공학석사학위논문

# Delivery of BMP-2 and Substance P Using Graphene Oxide for Bone Regeneration

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Abstract

# Delivery of BMP-2 and Substance P Using Graphene Oxide for Bone Regeneration

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In the previous study, we used graphene oxides (GO) coated titanium (Ti) substrate for successful BMP-2 delivery vehicle for bone regeneration. A therapeutic protein (bone morphogenetic protein-2, BMP-2) was loaded on the GO-coated Ti substrate. The GO coating on Ti substrate enabled loading of large doses and the sustained release of BMP-2 with preservation of the structure and bioactivity of the drug. For enhancing the bone regeneration efficacy, we combined the

dual delivery with substance P (SP). SP was induced the in vitro mesencymal stem cell migration, which is CD29+ cell. The dual delivery of BMP-2 with SP was expected the in situ cell recruitment to defect site by SP and enhanced bone regeneration both delivered BMP-2. The dual delivery of SP and BMP-2 from the GO coated Ti substrate showed the most bone regeneration efficacy than other groups. However, SP and BMP-2 dual delivery from pristine Ti substrate did not enhance the bone regeneration efficacy than BMP-2 alone delivered from Ti. The appropriate BMP-2 delivery vehicle with SP, has potential as an effective bone regeneration strategy.

keywords : Bone morphogenetic protein-2, Bone regeneration, Graphene oxides, Stem cell recruitment, Substance P

*Student Number* : 2011-21077

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# 1. Introduction

## 1.1. BMP-2 as an osteoinductive growth factor

Bone morphogenetic proteins (BMPs) are the most potent osteoinductive growth factors for bone regeneration. Among the BMPs, bone morphogenetic protein-2 (BMP-2) is a well-known growth factor for inducing osteogenic differentiation of stem cells, and BMP-2 has been used clinically since it got approved by food and drug administration.[1-6] In spite of the great osteoinductivity of BMP-2, several limitations for clinical use of BMP-2 still remain; 1) clinical treatment requires large dose of BMP-2, which possibly leads to several side effects such as overgrowth and uncontrolled bone formation, 2) rapid systemic diffusion of loaded BMP-2 from protein carriers occurs, 3) patients have to bear high cost for large quantity of growth factors, and 4) BMP-2 might provoke immune response.[6-8] To overcome these limitations, appropriate delivery platform should be chosen that makes possible for local delivery of BMP-2 to the sites of interest with sustained release from the carrier.[6,9,10,11]

## 1.2. Substance P; a candidate agent for stem cell recruitment

Osteoregeneration process depends not only on the osteoinductive BMP-2 and the protein carrier but also on osteoregenerative cells such as mesenchymal stem cells (MSCs) or osteoprogenitor cells.[2] To improve regeneration efficiency of defect sites, it is useful to recruit stem cells to the defect area.[19,20] Substance P (SP) is a highly conserved 11-amino acid neuropeptide which mediates pain perception.[12] It has been reported that SP involves many reactions like regulating inflammation, wound healing, and angiogenesis.[13-17] According to the recent study, it was demonstrated that SP promotes recruitment and mobilization of MSCs to the circulation blood.[18] SP has been reported to be effective in tissue regeneration by promoting recruitment of MSCs to damaged tissues, which makes it an ideal candidate agent for promoting endogenous stem cell mobilization/ migration.[16,20,21]

## 1.3. Necessity of appropriate protein carrier

As mentioned earlier, for bioimplants, to choose proper protein carrier is important to achieve effective regeneration in the manner of local delivery and sustained release of protein growth factors. Titanium (Ti) has been widely used for dental implant for its biocompatibility and good mechanical properties.[22-25] Titanium implant itself, however, is lack of integration with bone tissue,[22] many researches proposed titanium implant coated with various inorganic materials such as hydroxyl apatite or calcium phosphate.[22-25] Even though use of inorganic material-coated titanium implants shows improvement of efficiency, there are some problems that loading growth factors could lose their biological activities during inorganic coating steps or could undergo rapid degradation.[26] Using BMP-2 instead of inorganic materials could overcome those problems by inducing osteogenic differentiation of stem cells which could enhance osteointegration of implants by forming bone at the space between the implants and implantation site.[26-28]

## 1.4. Characteristics of GO for protein delivery

In the previous study, we used GO-coated Ti implants for BMP-2 delivery vehicle for bone regeneration.[29] With unique chemical and physical characteristics, GO could be used in biological application.[30-32] Ionized groups on the surface make GO possible for a protein delivery carrier by binding through electrostatic interaction.[29,33,34] Also, GO's hydrophobic domains can interact with protein by hydrophobic π-π stacking.[35-37] Therefore, GO can provide the sustained release kinetics of loaded protein, which is critical for clinical use of protein delivery.

