The effect of fibronectin derived fibrin-binding peptide fragments on periodontal ligament cell migration : an in vitro wound healing model

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Abstract

One of the major components of ECM is fibronectin (FN), a large glycoprotein, which interacts with cells and transmits signals through numerous receptors. FN mediates a wide variety of cellular interactions with ECM and plays important roles in cell adhesion, migration, proliferation, and differentiation. By means of Scratch wound assay using synthetic oligopeptide, the effect of each fragments on cell migration was investigated.

Background and Aim

Aim of the study is to examine the effect of synthetic FN fragments including fibrin binding sites on migration of osteoblast-like cells via *in vitro* scratch assay.

Methods and Materials

- MC3T3-E1 osteoblast-like cells
- Synthetic oligopeptide
- FF1**(CYDNGKHYQ), **FF3**(CFDKYTGNTYR), **FF5**(CTSRNRCNDQ)
 - diluted to **50, 100** and **150 μM**
 - coated to 4-well cell culture plate
- Scratch wound assay
 - 1 × 10⁵ cells/well
 - parallel scratches with 200μl plastic pipette tips
 - pretreated with mitomycin C(30μg/ml) 1 hr before wounding
- Fixation and Staining
 - 14, 20hr after wounding with Multiple stain
- Migration rate (%)
 - = area of migrated cells (A) / cell free area (A+B)

Results

- Scratched MC3T3 monolayers

- Migration rate (%)

peptide	conc.(μM)	14h(%)	p-value	20h(%)	p-value
Control	0	23.5±1.2		33.8±3.0	
FF1	50	26.1±1.6	0.038*	36.3±3.7	0.430
	100	29.6±2.3	0.000*	38.4±3.4	0.049*
	150	21.5±1.8	0.125	43.7±3.2	0.000*
FF3	50	31.7±1.4	0.000*	36.4±2.6	0.390
	100	35.1±1.5	0.000*	44.7±4.4	0.000*
	150	28.7±3.5	0.000*	34.0±2.5	0.999
FF5	50	33.6±2.1	0.000*	39.3±4.0	0.006*
	100	35.1±2.3	0.000*	47.6±2.6	0.000*
	150	25.2±1.6	0.274	29.6±2.1	0.042*

n=8, *significantly different from control(p<0.05)

All peptide groups except 150μM showed statistically significant increase in migration rate at 14 hr. At 20 hr all experimental groups of 100μM and FF1 of 150μM concentration were significantly improved the scratch closure.

Significantly increased migration was observed at type I repeats 2-5 fibrin-binding fragments of FN – FF3 and FF5 - added MC3T3-E1 cells. Optimum concentration was 100μM.

The biologic effect of fibrin-binding oligopeptide in this study supports the FN's essential contribution in bone regeneration as a temporary scaffold in FN-fibrin matrix.

Conclusions

Fibrin-binding oligopeptides, 100μM of FF3 and FF5, derived from FN enhanced the migration rate of MC3T3-E1 osteoblast-like cells in *in vitro* wound healing model and these peptdies can be applied to enhance the early wound healing capacity of biomaterials inserted into a bone.

References

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