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Multigrowth Factor Delivery via Immobilization of Gene Therapy Vectors

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The fundamental goal of tissue engineering and regenerative medicine (TE/RM) is to promote formation, organization, and integration of tissue formation to produce functional organs or structures. Key components of TE/RM for clinical application include putative regenerative cells, signaling growth factors, 3D scaffolding matrices, and a vascular or nutrient supply to allow tissue neogenesis and homeostasis.^[1] Of these, growth factors play critical roles in regulating all the biological activities of cells, such as migration,

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Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

proliferation, differentiation, maturation, and apoptosis. A major clinical challenge for oral and craniofacial TE/RM is the controlled and efficient delivery of growth factors for repair of multitissue interfaces (i.e., bone, ligaments, and oral mucosal soft tissues) due to rapid degradation of the proteins and safety considerations of gene-mediated local delivery platforms. In this study, we demonstrate for the first time the efficacy of multigrowth factor gene delivery using adenoviral vectors chemically conjugated onto the surfaces of poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), and titanium, which indicates the feasibility of currently available U.S. Food and Drug Administration (FDA)-approved biomaterials in TE/RM clinical applications.

Biodegradable and biocompatible scaffold materials, such as PCL and PLGA, are approved by the FDA for several clinical applications and promote tissue regeneration in numerous biomedical studies.^[2,3] Several methods for attachment of biomolecules to material surfaces depend on physical adsorption, which lacks the ability to target and immobilize biomolecules or release them in a controlled manner.^[4] Reactive polymer coating prepared via chemical vapor deposition (CVD) polymerization of substituted paracyclophanes presents a broadly applicable strategy for covalent immobilization of biomolecules onto a variety of solid materials.^[5] In addition, the polymer coatings can be applied conformally onto 3D complex structures, such as tissue scaffolds, which enables direct conjugation of biomolecules on tissue scaffolds and allows for localized delivery of genes and proteins to cells, and minimizing infection of surrounding tissues.^[4,6] Previous studies have reported the potential of CVD polymerization for immobilization of adenovirus to polymer films for up-regulated growth factor expression and cellular differentiation.^[7,8]

For clinical use, bone morphogenetic protein-7 (BMP7) for bone regeneration and platelet-derived growth factor-BB (PDGF-BB) for soft-tissue regeneration (two FDA-approved growth factors used for craniofacial applications) provide a matrix for restoring tissue lost due to oral disease. In contrast to direct protein-based or plasmid therapies, virus-mediated gene therapy has the advantage of sustained and localized production of therapeutic proteins. One of the main benefits of utilizing adenoviral vectors for gene delivery is that, in theory, adenovirus can infect many human cells due to the presence of the classical adenoviral receptor, coxsackie and adenovirus receptor (CAR), which is present in fibroblasts, endothelial, epithelial and smooth muscle cells. The CVD coating methodology tethers the virus to the material, thereby reducing the acute toxicity and hepatic pathology caused by direct systemic administration of adenoviral vectors.^[9] In the present study, we utilized CVD coatings on three distinct materials for direct gene delivery to orally derived progenitor cells (primary human periodontal ligament (hPDL) fibroblasts). Our findings revealed that the immobilized adenovirus transduced hPDL cells and induced sustained production of bioactive PDGF-BB and BMP7 protein on all tested materials (PCL, PLGA, and titanium). No cytotoxic effects were observed due to the polymer coating. We found that the adenoviral load could easily be modified to ensure that the resulting growth factor concentration is optimal for tissue formation for subsequent in vivo applications. This work establishes a general strategy for safe and efficient cell transduction and growth factor protein production because it is immobilized and controllable.