In this study, we delivered SP and BMP-2 to calvarial defects in mice by using the GO-coated titanium implant for effective bone regeneration. In that cell adhesiveness on the GO has been demonstrated,[31,34] recruited stem cells could be well mobilized to the defect sites. Followed in situ osteogenic differentiation of the recruited cells by BMP-2 stimulation enhances bone regeneration efficiency. The bone regeneration efficacy was compared among experimental groups by various analytical methods.

# 2. Materials and Methods

## 2.1. Preparation of GO

Graphite oxide was prepared from graphite using the modified Hummers method.[33] Prepared graphite oxide was exfoliated onto each GO-$COO^-$ sheet by ultrasonification in an ice bath. The resultant brown dispersion was centrifuged at 3470 g for 5 min to remove any unexfoliated powder. Positively charged graphene oxide (GO-$NH_3^+$) was prepared via the 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methyliodide and ethylene diamine (EDC)-mediated amine exchange reaction. EDC was added to the prepared GO-$COO^-$. The solution was rapidly stirred for 12 h, and then dialyzed for 4 days in a membrane tube (MWCO = 12,000-14,000 Da) to remove any residual chemicals after the reactions.

## 2.2. Multilayer Coatings of Ti substrates with GO

The concentration of GO in solutions used in all of the GO coating experiments was fixed at 0.05 % (w/v) without any ionic salts. The GO-$NH_3^+$/GO-$COO^-$ multilayer coating was administered by first treating Ti substrates with a plasma cleaner (Harrick Scientific Product, NY, USA) with O2 plasma for 5 min. The substrates were then first dipped for 10 min in the cationic GO-$NH_3^+$ solution, washed three times by dipping in deionized water for 1 min, and then dried with a gentle stream of nitrogen. The negatively charged GO-$COO^-$ was subsequently deposited onto the GO-$NH_3^+$ coated films using the same adsorption, washing, and drying procedures described above.[29]

## 2.3. Release kinetics of SP and BMP-2

The in vitro release profiles of SP (Millipore Corp., Billerica, MA, USA) and BMP-2 (Cowell Medi Co., Busan, Korea) from Ti or GO-coated Ti substrate (Ti/GO) for various periods were determined by enzyme-linked immunosorbent assays (ELISA; R&D Systems Inc., Minneapolis, MN, USA). The SP- or BMP-2-loaded Ti and Ti/GO was immersed in PBS at 37 °C. At various time points, the supernatant was collected and the concentrations of SP and BMP-2 in the supernatants were determined by ELISA (n = 5 each). $*p < 0.05$ compared with any group.

## 2.4. Bioactivity of *in vitro*-released BMP-2

The bioactivity of BMP-2 released from Ti or Ti/GO was determined by measuring alkaline phosphatase (ALP) activity of osteoblasts. Calvarial osteoblasts were isolated from the calvaria of neonatal (less than 1 day old) Sprague-Dawley rats (SLC, Tokyo, Japan) by a digestive enzymatic process. The bioactivity of the BMP-2 released from the delivery systems in vitro was assessed by determining the ability of BMP-2 to stimulate ALP activity in the cultured rat calvarial osteoblasts. Rat calvarial osteoblasts ($3\times10^4$ cells per well) were plated

in the wells of six-well tissue culture plates (Corning, Corning, NY, USA). Each delivery vehicle containing BMP-2 (2 μg) was placed on a culture insert (Transwell®; Corning) in the culture plates. The culture medium was Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Gaithersburg, MD, USA) containing 10% (v/v) fetal bovine serum (FBS; Gibco BRL) and 1% penicillin/streptomycin (Pen Strep®; Gibco BRL). The medium was changed every 3 days. The ALP activity was determined using p-nitrophenol phosphate (Anaspec®, San Jose, CA, USA) as the substrate. The cultured rat calvarial osteoblasts were rinsed twice with phosphate buffered saline (PBS) and lysed in alkaline lysis buffer, followed by three freeze-thaw cycles that involved serial exposure to -70°C and 37°C. The aliquots were incubated in glycine buffer containing 2 mg/mL of p-nitrophenol phosphate. After 30 min, 3 N NaOH was added to stop the reaction. The absorbance of p-nitrophenol was measured at 405 nm. The total amount of cellular protein was determined using the Bradford reagent (Sigma, St. Louis, MO, USA). The enzyme activity was then normalized relative to the total amount of cellular protein. The experiments were performed in triplicate.

