Nano controlled dual release of bone morphogenic protein-2 and insulin-like growth factor-1 and titanium surface microgrooves enhance osteoblastic differentiation of human mesenchymal stem cells

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Abstract

We aimed to demonstrate that nano controlled dual release of bone morphogenic protein-2 (BMP-2) and insulin-like growth factor-1 (IGF-1) by catechol-functionalized adhesive polymer nanoparticles on microgrooved titanium (Ti) surface enhances *in vitro* osteoblastic differentiation of human mesenchymal stem cells (MSCs). Photolithography was used to fabricate the microgrooved Ti, and catechol-functionalized adhesive nanoparticles were immobilized onto Ti surfaces for controlled dual release of BMP-2 and IGF-1. After characterizing the release profiles of BMP-2 and IGF-1 from the nanoparticle-immobilized Ti substrata, we assessed the alkaline phosphatase activity and osteoblastic differentiation and confirmed the result by determining the expression of major osteoblast marker genes and proteins. The proposed combined surface of microgrooves and growth factor release system can be used as a strong osteogenic promoter on biomaterial surfaces. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Republic of Korea (NRF-2015R1D1A1A01057956).

Background and Aim

- To establish the Catechol-poly(L-aspartic acid)-b-poly(L-phenylalanine) (Cat-PASP-PPhe-NP) nanoparticle as the dual-releasing system of BMP-2 and IGF-1.
- To investigate the synergistic effect of microgrooved surface structures and the combined release of BMP-2 and IGF-1 on osteoblastic differentiation of human MSCs.

Methods and Materials

- Photolithography was used to fabricate the microgrooved Ti.

- Catechol-functionalized Ti-adhesive nanoparticle and microgrooved Ti substrata with surface-immobilized nanoparticles incorporating BMP-2 and IGF-1 for inducing combined dual release of the growth factors.

Fig. 1. Schematic illustration for immobilization of adhesive nanoparticles on microgrooved Ti, and incorporation and sequential release of BMP-2 and IGF-1.

- The anionic PAsp shell of immobilized Cat-PASP-PPhe-NPs offers a loading area of positively charged BMP-2 and IGF-1.

Fig. 2. Self-assembly of Catechol-PASP-b-PPhe to form Ti-adhesive nanoparticles for BMP-2 loading/release.

- Control & Experimental groups

Table 1. Titanium (Ti) substrata with various surface topographies used in this study (μm).						
	Groove (ridge) width	Groove depth	Bottom width	Acid-etching	NP application	Growth factor
NE0	0	0	0	Non-etched	N/A	N/A
E0	0	0	0	Acid-etched	N/A	N/A
E60/10	60	10	40	Acid-etched	N/A	N/A
NE0NP	0	0	0	Non-etched	NP applied	N/A
E0NP	0	0	0	Acid-etched	NP applied	N/A
E60/10NP	60	10	40	Acid-etched	NP applied	N/A
NE0BMP2	0	0	0	Non-etched	NP applied	BMP-2 only
E0BMP2	0	0	0	Acid-etched	NP applied	BMP-2 only
E60/10BMP2	60	10	40	Acid-etched	NP applied	BMP-2 only
NE0IGF1	0	0	0	Non-etched	NP applied	IGF-1 only
E0IGF1	0	0	0	Acid-etched	NP applied	IGF-1 only
E60/10IGF1	60	10	40	Acid-etched	NP applied	IGF-1 only
NE0BMP2IGF1	0	0	0	Non-etched	NP applied	BMP-2/IGF-1
E0BMP2IGF1	0	0	0	Acid-etched	NP applied	BMP-2/IGF-1
E60/10BMP2IGF1	60	10	40	Acid-etched	NP applied	BMP-2/IGF-1

NE0: ground Ti substrata.
E0: NE0 with subsequent HF etching.
E60/10: Ti substrata with surface microgrooves of 60 μm width and 10 μm depth, and with subsequent HF etching.
BMP2: Ti substrata (a) with controlled nanoparticle-encapsulated BMP-2 releasing system.
IGF1: Ti substrata (b) with controlled nanoparticle-encapsulated IGF-1 releasing system.
BMP2IGF1: Ti substrata (c) with controlled sequential nanoparticle-encapsulated dual growth factor (BMP-2 and IGF-1) releasing system.
HF: hydrofluoric acid.
NP: nanoparticle.
N/A: non-applicable.

Results

FE-SEM

- The surface morphology of the Cat-PASP-PPhe-NPs on the microgrooved Ti substrata was observed by FE-SEM (JSM-6700F, JEOL, Tokyo, Japan).
- Well defined nanoparticles with even distribution on Ti surfaces were observed.

Microgroove-Growth Factors

- Green-fluorescent FITC-labeled BMP-2 (FITC-BMP-2) and red-fluorescent TRITC-labeled IGF-1 (TRITC-IGF-1) were imaged to visualize the surface loading of BMP-2 and IGF-1 on E60/10.

Growth Factor release kinetics

- Release kinetics of BMP-2 and IGF-1 were analyzed by ELISA (PeproTech for BMP-2 and R&D System for IGF-1).
- Sustained release of both BMP-2 and IGF-1 was determined.

ALP activity

- Alkaline phosphatase (ALP) activity and Extracellular calcium deposition assays.
- E60/10BMP2IGF1 significantly induced the highest ALP activity.

Quantitative real-time PCR

- Quantitative real-time PCR of major osteoblast marker genes such as alkaline phosphatase (ALP), bone sialoprotein II (BSP2), type I collagen α1 (COL1A1), osteocalcin (OCN), osteonectin (ON), osteopontin (OPN), osteix (OSX), runt-related transcription factor 2 (RUNX2).
- E60/10BMP2IGF1 significantly induced the highest level of major osteoblast marker-gene expression.

Western blotting and protein quantification

- E60/10BMP2IGF1 significantly induced the highest level of major osteoblast marker expression.

Conclusion

- Synergistic promoter effect of surface microgrooves and a dual growth factor-releasing system on the osteoblastic differentiation of MSCs.
- The Ti-adhesive nanoparticles were successfully immobilized on the groove-bottom surface as well as the ridge-top surface with a high uniformity.
- BMP-2 and IGF-1 were efficiently loaded on the shell of the nanoparticles immobilized on the microgrooved Ti surfaces, and released in a sustained manner for up to 40 days.
- The microgrooved Ti substrata equipped with a dual growth factor-releasing function provided a favorable surface environment for increasing the expression of various osteoblast marker genes.
- The proposed surface of microgrooves with dual growth factor-release could be used for various biomaterials as a potent osteogenesis promoter.