

1 . 2 . 2 . 1 . 1 . 1 . 1 . 1

1
2

I.

1976 Melcher가

가

가
가 ¹⁾

2-4)

, Nyman ⁵⁾

Gottlow ⁶⁾
(Guided Tissue

Regeneration: GTR) '

가 가 가

polylactide

polytetrafluoroethylene

expanded

가

가

가

가

(ion beam irradiation)

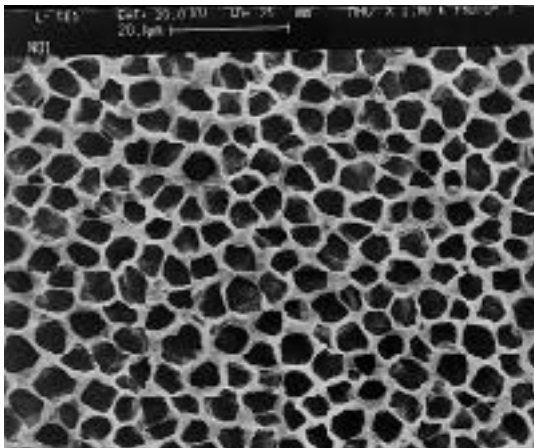


Figure 1. Scanning electron micrograph of PLLA membrane

376,000 poly(lactide)(PLLA)
24

PLLA

가 150μm

8μm

(Figure 1).

2. PLLA

30 - 400 KeV

가

(ion implanter, High Voltage Inc, USA)

PLLA

35 KeV

(Ar⁺)

5 × 10¹³, 5 ×

10¹⁴, 5 × 10¹⁵ ions/cm²

PLLA

3.

PLLA

II.

1. (Poly L-lactic acid, PLLA)

10μl

PLLA

PLLA

(Kernco Ins. Co., TX, USA)

5.2g/d, 20%
polyglycolic acid(PGA,) mesh

4. PLLA coating gold - palladium

18 - 19

Sprague - Dawley

Bellows ¹⁷⁾ 24 PLLA

heamocytometer HBSS(Gibco) 3 - 4

10% FBS(Gibco, NY, USA), 1% antibiotic - antimycotic solution(Gibco)가 - MEM(Gibco) trypsin - EDTA(Gibco) , 0.25%

5 × 10⁶ cell/ml 가

24 - well plate(Nunc, NY, USA) heamocytometer

vacuum grease , flow cytometer (FACS Caliber, Bectron Co., CA, USA)

PLLA , Ar⁺ PLLA

50ul

wetting 20μl

가 가 cell 가 1cm x 7. Alkaline phosphatase(ALPase)

1cm 10⁵cells/cm²가

37 , 5% CO₂ cell

3 1ml

가 가 , 24

5. 0.1 ml 0.1ml 0.1 M glycin - NaOH buffer, 0.1 ml 15 mM para - nitrophenol phosphate(PNPP), 0.1% Triton X - 100 0.1 ml 30 37 °C

(Stereoscan 360, Cambridge Ins., Cambridge, UK) 2.5 ml 0.1 N NaOH

가

HBSS(Gibco) 3 - 4 ELISA reader 405 nm

0.1M PBS(pH 7.4) para -

2.5% glutaraldehyde 4 40 nitrophenol

0.1M PBS

0.1M PBS 1% 8.

osmium tetroxide 0 40

-70 24

Table 1. Contact angles in each dose of ion beam irradiated PLLA membranes

	Control	5×10^{13}	5×10^{14} (ions/cm ²)	5×10^{15}
contact angle ^a ($^{\circ}$)	133.0 ± 1.0	$81.0 \pm 5.6^*$	$68.3 \pm 2.9^*$	$82.0 \pm 3.0^*$