## 2.5. *In vitro* cell migration assay

To examine the effect of SP on in vitro cell migration, collagen gel assay was performed.[38] For tracking the cells in collagen gel, the cytoplasmic membranes of MSCs were prelabled with fluorescent probe Cell Tracker, 1,1-dioactadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Invitrogen, Carlsbad, CA, USA). Human dermal fibroblast cells (hDFs) were used as a control. Cells were incubated in culture medium containing DiI dye (6.25 μg/mL) at room temperature for 2 h. The labeled cells were washed twice with PBS.

Collagen gel was prepared according to Farhat's protocol. Type I collagen (sigma) is dissolved in water with acetic acid added to pH 3.0. Collagen solution was neutralized with PBS and NaOH to a pH around 7.4 which is isotonic with the cells. The collagen solution was then allowed to gel at 37°C for about an hour with atmospheric humidity.

For testing the migration of cells under the influence of SP, $5\times10^3$ DiI-labeled cells were seeded on the one side of the collagen gel. SP was loaded on the other side of the gel. The culture medium was high-glucose DMEM (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Gibco). After 24 hours of culture, the migrated cells that moved through the collagen gel was visualized and photographed using a fluorescence microscope (IX71 inverted microscope; Olympus, Tokyo, Japan). The labeled cells were counted at

high magnification (×40). Cells were counted in multiple fields (n=5) per sample, and the counts were averaged.

## 2.6. Implantation of the Ti or Ti/GO device into the mouse calvaria

Six-week-old mice from the Institute of Cancer Research (Orient Bio Co., Sungnam, Kyunggi-do, Korea) were anesthetized using xylazine (20 mg/kg) and ketamine (100 mg/kg). After shaving the scalp hair, a longitudinal incision was made in the midline of the cranium from the nasal bone to the posterior nuchal line, and the periosteum was elevated to expose the surface of the parietal bones. A 1.2-mm diameter twist drill was used to make one hole through the calvaria.39 Then, a Ti or Ti/GO ring implant (4 mm in outer diameter, 1.2 mm in inner diameter) with or without SP and/or BMP-2 loading was placed beneath the periosteum, directly on the hole of occipital bone. The implant was stabilized using a screw (2 mm in diameter). The drilling site was irrigated with saline, and bleeding points were electrocauterized. Each animal had one implant, and five animals were used for each group. The animals were divided into eight groups: pristine Ti (Ti), GO-coated Ti (Ti/GO), SP-loaded Ti (Ti/SP), BMP-2-loaded Ti (Ti/BMP-2), SP-loaded Ti/GO (Ti/GO/SP), BMP-2-loaded Ti/GO (Ti/GO/BMP-2), SP and BMP-2-loaded Ti (Ti/SP/BMP-2), and SP and BMP-2-loaded Ti/GO (Ti/GO/SP/BMP-2). The amount of SP loaded on with or without GO-coated titanium plates was 0.2 μg, and the amount of loaded BMP-2 on each delivery system was 2 μg. The animal study was approved by the Institutional Animal

Care and Use Committee of Seoul National University (SNU-120711-4).

## 2.7. Micro-CT analysis

Eight weeks after the implantation, the mice were sacrificed and the implants were retrieved and fixed in 4% paraformaldehyde. Bone formation was evaluated by using microcomputed tomography (micro-CT) scanning (SkyScan-1172; Skyscan, Kontich, Belgium) and histological analysis. The new bone volume was determined using a CT analyser program (CT-An, Skyscan).

## 2.8. Histology

After micro-CT imaging, the specimens were prepared for histological and histomorphometric analysis. The specimens were immersed in 10% (v/v) buffered formalin solution, dehydrated in alcohol solutions of increasing concentrations, clarified in xylene, and embedded in polymethylmethacrylate. One sagital and one frontal section from each of two specimens per animal were obtained by a microcutting and

grinding technique. The sections were stained with Goldner's trichrome stain. The area of bone formation was determined using Adobe Photoshop software (Adobe Systems, Inc., San Jose, CA, USA) by the percentage of newly formed mineralized bone, excluding marrow and fibrovascular tissue in the original bone defect area [new bone area / defect area x 100, n = 5]

## 2.9. Statistical analysis

Quantitative data were expressed as the means ± standard deviations. The statistical analysis was performed using one-way analysis of variance (ANOVA) with the Tukey significant difference post hoc test using SPSS software (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered to denote statistical significance

