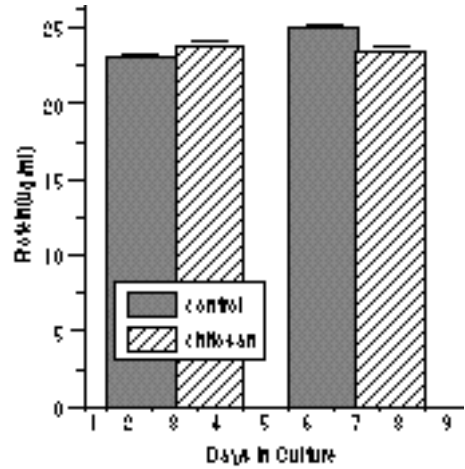
#### 6 Effect of Chitosan on the Protein Level in Human Gingival Fibroblast

Group	protein( $\mu$ g/ml)	
	3 day	7 day
control	23.157 $\pm$ 0.259	25.347 $\pm$ 0.141
chitosan	24.122 $\pm$ 0.330	23.934 $\pm$ 0.188

Values are mean  $\pm$  S. E., n=3



5 Effect of chitosan on the protein level in rat calvaria cell



6 Effect of chitosan on the protein level in human fibroblast

7 Effect of Chitosan on Alkaline Phosphatase Activity in Human Periodontal Ligament Cell

Group	ALP activity (IU)	
	3 day	7 day
control	1.414 ± 0.496	1.984 ± 0.123
chitosan	2.114 ± 0.139*	5.547 ± 0.135*

Values are mean ± S. E., n=3

\* : Statistically significant difference from control group (P<0.01).

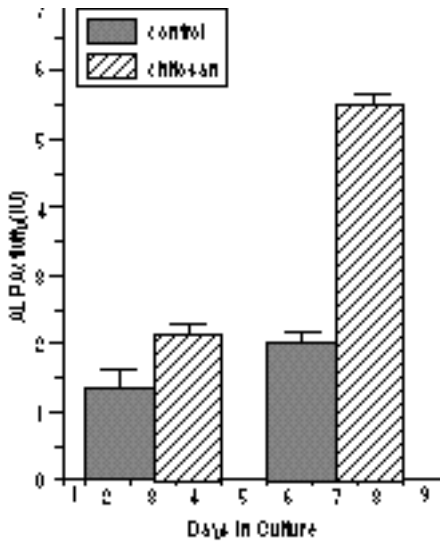
8 Effect of Chitosan on Alkaline Phosphatase Activity in Rat Calvaria Cell

Group	ALP activity (IU)	
	3day	7 day
control	3.346 ± 0.2	5.232 ± 0.04
chitosan	6.232 ± 0.48*	5.492 ± 0.17

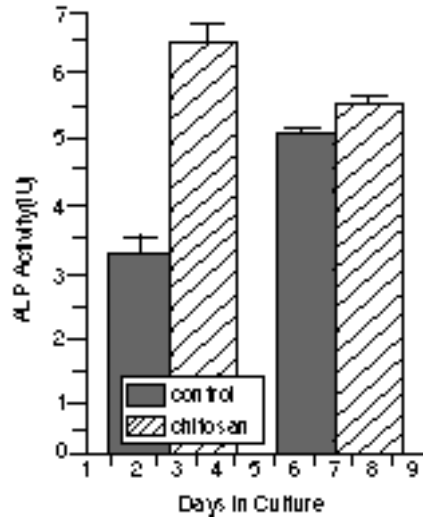
Values are mean ± S. E., n=3

\* : Statistically significant difference from control group (P<0.01).

3.35IU, 6.23IU ( 8, 8).  
 5.49IU, 7 5.23IU, 3 (2.09IU,  
 가 1.86IU), 7 (3.64IU, 4.26IU)



7 Effect of chitosan on alkaline phosphatase activity in human periodontal ligament cell

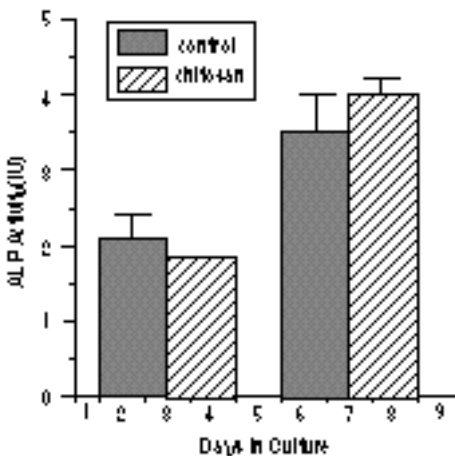


8 Effect of chitosan on alkaline phosphatase activity in rat calvaria cell

9 Effect of Chitosan on Alkaline Phosphatase Activity in Human Gingival Fibroblast

Group	ALP activity (IU)	
	3 day	7 day
control	2.087 ± 0.232	3.640 ± 0.488
chitosan	1.855 ± 0	4.260 ± 0.179

Values are mean ± S. E., n=3



9 Effect of chitosan on alkaline phosphatase activity in Human fibroblast

(P<0.01) ( 9, 9) 가

4.