Several scaffold materials are available, but currently a technique to customize polymer surfaces for specific gene delivery applications is needed.^[10] PLGA is a copolymer prepared from different ratios of lactic (LA) and glycolic acid (GA) monomers. There is great interest in PLGA due to its modifiable surface properties, biodegradation rate, and biocompatibility and it has been widely used for tissue engineering as scaffolds, fibers, hydrogels, microspheres, and in composite constructs.^[11] Titanium is the standard material for dental and biomedical implant applications requiring osseointegration. We will use these materials to mimic the normal periodontal structure (bone–ligament–cementum). Bioactive agents have been applied to titanium surfaces to further improve bone-to-implant contact and accelerate healing.^[12] Our strategy could be applied to a multilayered scaffold that aims to regenerate several tissues simultaneously by immobilizing different viral vectors for gene therapy onto different regions of the scaffold, thereby reducing interference between different biomolecules compared to other methods of delivery in which unbound virus can dissipate into surrounding tissue.

A schematic illustrating the polymer coating and conjugation of antibody and adenovirus on the three materials is shown in Figure 1a. First, a thin layer of polymer film containing pentafluorophenol (PFP)-ester groups was coated on the three materials via CVD polymerization. Goat antiadenovirus antibody was immobilized on the polymer-coated materials. Subsequently, adenoviruses were immobilized on the materials via antibody–antigen interaction. X-ray photoelectron spectroscopy (XPS) and immunofluorescence were used to verify the presence of polymer and binding of antiadenovirus antibody on the three polymer-coated materials. XPS is capable of detecting the elemental composition of the top 10 nm of a specimen. Figure 1b summarizes the elemental composition of the three types of materials coated with the polymer. The characteristic elements of the polymer coating were detected on all three materials and the composition of these elements are in good agreement with the calculated elemental composition of the polymer coating based on the structure of the polymer, confirming the presence of the polymer coating on all three materials. AlexaFluor647-conjugated anti-Goat IgG secondary antibody was used to verify binding of antiadenovirus antibody on the three polymer-coated materials via immunofluorescence. Mean fluorescence intensity on the polymer-coated materials was higher than on the uncoated materials, confirming successful binding of antiadenovirus antibody on the polymer-coated materials (Figure 1c).

To ensure successful covalent immobilization of adenovirus to the three material surfaces after CVD coating, a green fluorescent protein (GFP)-expressing adenovirus was conjugated to antibody and viral particles were quantified using scanning electron microscopy (SEM) images (Figure 1d,e). Two concentrations of virus were used for immobilization to the respective surfaces. For transduction, the hPDL cells were directly seeded onto the coated samples. Fluorescence microscopy showed that maximal GFP protein expression was seen at 72 h for all coated materials, indicating excellent transduction efficiency of the surface-bound adenovirus (Figure S2, Supporting Information). To highlight the potential uses of CVD in oral-tissue engineering, we tested three cell types in addition to ligament progenitor cells (Figure S3, Supporting Information).

Recombinant proteins have been clinically approved and shown to improve tissue regeneration. However, degradation of proteins over a period of hours to days greatly limits their effects in vivo.^[13] Therefore, we hypothesized that the CVD-based transduction strategy could improve delivery and production of these proteins. PDGF-B and BMP7 encoding adenoviruses were directly conjugated to PCL, PLGA, and titanium disc surfaces. Enzyme-linked immunosorbent assay (ELISA) analysis showed highly efficient transduction of PDGF-BB and BMP7 protein production over 14 d (Figure 2a and 3a). The optimal particle number (PN) noted was 10^{11} . In pilot studies, we tested a range from 50 to 400 MOI (translating to an optimal MOI = 300, data not shown). Maximum production of 650 ng mL^{-1} of PDGF-BB was shown at 9 d from 10^{12} PN on PLGA, compared to 325 and 180 ng mL^{-1} for titanium and PCL at days 7 and 9, respectively. 10^{11} PN resulted in maximal PDGF-BB protein production at 300, 35, and 95 ng mL^{-1} for PLGA, titanium, and PCL, respectively.