# 3. Results

## 3.1. Coating of Ti substrates with GO

To ensure full coverage of Ti substrates with GO sheets (single GO sheet area = 0.75 ± 0.12 $\mu m^2$, Figure 1a), the Ti substrate was alternatively coated with positively and negatively charged GO sheets [GO-$NH_3^+$ and GO-$COO^-$, respectively] by LbL assembly. Coating with multiple layers of GO resulted in full coverage of the Ti substrate, whereas coating with the number of GO-$COO^-$/GO-$NH_3^+$ bilayers of 1 enabled only partial coverage (Figure 1a). Atomic force microscopy (AFM) revealed that, unlike the bumpy surface of an uncoated Ti substrate, the GO-coated Ti surfaces became much smoother after multiple cycles of LbL deposition. The full coverage of the GO sheets on Ti substrates was realized by LbL assembly of 15 bilayers. The GO coating on Ti was also confirmed by X-ray photoelectron spectroscopy (XPS) (Figure 1b). The C(1s) peak, which normally originates from GO, is clearly evident at approximately 285 eV in the spectrum of the GO coating on Ti. The increased number of GO bilayers showed the increased C(1s) peak, whereas the characteristic peaks of Ti(2p3/2) at 459 eV and Ti(2p1/2) at 463 eV nearly disappear after coating Ti with GO sheets. Coating with GO eliminated the relatively hydrophobic nature of Ti, and Ti/GO with 15 bilayers was more hydrophilic than

unmodified Ti  owing to the polar modification with the GO sheets (Figure 1c). This is consistent with the decrease in the contact angle after coating a hydrophobic polymer with GO.

## Figure 1.

Coating of GO multilayers onto Ti substrates and surface morphologies.