11 Alizarin red S  
chitosan

가

가

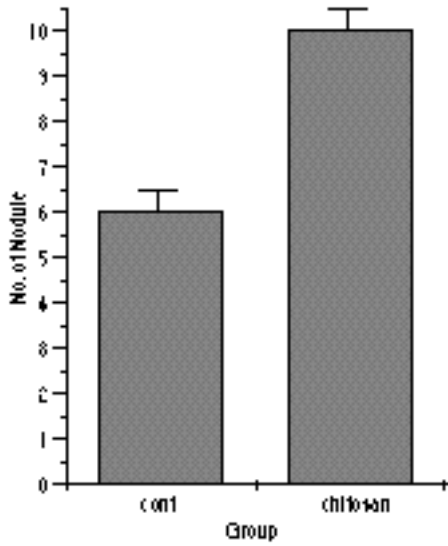
. chitosan

6 ,

10 ,

chitosan





10 Effect of chitosan on formation of mineralized nodule in rat calvaria cell

( $P < 0.01$ ) ( 10).

IV.

fibronectin  
가  
가  
가  
가  
가  
가  
가  
가  
가  
가  
가  
가

24-26).

가

3 chitosan  
7 ( 1,  
2, 4, 5). chitosan

Polypeptide Growth Factor  
, Piche Graves(1989)<sup>27)</sup>  
Platelet- Derived Growth  
Factor(PDGF) 가  
, Lynch PDGF 1ml 2mg chitosan chi-  
Insulin-like Growth Factor(IGF-I) tosan 200 μ가  
, 200 μ chitosan

가 acetic acid chitosan  
Lynch (1991)<sup>28)</sup> titanium  
implant PDGF IGF-I Klokkevold 5)  
implant 가 chitosan  
chitin chitosan cellulose 가 0.2% acetic acid 1ml 200 μg  
, , 가 1ml 40 μg  
chitin chitosan 1, 3 7

chitosan(1-4, 2-amino-2-deoxy- -D-glucan) ( 3). Van der  
hyaluronic acid polycat- Lei Wildevuur(1989)<sup>17)</sup> , chi-  
tionic complex carbohydrate , tosan  
800-1,500Kd 5). chitosan ,

가 , lysozyme  
(depolymerization) 6 , chitosan  
6). D-glu-  
cosamine 가 40%  
N-acetyl-D-glucosamine residues Klokkevold (1997)<sup>5)</sup> 7  
7, 11). 가 chi-  
chitosan tosan  
9 , 12 ,  
chitosan

, chitosan (1996)<sup>32)</sup>  
 organized tissue reconstruction  
 . (ALPase)  
 , ALPase  
 ,  
 ,  
 가  
 가 ALPase  
 .  
 가 가  
 3, 7 chitosan  
 ( . 7), chi- 가  
 tosan calcium phosphate  
 Namita (1988) . De Bernard  
 6). (1982) 가 가  
 가  
 , 95% 1 가  
 cyclic AMP가 가 , osteonectin  
 biglycan 33-35).  
 marker가 가  
 , 3, 7 chitosan  
 가  
 . in vitro 7 가  
 가 7  
 가  
 가  
 29-31). Arceo (1991)<sup>29)</sup> in vitro  
 가  
 chitosan in  
 vivo , Kawakami (1991)<sup>19)</sup>  
 Ito (1991)<sup>22)</sup>  
 , in vitro chitosan-bonded hydroxyapatite self-  
 가 hardening paste

, 1992  
 , paste . Klokkevold (1997)<sup>5)</sup> in vitro  
 가 chitosan (

1.5g quick-hardening paste

chitosan partially  
 deacetylated chitin ,  
 chitosan-bonded hydroxyapatite selhardening biostimulating activity  
 paste가 가 ,

19). Muzzarelli (1993)<sup>6)</sup> 10 , chitin  
 3 가 , ,

freeze-  
 dried methylpyrrolidinone chitosan(soft sponge ,  
 material) 가 chitosan .

lysozyme 6 , chitosan

, chitosan

1 William (1983) chitosan  
 in vivo methylpyrrolidinone chitosan

Muzzarelli (1993)<sup>20)</sup> freeze- chitosan  
 dried methylpyrrolidinone chitosan  
 , 60 Reynolds(1960)  
 , chitosan N-acetylglucosamine  
 chelating ability , - , Muzzarelli (1988)  
 endosteal-periosteal and bone marrow N-carboxyalkyl chitosan  
 osteoblast-like precursors가 glycosaminoglycans ,

