

Glycosaminoglycan

I.

Fardal

8), Adelson

9).

10),

11),

12),

13),

14)

1-3).

가

가

가 4,5),

가

15).

Glycosaminoglycan

N - acetyl - hexosamine

가

hexose

hexuronic acid

가

6,7).

가

chondroitin sulfate, dermatan

sulfate, heparan sulfate, heparin, keratan sulfate, hyaluronic acid

dermatan sulfate, chondroitin sulfate, heparan sulfate

glycosaminoglycan

0.3%

16), 17), 18), 19)

glycosaminoglycan

가

dermatan sulfate

dermatan sulfate 60%

, 5% heparan sulfate

hyaluronic acid, chondroitin sulfate

chondroitin sulfate가 가

dermatan

sulfate, chondroitin sulfate가 가

glycosaminoglycan 20).

glycosaminoglycan 가

. Dermatan sulfate

chondroitin sulfate

heparan sulfate

glucuronidase,

collagenase, elastase, hyaluronidase, neutral proteinase, heparinase, chondrosulfatase

gly -

cosaminoglycan

21),

dermatan sulfate

, chondroitin sulfate 가

22). dermatan sulfate

chondroitin sulfate 가

23).

가

chondroitin

sulfate, heparan sulfate, dermatan sulfate, hyaluronic acid

24).

glycosaminoglycan

25).

glycosaminoglycan

dermatan sulfate, chondroitin sulfate, heparan sulfate

II.

1. Glycosaminoglycan

Glycosaminoglycan

가

dermatan sulfate(Sigma chemical Co., USA), chondroitin sulfate(Sigma chemical Co., USA), heparan sulfate(Sigma chemical Co. USA), 10⁻⁷g/ML, 10⁻⁶g/ML, 10⁻⁵g/ML, 10⁻⁴g/ML

2.

Dulbecco ' s Modified Eagle ' s Medium(DMEM, Gibco Co., USA)

15ML tube

3

, 10% FBS(fetal bovine serum, Gibco Co., USA) 1%

(Penicillin G 10,000 units/ml, Amphotericin B 25µg/ML, Gibco Co., USA)가

가 DMEM 100mm
 No. 15 blade 1mm²
 , 60mm 5 - 6
 . 30 37 , 100%
 , 5% CO₂
 10% FBS 1% 가
 DMEM 3Mℓ 가
 2 - 3
 2 , 0.25% Trypsin/EDTA (1 × ,
 Gibco Co., USA)
 60mm
 2 - 3
 1:3 - 4
 5 - 8
 3. 가
 3 - (4,5 - dimethylthiazol - 2 - y) -
 2,5 - diphenyl tetrazolium bromide (MTT)
 . MTT
 dehydroge -
 nase formazan
 0.25% trypsin/EDTA
 trypan blue hemocy -
 tometer 24 - well plate
 well 1 × 10⁴ 가
 , dermatan sulfate, heparan
 sulfate, chondroitin sulfate 10⁻⁷g/Mℓ, 10⁻
⁶g/Mℓ, 10⁻⁵g/Mℓ, 10⁻⁴g/Mℓ 가
 90 , 3 , 12

DMEM

DMEM 1

Mℓ well 가
 PBS(10 × phos -
 phate buffered saline, Gibco Co., USA)
 MTT (Sigma Co. USA) 가
 formazan 500μℓ
 DMSO (Dimethyl sulfoxide, Sigma, USA)
 가 96 - well plate
 ELISA analyser (Spectra. MAX 250,
 Molecular Devices Co. USA) 570 nm
 4. 가
 trypsin -
 EDTA trypan blue
 hematocytometer 24 - wall
 plate well 1 × 10⁴ 가 가
 1
 37 , 5% CO₂, 100%
 DMEM 450μℓ well
 가 dermatan sulfate, chon -
 droitin sulfate, heparan sulfate 10⁻⁷g/Mℓ,
 10⁻⁶g/Mℓ, 10⁻⁵g/Mℓ, 10⁻⁴g/Mℓ 가
 48 , 72
 DMEM . 48 72
 PBS 300μℓ MTT
 well formazan
 500μℓ DMSO 가 formazan
 96 - well plate
 ELISA analyser (Spectra. MAX 250,
 Molecular Devices Co. USA) 570 nm
 5.