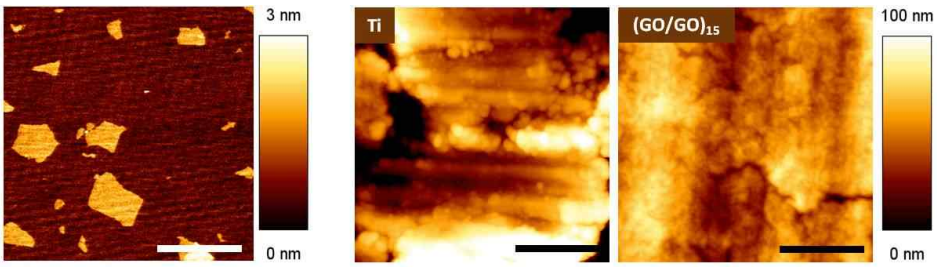



**Fig 1. a.** AFM images of GO, Ti, and $(GO\text{-}NH_3^+/GO\text{-}COO^-)_{15}$-Ti substrates. Scale bars = 1 μm.

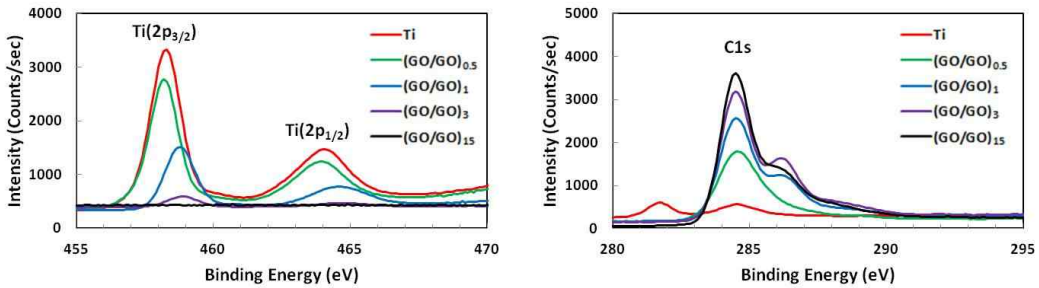



**Fig 1. b.** High-resolution XPS Ti(2p3/2), Ti(2p1/2), and C (1s), spectra of (GO-$NH_3^+$/GO-$COO^-$)n-Ti substrates.

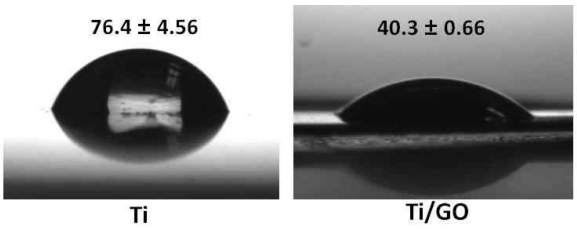
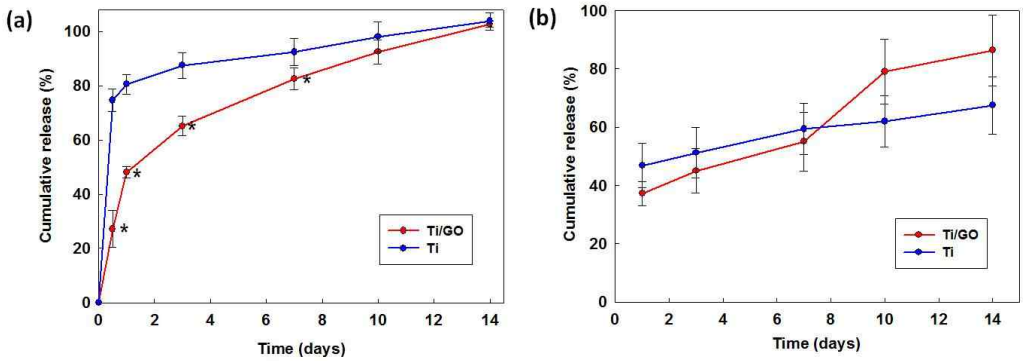



**Fig 1. c.** Contact water angles on bare Ti and Ti/GO, showing the change of the relatively hydrophobic nature of Ti to the more hydrophilic nature of $GO^{-}$.

## 3.2. Sustained release of BMP-2 and SP from Ti or Ti/GO substrates

The in vitro release of BMP-2 from the Ti/GO substrate was maintained for 14 days (Figure 2a). In contrast, Ti exhibited an initial burst of BMP-2 release. More than 72% of the initially loaded BMP-2 was released from Ti within a day. The in vitro release of SP also measured by ELISA (Figure 2b). The less than 80% of SP was released in 14 days steadily without initial burst. There was no significant difference of released SP from both Ti and Ti/GO.




## Figure 2.

*In vitro* release profiles of BMP-2 and SP from Ti and Ti/GO substrates. The BMP-2- or SP-loaded Ti and Ti/GO was immersed in PBS at 37 °C. At various time points, the supernatant was collected and the concentrations of SP and BMP-2 in the supernatants were determined by ELISA (n = 5 each). *p < 0.05 compared to Ti.

## 3.3. The bioactivity of released BMP-2.

We compared the bioactivities of BMP-2 released from bare Ti and Ti/GO (Figure 3). Bioactive BMP-2 released from both carriers increased the ALP activity of cultured osteoblasts. The ALP activity of osteoblasts in the Ti/GO/BMP-2 group was significantly higher than that in the Ti/BMP-2 group for 14 days of incubation, and similar to the group with daily addition of BMP-2 to the culture medium.

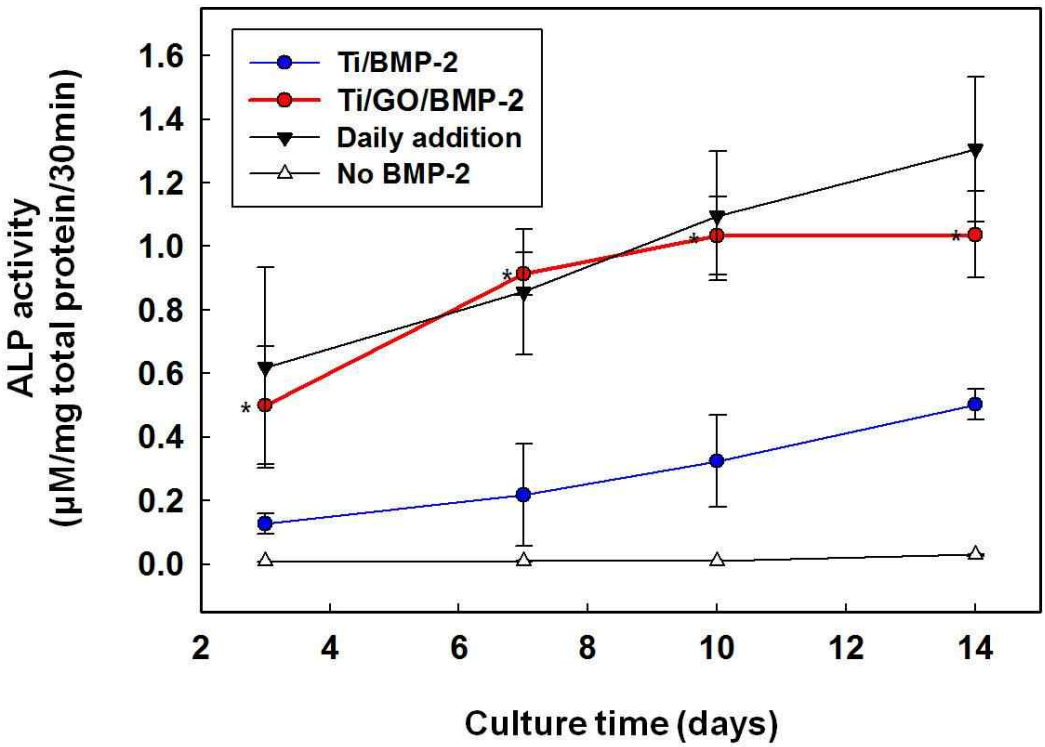



Figure 3.