^amean \pm s.d., N=4; *:P<0.01, as compared with control,

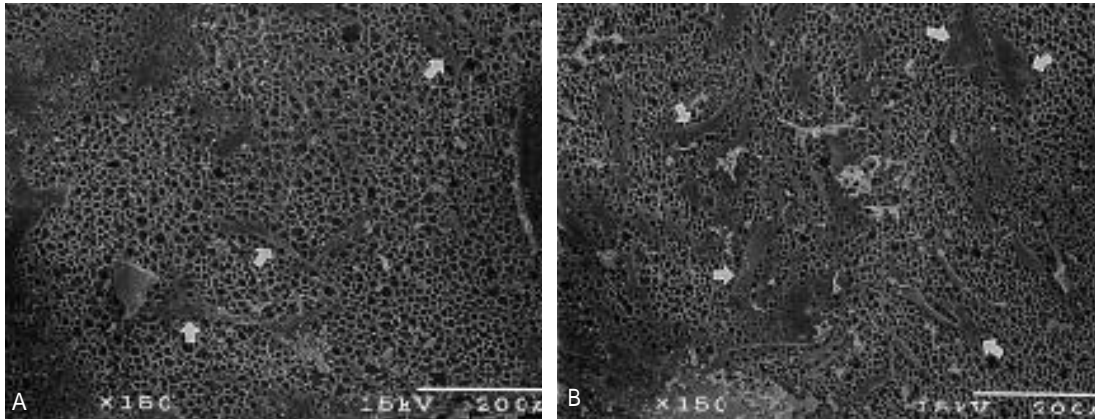


Figure 2. Scanning electron micrograph of osteoblasts attached on the PLLA membrane. Arrows indicate osteoblasts. A: control PLLA membrane, B: ion beam(5×10^{15} ions/cm²) irradiated

ALPase, 5×10^{14} ions/cm², $68.3 \pm 2.9^{\circ}$
 unpaired t - test, P<0.01, 5×10^{15} ions/cm², $82.0 \pm 3.0^{\circ}$
 (Table 1, Figure 2).

III.

2.

1.

(Figure 3A).

가

5×10^{14} ions/cm² 가
 $133.0 \pm 1.0^{\circ}$
 5×10^{13} ions/cm²
 $81.0 \pm 5.6^{\circ}$, 5