Muzzarelli (1994)<sup>21)</sup> Muzzarelli (1989)<sup>15)</sup>  
 imidazole modified chitosan(IMIC) chitosan ascorbate gel

good structural organization wound dressing regeneration ordered , 가

가 Tarsi (1997)<sup>9)</sup> hydroxyapatite modified chitosan Streptococcus mutans가 HA chitosan S. mutans가

freeze-dried N-carboxybutyl chitosan soft pad

가 , N-carboxybutyl chitosan Hirano (1989)<sup>36)</sup> lysozyme chitinase 가 N-acetyl group , chitosan

Biagini (1991)<sup>16)</sup> Biagini (1991)<sup>18)</sup> N-carboxybutyl chitosan expander , chitosan in vitro chitosan 가

( 10), chi-tosan 0.2% acetic acid , chitosan

Muzzarelli (1990)<sup>8)</sup> N-carboxybutyl chitosan 6.2 ± 1.2 Klokkevold (1997)<sup>5)</sup> 3.6 ± 0.6 in vitro

chitosan ascorbic acid -glycerophosphate, dexamethasone . ascorbic acid (procollagen molecule) proline hydroxylation

2 ATPase, N-carboxybutyl chitosan wound dressing synthetic glucocorticoid -glycerophosphate . N-carboxybutyl chitosan 가

. dexamethasone in vitro ,  
가  
가 ,  
dexam- 1. chitosan  
ethasone 가,  
osteopontin, 9 , 12  
osteocalcin 가 .  
dexamethasone .  
in vitro . 2. chitosan 3 가  
, , 가, 7  
cytokines (37-39).  
Alizarin red S anthraquinone 3. chitosan 3 , 7  
calcium chelate 가  
magnesium, ,  
mangan, barium, strontium, iron 7  
calcium  
calcium  
가  
chitosan  
가 in 4. chitosan 가 (P<0.01).  
vivo chitosan  
chitosan  
chitosan  
가 chitosan

V.

VI.

chitosan(Poly-N-Acetyl Gluco-  
saminoglycan) ,

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Cohen, D. W. : Contempory  
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## Effects of chitosan on the characteristics of periodontal ligament, calvaria cells and gingival fibroblasts

Sun-Hee Kim, Young-Hyuk Kwon, Man-Sup Lee, Joon-Bong Park, Yeek Herr  
Department of Periodontology, College of Dentistry, Kyung-Hee University

Chitosan, with a chemical structure similar to hyaluronic acid, has been implicated as a wound healing agent. The purpose of this research was to evaluate the effects of chitosan on the characteristics of periodontal ligament cells, calvaria cells and gingival fibroblasts and to define the effects of chitosan on bone formation in vitro.

In control group, the cells were cultured alone with Dulbecco's Modified Eagle's Medium contained with 10% Fetal bovine serum, 100unit/ml penicillin, 100 µg/ml streptomycin, 0.5 µg/ml amphotericin-B. In experimental group, chitosan(40 µg/ml) is added into the above culture condition. And then each group was characterized by examining the cell proliferation at 1, 3, 5, 7, 9, 12, 15 day, the amount of total protein synthesis, alkaline phosphatase activity at 3, 7 day and the ability to produce mineralized nodules of rat calvaria cell at 11 day.

The results were as follows :

1. At early time both periodontal ligament cells and calvaria cells in chitosan-treated group proliferated more rapidly than in non-treated control group, but chitosan-treated group of periodontal ligament cells at 9 days and calvaria cells at 12days showed lower growth rate than control group.  
Gingival fibroblast in chitosan-treated group had lower growth rate than in control group but the difference was not statistically significant ( $P < 0.01$ ).
2. Both periodontal ligament cells and calvaria cells in chitosan-treated group showed much protein synthesis than in control group at 3 days, but showed fewer than in control group at 7 days. Amount of total protein synthesis of gingival fibroblast didn't have statistically significant difference among the two groups( $P < 0.01$ ).
3. At 3 and 7 days, alkaline phosphatase activity of periodontal ligament cells and calvaria cells was increased in chitosan-treated group, but at 7 days there was not statistically significant difference among the two groups of calvaria cells ( $P < 0.01$ ). Alkaline phosphatase activity of gingival fibroblast didn't have statistically significant difference among the two groups( $P < 0.01$ ).
4. Mineralized nodules in chitosan-treated group of rat calvaria cells were more than in control

group.

In summery, chitosan had an effect on the proliferation, protein systhesis, alkaline phosphatase activity of periodontal ligament cells and calvaria cells, and facilitated the formation of bone. It is thought that these effects can be used clinically in periodontal regeneration therapy.