Bioactivity of BMP-2 released from Ti and Ti/GO substrates. Evaluated by measuring the alkaline phosphatase (ALP) activity of osteoblasts cultured on tissue culture plastics with or without the BMP-2 delivery device. Cultures with or without a daily addition of BMP-2 to the culture medium served as controls. *$p<0.05$ compared to Ti/BMP-2.

## 3.4. SP stimulated MSCs migration *in vitro*

The potency of SP for inducing migration of MSCs that are known to contain CD29+ cells was evaluated (Figure 4). The test scheme is depicted in Figure 2A. BMP-2 treatment was included to determine the possible positive or negative effects of using BMP-2 with SP in terms of cell migration. After 24h, SP induced obvious cell migration regardless of BMP-2 treatment (Figure 4). CD29- cell of hDFs did not migrate.

## Figure 4.

*In vitro* cell migration stimulated by SP.

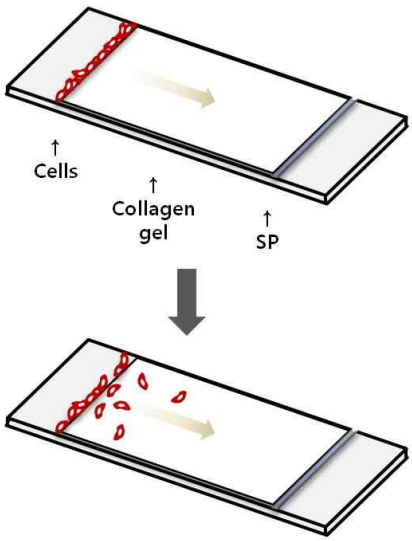



**Fig 4. a.** Schematic diagram of cell migration in collagen gel. DiI labeled cells were seeded on the one side of the collagen gel, and SP was loaded on the other side of the gel.

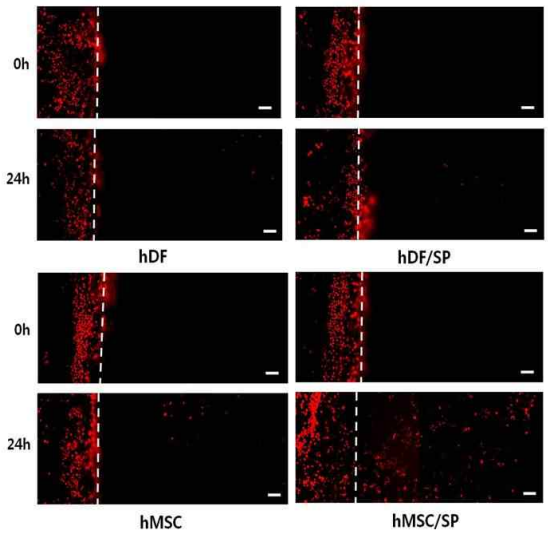



**Fig 4. b.** Fluorescence images of the location of DiI-labeled cells inside the gel a day after seeding and SP release. Scale bar = 200μm.

## 3.5. Enhanced *in vivo* bone formation by BMP-2 delivery using Ti/GO substrate in a mouse calvarial defect model.

The therapeutic efficacy of BMP-2 and SP delivered by Ti or Ti/GO substrate was investigated in vivo by evaluating bone formation after implantation of Ti or Ti/GO substrates, with or without BMP-2 and SP, into mice with calvarial defects, and monitoring the effects after 8 weeks (Figure 5). Micro-CT and histological analysis with Goldner's trichrome staining revealed BMP-2- and carrier-dependent formation of new bone (Figure 5a and b). Negligible bone formation on Ti and Ti/GO implants was observed in the groups without BMP-2 groups (Ti, Ti/SP, Ti/GO and Ti/GO/SP). The GO coated implants with BMP-2 groups showed much extensive bone formation compared to bare Ti implants with BMP-2. Comparison with Ti/BMP-2 and Ti/SP/BMP-2, there was no significantly difference. However, Ti/GO/SP/BMP-2 implants showed much more extensive bone formation than Ti/GO/BMP-2 implants. The volumes and areas of bone formation were quantified using micro-CT and histological data, respectively. These analyses revealed higher therapeutic efficacy (scored as more extensive in vivo bone formation) of the BMP-2 delivery platform of GO-coated Ti substrate compared with BMP-2 delivery from bare Ti substrate. Also SP delivered with the GO and BMP-2 enhanced the

new bone formation compared to the no SP-delivered groups.