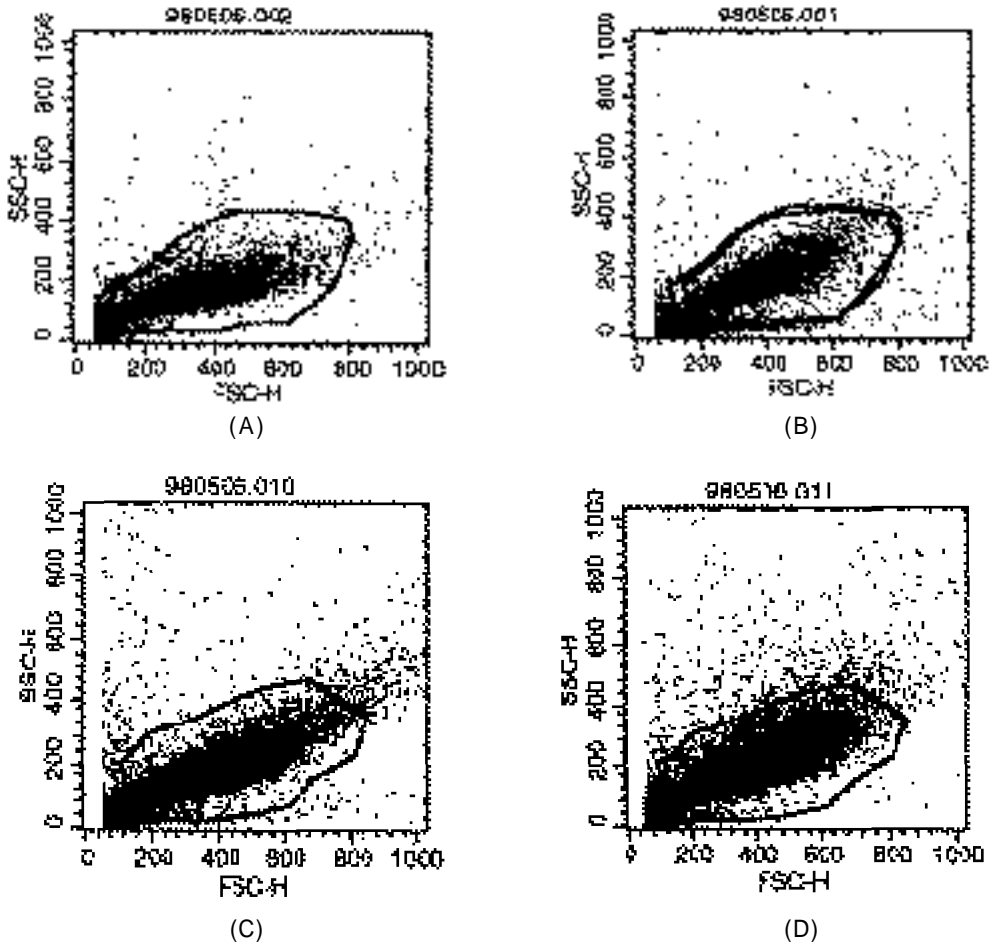


Figure 3. Viability of adhered osteoblasts onto ion beam irradiated PLLA membranes (findings by flow cytometer). A: control PLLA membrane, B: 5×10^{13} ions/cm² irradiated PLLA membrane, C: 5×10^{14} ions/cm² irradiated PLLA membrane, D: 5×10^{15} ions/cm² irradiated PLLA membrane.

Table 2. Cellular attachment in each dose of ion beam irradiated PLLA membranes

	Control	5×10^{13}	5×10^{14} (ions/cm ²)	5×10^{15}
Number of attached Osteoblasts ^a (10 ⁴ cell/cm ²)	5.53 ± 0.51	7.25 ± 0.65*	8.48 ± 0.46*	7.75 ± 0.73*

^amean ± s.d., N=4; *:P<0.01, as compared with control

(Figure 3B).

3.

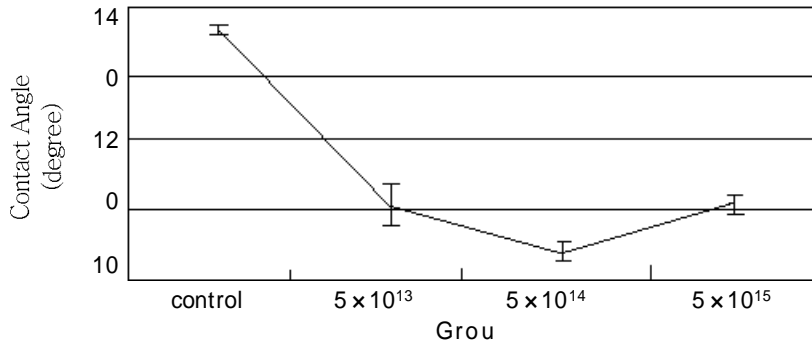


Figure 4. Contact angles in each dose of ion beam irradiated PLLA membranes

Table 3. ALPase activities of osteoblasts attached on each dose of ion beam irradiated PLLA mem -

	Control	5×10^{13}	5×10^{14} (ions/cm ²)	5×10^{15}
ALPase activity ^a (nM of PNP/30 min/10 ⁴ cells)	5.52 ± 1.17	5.56 ± 0.84	6.50 ± 0.62	6.06 ± 0.27

^amean ± s.d., N=4; all test groups were not significantly different from control group.

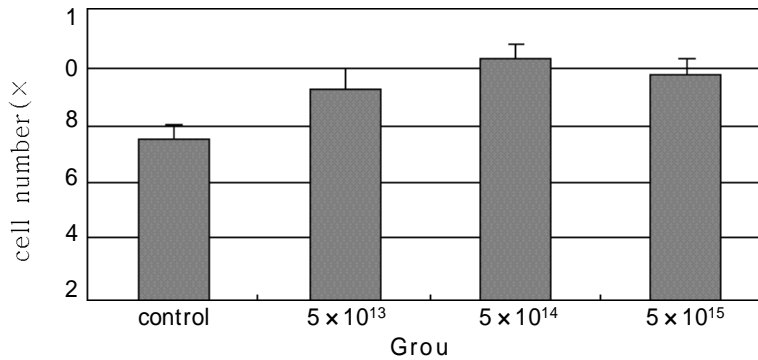


Figure 5. Cellular attachment in each dose of ion beam irradiated PLLA membranes

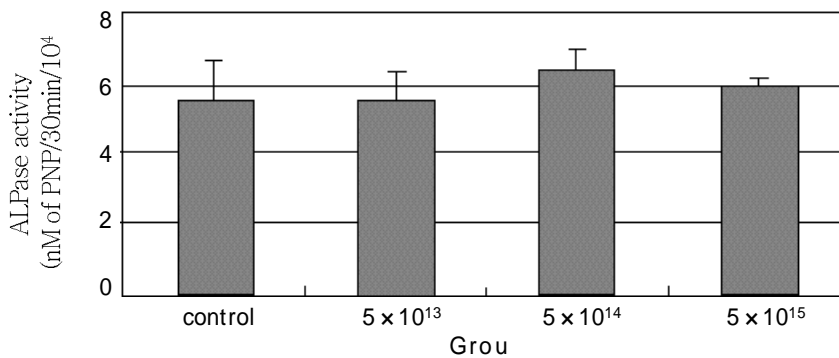


Figure 6. ALPase activities of osteoblasts attached on each dose of ion beam irradiated PLLA mem -