SPSS WIN 8.0

glycosaminoglycan

Table 1. Attachment of gingival fibroblasts after dermatan sulfate application (mean±S.D.)

time	control	10 ⁻⁷ g/Mℓ	10 ⁻⁶ g/Mℓ	10 ⁻⁵ g/Mℓ	10 ⁻⁴ g/Mℓ
90 minute	0.531 ± 0.005	0.534 ± 0.010	0.523 ± 0.009	0.522 ± 0.007	0.520 ± 0.017
3 hour	0.541 ± 0.010	0.551 ± 0.009	0.528 ± 0.015	0.521 ± 0.002	0.517 ± 0.025
12 hour	0.641 ± 0.017	0.653 ± 0.016	0.645 ± 0.012	0.645 ± 0.021	0.636 ± 0.009

Table 2. Attachment of gingival fibroblasts after chondroitin sulfate application (mean±S.D.)

time	control	10 ⁻⁷ g/Mℓ	10 ⁻⁶ g/Mℓ	10 ⁻⁵ g/Mℓ	10 ⁻⁴ g/Mℓ
90 minute	0.546 ± 0.012	0.546 ± 0.022	0.552 ± 0.024	0.543 ± 0.017	0.542 ± 0.020
3 hour	0.554 ± 0.006	0.548 ± 0.014	0.559 ± 0.016	0.547 ± 0.018	0.546 ± 0.010
12 hour	0.667 ± 0.010	0.664 ± 0.004	0.671 ± 0.018	0.659 ± 0.020	0.659 ± 0.015

Table 3. Attachment of gingival fibroblasts after heparan sulfate application (mean±S.D.)

time	control	10 ⁻⁷ g/Mℓ	10 ⁻⁶ g/Mℓ	10 ⁻⁵ g/Mℓ	10 ⁻⁴ g/Mℓ
90 minute	0.538 ± 0.008	0.565 ± 0.005	0.562 ± 0.008	0.568 ± 0.008	0.581 ± 0.013
3 hour	0.547 ± 0.009	0.571 ± 0.010	0.578 ± 0.008	0.584 ± 0.014	0.588 ± 0.011
12 hour	0.648 ± 0.008	0.638 ± 0.016	0.651 ± 0.035	0.655 ± 0.018	0.667 ± 0.030

: Significantly different from the control(p<0.05), (N=5)

Table 4. Proliferation of gingival fibroblasts after dermatan sulfate application (mean±S.D.)

hours	control	10 ⁻⁷ g/Mℓ	10 ⁻⁶ g/Mℓ	10 ⁻⁵ g/Mℓ	10 ⁻⁴ g/Mℓ
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one way ANOVA t - test
95%

(p<0.05).

III.

2. Chondroitin sulfate가

1. Dermatan sulfate가

가 10⁻⁶g/Mℓ 90 , 3
, 12
, 10⁻⁷g/Mℓ
가 , 10⁻

Table 5. Proliferation of gingival fibroblasts after chondroitin sulfate application (mean±S.D.)

hours	control	10 ⁻⁷ g/Mℓ	10 ⁻⁶ g/Mℓ	10 ⁻⁵ g/Mℓ	10 ⁻⁴ g/Mℓ
48	0.897±0.017	0.895±0.015	0.883±0.050	0.900±0.037	0.902±0.052
72	1.045±0.031	1.050±0.005	1.093±0.018	1.102±0.015	1.119±0.013

Table 6. Proliferation of gingival fibroblasts after heparan sulfate application (mean±S.D.)

hours	control	10 ⁻⁷ g/Mℓ	10 ⁻⁶ g/Mℓ	10 ⁻⁵ g/Mℓ	10 ⁻⁴ g/Mℓ
48	0.928±0.015	0.975±0.018	0.954±0.011	0.981±0.028	1.127±0.036
72	1.034±0.039	1.091±0.013	1.098±0.044	1.141±0.032	1.492±0.010

: Significantly different from the control(p<0.05), (N=5)

3. Heparan sulfate

가 가
가 , 90
10⁻⁴g/Mℓ 3 10⁻⁵g/Mℓ, 10<sup>-
4g/Mℓ 가 . 72</sup>

6. Heparan sulfate가

가 가
가 , 48 10⁻⁴g/Mℓ
72 10⁻⁵g/Mℓ, 10⁻⁴g/Mℓ
가 .

4. Dermatan sulfate가

48 가
. 72
가

IV.

5. Chondroitin sulfate가

48 10⁻⁷g/Mℓ, 10⁻⁶g/Mℓ 가
. 10⁻⁵g/Mℓ, 10⁻⁴g/Mℓ 가
가 ,
. 72

glycosaminoglycan

cosaminoglycan

cosaminoglycan

gly -

gly -

dermatan sulfate, chondroitin sulfate, heparan sulfate
 Dermatan sulfate 20 - 50 KDa

glycosaminoglycan
 60% . Chondroitin sulfate
 가 gly -
 cosaminoglycan , , ,
 . 150 - 200 KDa
 . Heparan sulfate
 , 4 , FGF
 - , -
 ,
 26).

glycosaminoglycan
 . Takagi
 chondroitin sulfate dermatan sulfate
 gly -
 cosaminoglycan chondroitin sulfate
 dermatan sulfate가
 , chondroitin sulfate
 27). Kirkham
 glycosaminoglycan
 gly -
 cosaminoglycan ,
 28).