## Figure 5.

Regeneration of mouse calvarial defect using Ti implant modified by GO.

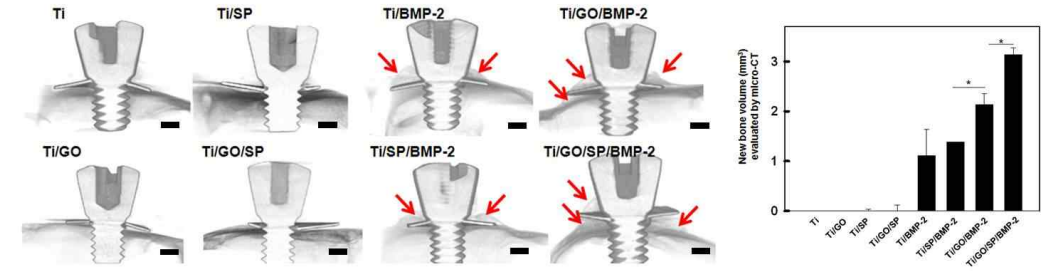



**Fig 5. a.** Micro-computed tomography (micro-CT) images of the calvarial defects with implants 8 weeks after implantation. Red arrows indicate newly formed bone. New bone volume was measured using a micro-CT analyzer program (n = 5). *p < 0.05 compared between two groups. Scale bars = 1 mm.

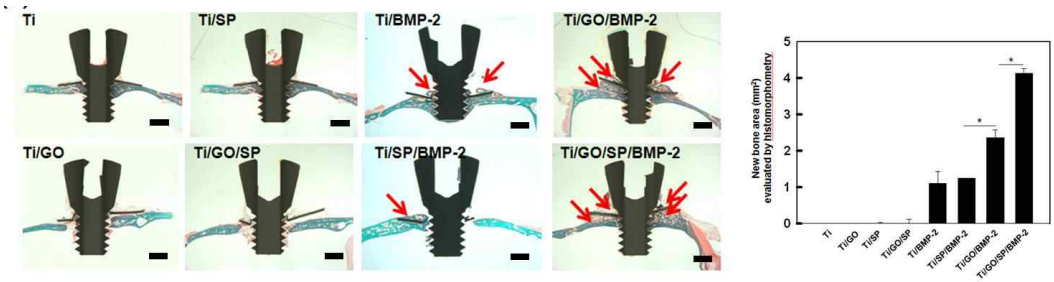



**Fig 5. b.** Histological analysis with Goldner's trichrome staining of mouse calvarial defects with implants 8 weeks after treatment. Red arrows indicate newly formed bone. The area of new bone was determined using histomorphometry analysis. Scale bars = 1 mm. (n = 5). *p < 0.05 compared between two groups.

# 4. Discussion

In this study, dual delivery of BMP-2 and SP by using the GO coated Ti implants significantly enhanced bone regeneration in a mouse calvarial model. Clinical stem cell implantation to induce bone regeneration required ex vivo cell manipulation. The cell culture processes make it costly and it needs more than 3-4 weeks due to cell manipulation time. To overcome such obstacles of stem cell therapy, there is another promising therapy. The in situ cell therapy is the recruitment of circulating host cells to the site for regenerating damaged tissue, which induces mobilization of host stem cells and controls cell differentiation into tissue-specific cell types.[40,41] Unlike stem cell implantation, this strategy does not include donor cell procurement and in vitro cell manipulation.[40,41] Second, since using BMP-2 which is used clinically for bone regeneration has been reported various side effects,[2,6] use of decreased dose of BMP-2 is required. Therefore combined with other therapy such as in situ therapy is required for enhancing bone regeneration with BMP-2. The *in situ* tissue regeneration we expected in the present study involves endogenous stem cell mobilization/homing and subsequent differentiation into appropriate lineages for the regeneration bone defect. In the present study, SP, which is known to recruit MSCs from bone marrow to blood circulation,[42] was used as a chemotactic agent. Additionally, we used BMP-2, which has been reported for potency of

osteoinduction, to induce osteogenic differentiation of endogenous stem cells recruited by SP to the bone defects.