Flowcytometer

(Figure 4).

cell/cm²) 5.53 ± 0.51 (10⁴
5 × 10¹³, 5 × 10¹⁴, 5
× 10¹⁵ ions/cm² 7.25 ± 0.65, 8.48
± 0.46, 7.75 ± 0.73

가

5 × 10¹⁴ ions/cm² 가
(Table 2, Figure 4).

4.

ALPase

ALPase

Table 3

ALPase

5.52 ±

1.17 (nM of PNP/30 min/10⁴ cells)

5 × 10¹³, 5 × 10¹⁴, 5 × 10¹⁵ ions/cm²

5.56 ± 0.84, 6.50 ± 0.62, 6.06 ± 0.27

, 5 × 10¹⁴ 5 × 10¹⁵ ions/cm²

가

(Table 3,

Figure 5).

IV.

Hardwick ¹⁹⁾

가

Suzuki

가

가

10, 11).

가

가

가
 , Ar⁺
 가 PLLA
 Suzuki ^{10, 11}), Nakao
 flow cytometry
 12) Pignataro ¹⁶)
 , ALPase
 ,
 가 PLLA

가
 ,
 ,
 ,
 (implantation)
 ,
 .

V.
 가
 ,
 가
 5 × 10¹⁴ Ar⁺ 가
 가 5 × 10¹⁵ 가
 가
 가 (Ar⁺)

PLLA
 가
 5 × 10¹³ 5 × 10¹⁴ Ar⁺ 가
 1.
 ,

가
 가
 , 1 × 10¹⁶ 가
 10¹⁴ Ar⁺ ions/cm² 가 5 ×
 2.
 ,

(carbonized phase)
¹⁰⁻¹²),
 3.
 (surface free energy)

¹³⁻¹⁵). Pignataro
 (10¹⁵ ions /cm²)
 가
 10¹⁴ Ar⁺ ions/cm² 가 , 5 ×
 16),
 가

4. ALPase
 5×10^{14} , 5×10^{15} Ar⁺ ions/cm²
 가

VI.

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- Abstract -

Improved cell adhesion to ion beam-irradiated biodegradable membranes

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Ion irradiation is a very promising tool to modify the chemical structure and physical properties of polymers. This study was aimed to evaluate the cellular adhesion to ion beam - irradiated surface of biodegradable poly - l - lactide(PLLA) membrane. The PLLA membrane samples were irradiated by using 35 KeV Ar⁺ to fluence of 5×10^{13} , 5×10^{14} and 5×10^{15} ion/cm². Water contact angles to control and each dose of ion beam - irradiated PLLA membranes were measured. Cultured fetal rat calvarial osteoblasts were seeded onto control and each dose of ion beam - irradiated PLLA membranes and cultured. After 24 hours, each PLLA membranes onto which osteoblasts attached were examined by scanning electron microscopy (SEM). Osteoblasts were removed from each PLLA

membrane and then, the vitality and the number of cells were calibrated. Alkaline phosphatase of detached cells from each PLLA membranes were measured. Ion beam - irradiated PLLA membranes showed no significantly morphological change from control PLLA membranes. In the measurement of water contact angle to each membrane, the dose range of ion beam employed in this study reduced significantly contact angles. Among them, 5×10^{14} ion/cm² showed the least contact angle. The vitalities of osteoblasts detached from each membranes were confirmed by flow cytometer and well attached cells with their own morphology onto each membranes were observed by SEM. A very strong improvement of the cell adhesion and proliferation was observed for ion beam - irradiated surfaces of PLLA membranes. 5×10^{14} ions/cm² exhibited the most strong effect also in cellular adherence. ALPase activities also tended to increase in ion beam - irradiated membranes but statistical differences were not found. These results suggested that ion beam irradiation is an effective tool to improve the adhesion and spreading behaviour of the cells onto the biodegradable PLLA membranes for the promotion of membrane - tissue integration.

Key Words: ion beam irradiation, cell adhesion, biodegradable poly - l - lactide(PLLA) membranes, tissue integration