Dermatan sulfate
 ,
 가 . Dermatan sulfate
 가
 glycosaminoglycan ,
 5%
 가 , dermatan sulfate
 가 chondroitin sulfate heparan
 sulfate 29). Chondroitin sulfate
 glycosaminoglycan 10%
 ,

가 가 .
 Barthold Page
 29).
 glycosaminoglycan
 Dahllorf phenytoin
 der -
 matan sulfate 가 30).
 Matsukawa
 glycosaminoglycan
 dermatan sulfate, chondroitin sulfate가
 , chondroitin sulfate
 , dermatan
 sulfate
 ,
 31). gly -
 cosaminoglycan
 ,
 . Penc
 dermatan
 sulfate, heparan sulfate가 FGF - 2
 , chondroitin
 sulfate
 . FGF - 2 ,
 glycosaminoglycan
 ,
 glycosaminoglycan
 FGF - 2 85% ,
 가 glycosamino -
 glycan
 32). Nagasawa Tawa
 48 heparan
 sulfate $10^{-5}g/Ml$, dermatan sul -
 fate $10^{-4}g/Ml$ 가
 , chondroitin sulfate
 , 48 heparan
 sulfate $10^{-4}g/Ml$, dermatan sulfate

10⁻⁵ g/Mℓ 가 ,
chondroitin sulfate
가 33).

Tawa
Nagasawa
, dermatan sulfate chondroitin
sulfate

heparan sulfate 10⁻⁴g/Mℓ
48 72 , 10⁻⁵g/Mℓ 72
가

가 Penc
heparan sulfate
dermatan sulfate chondroitin
sulfate

glycosaminoglycan
가

가 .
glycosaminoglycan ,
가 가
glycosaminoglycan chondroitin sulfate,
heparan sulfate가 , chondroitin
sulfate dermatan sulfate

,
, Caffesse

가 가 34). Pitaru

heparan sulfate 가

12).
dermatan sulfate 90 ,
3 , 12 10⁻⁷g/Mℓ, chondroitin
sulfate 10⁻⁶g/Mℓ
가 가 ,

, heparan sul -
fate
가 가 , 90 10⁻⁴g/Mℓ,
3 10⁻⁵g/Mℓ, 10⁻⁴g/Mℓ
가 .

glycosaminoglycan , 가

heparan sulfate가

가

V.

가

glycosaminoglycan dermatan sulfate, chondroitin sulfate, heparan sulfate가

가

1. Dermatan sulfate

90 3 , 12

48 72

(p<0.05).

2. Chondroitin sulfate

90 3 , 12

48 72

(p<0.05).

3. Heparan sulfate

90 10⁻⁴g/Mℓ 3

10⁻⁵g/Mℓ, 10⁻⁴g/Mℓ

가 , 48

10⁻⁴g/Mℓ 72 10⁻⁵g/Mℓ, 10⁻⁴g/Mℓ 가

(p<0.05).

heparan sulfate가

가

, dermatan

sulfate chondroitin sulfate

VI.

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- Abstract -

Effects of Glycosaminoglycan on the Growth of Human Gingival Fibroblast

Yong - Bae Lee, Sung - Hee Pi, Tak kim,
Kwang - Soo Lee, Hyung - Keun You,
Hyung - Shik Shin

Department of Periodontology, School of
Dentistry, Wonkwang University

Gingival fibroblasts are embedded in an extracellular matrix. The matrixes have influence on the development, polarity, and behavior of nearby cells. The major component of periodontal extracellular matrix is a glycosaminoglycan. The glycosaminoglycans are large carbohydrates that are composed of repeating disaccharide units and exist in three main forms: dermatan sulfate, chondroitin sulfate, heparan sulfate. The purpose of present study is to examine the biologic effects of glycosaminoglycan on human gingival fibroblast. Human gingival fibroblasts were supplemented with each glycosaminoglycan, and cellular attachment and proliferation was determined by MTT assay. Dermatan sulfate and chondroitin sulfate did not stimulate the attachment and proliferation of human gingival fibroblasts, but heparan sulfate increased the prolifera -

tion and attachment in a time - and dose - dependent manner.

These results indicated that heparan sulfate seems to have a high potential for gingival regeneration and root surface attachment.