In this study GO coated Ti implant was used for bone defect healing. GO can provide the prolonged BMP-2 release profile and protect the BMP-2 bioactivity.[29] The GO essentially has hydrophobic π domains mainly located on the basal plane and ionized carboxylic acids at their edges.[35,37] Thus π-electron clouds in the GO sheets are capable of interacting with the inner hydrophobic cores of BMP-2 protein. Also, the negatively charged $COO^-$ domains of GO-$COO^-$ could also bind with positively charged BMP-2 through the electrostatic interactions.[35,37] The enhanced BMP-2 bioactivity was measured by cultured osteoblast ALP activity (Figure 3). BMP-2 adsorbed on Ti/GO was prevented from the protein denaturation through the electrostatic interactions with the hydrophilic terminal functional groups attached to the GO sheets.[29]

Our previous study showed the enhanced *in vivo* new bone formation through the BMP-2 delivered from the GO coated Ti substrate.[29] Compared to the previous study, we co-delivered the SP with BMP-2 to enhance the in situ tissue regeneration efficacy. The Ti/GO/SP/BMP-2 group showed much more extensive bone formation compared to Ti/GO/BMP-2. It might be due to the enhanced MSC recruitment by SP to the defect site. The recruited MSC by SP maybe

promoted the bone regeneration combined with delivered BMP-2. However, Ti/SP/BMP-2 group did not show the enhanced bone formation compared to Ti/BMP-2. Furthermore Ti/SP/BMP-2 showed significantly less bone formation than Ti/GO/BMP-2. This result indicated that prolonged sustained BMP-2 release is more essential than stem cell recruitment by SP. The in situ bone tissue regeneration can be achieved only with the appropriate BMP-2 delivery vehicle. Additionally, it might be due to recruited cell adhesion ratio was increased by GO coated substrate.[34]

The high dose of each BMP-2 or SP implantation reported many side effects. Therefore, the methods of decreasing dose are required for successful clinical bone regeneration therapy. The present study showed successful combined bone regeneration therapy using dual delivery of SP and BMP-2. Further study for decreasing each dose of BMP-2 or SP is necessary to prevent clinical side effects.

# 5. Conclusion

In the present study, we used a GO coated Ti substrate for BMP-2 and SP delivery vehicle for local delivery of a stem cell recruitment agent and an osteoinductive agent as for in situ bone regeneration. GO can be used for successful BMP-2 delivery vehicle due to electrostatic and π-π interaction with BMP-2 protein. Also SP was delivered with BMP-2 to the defect site. The delivered SP and BMP-2 from the GO coated Ti substrate increased the bone regeneration compared to each SP or BMP-2 delivered alone from the GO coated Ti substrate. The present study represented the enhanced bone regeneration therapy using BMP-2 delivery from the GO combined with SP dual delivery system.

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요약(국문초록)

# 뼈 재생을 위한 그래핀 옥사이드를 이용한 골형성단백질-2와 물질P 전달

골 재생에 있어서 그래핀 옥사이드(GO)가 코팅된 티타늄(Ti) 임플란트가 골형성단백질-2(BMP-2)를 전달하는 새로운 전달체로서 효과가 있음이 알려져 있다. 본 연구에서는 효과적인 골 재생을 위해 줄기세포 동원인자인 물질 P(SP)와 골 분화 인자인 골형성단백질-2를 함께 전달하는 시스템을 골 재생 치료법으로서 제시하고자 한다. 그래핀 옥사이드는 표면에 음전하를 띠는 작용기가 존재하여 단백질을 구조와 활성도의 변화없이 부착 가능하며, 많은 양을 전달할 수 있을 뿐만 아니라 단백질의 서방형 방출이 가능하게 함을 *in vitro* 방출 거동 실험 결과를 통해 확인하였다. 또한 *in vitro* 세포 이동 실험을 통해 물질 P가 중간엽 줄기세포의 이동을 촉진시킴을 확인하였다. 이에 기초하여, 그래핀 옥사이드를 코팅한 티타늄 판에 물질 P와 골형성단백질-2를 부착시켜 쥐의 두개골 결손 부위에 전달하였다. 8주 후 두개골 결손 부위의 Micro-CT와 조직학을 분석한 결과, 물질 P와 골형성단백질-2를 함께 전달한 실험군이 다른 군들에 비해 뛰어난 골 재생 능력을 보였다. 이러한 결과는 그래핀 옥사이드에 의한 물질 P와 골형성단백질-2의 전달이, 줄기세포를 골 결손 부위로 동원하고 해당 세포의 *in situ* 골 분화를 가능케 함으로써 효과적인 골 재생 치료법이 될 수 있음을 보여준다.

주요어 : Bone morphogenetic protein-2, Bone regeneration, Graphene oxides, Stem cell recruitment, Substance P

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