Researchers^[13,14] showed that Ad-BMP7 and Ad-PDGF-B significantly increased new bone formation around implants compared to control and although there are numerous reports on efficient cellular transduction and protein production using adenoviral vectors,^[15] there is a lack of studies showing whether these proteins have functional and biological activity. To address this limitation, we performed 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) bioassays to measure cellular proliferation in response to supernatants from Ad-PDGF-B transduced cells relative to recombinant PDGF protein. Figure 2b shows the results from a time-course experiment using hPDL cells in medium containing either 10% fetal bovine serum (FBS) (positive control), 0.1% FBS (negative control), 2% FBS (vehicle), or vehicle plus 50 ng mL^{-1} rhPDGF-BB or vehicle plus Ad-PDGF-B cell supernatant. The adenovirus-derived supernatants from each material were diluted to a concentration of 50 ng mL^{-1} . The results show that the effects on hPDL cell proliferation are almost identical following incubation with either rhPDGF-BB or PDGF-BB protein produced from adenovirus-conjugated PLGA, PCL, and titanium discs.

BMP7 can induce cell differentiation and promote bone formation. Using an ELISA assay, the maximum protein produced at day 7 from the Ad-conjugated titanium discs was determined to be 380 ng mL^{-1} (10^{12} PN) and 220 ng mL^{-1} (10^{11} PN). For the PLGA and PCL discs, 75 and 300 ng mL^{-1} of BMP7 and 40 and 200 ng mL^{-1} of protein were produced at day 7 for 10^{11} and 10^{12} PN (Figure 3a). Then, hPDL cells were incubated with supernatant from Ad-BMP7-transduced cells for 7, 14, and 21 d to determine whether this adenovirus-derived BMP7 protein could promote cell differentiation and induce mineralization. Gene expression data are shown in Figure 3b.

COL1A1 is the primary collagen component found in cementum, PDL, and bone, with type V collagen in the extracellular matrix. In our study, the extracellular matrix is primarily composed of type I collagen, showing the maximum gene expression on day 7 and a subsequent decrease thereafter. Type III and type V collagen reached the highest expression on day 14, with type V collagen showing almost fivefold greater gene expression than control groups.

Alkaline phosphatase (ALP), osteopontin (OPN), and osteocalcin (OCN) are well known factors involved in regulating mineralization of tissues.^[16] We found that rhBMP7 induced ALP expression that remained elevated over the entire period. Compared to the control group, expression of ALP increased threefold on day 7 and fivefold on day 21. This is in line with a previous study showing that rhBMP7 stimulates a fivefold increase in ALPase activity at 100 ng mL⁻¹.^[17] Our results showed expression of OPN and OCN peaked at day 14 for all groups except OCN vehicle, while expression was significantly higher for experimental groups compared to control groups, though still lower than the rhBMP7 group. Collagen matrix formation and mineralization indicated differentiation of hPDL cells. Previous studies reported increased gene expression of those markers in cells treated with Ad-BMP7 at 6–7 d.^[18] Researchers also found that treatment with rhBMP7 significantly increased expression of OPN and OCN in cementoblasts compared to a control after 72 h.^[19]

To demonstrate our next steps for applying the CVD strategy using an in vivo model for multitissue regeneration, we investigated the effects of combinatorial gene delivery in vitro to quantify the gene expression within hPDL cells after 72 h transduction with Ad-PDGFB and Ad-BMP7. Figure 4a,b illustrates the two CVD-coated PCL scaffolds, each conjugated with adenoviral vector for either PDGFB (red) or BMP7 (green) prior to direct seeding with hPDL cells. The results in Figure 4c verify that the gene delivery vectors remain fully compartmentalized such that the relative expression of PDGF is not significantly reduced when incubated with Ad-BMP7 versus Ad-empty vector control. Although the relative expression of BMP7 gene was reduced $\approx 60\%$ (9.89×10^5 units vs 3.54×10^5 units) when incubated with Ad-empty vector control versus Ad-PDGFB, the result is still a significant level of gene expression to elicit cellular levels of BMP7 protein for bone regeneration. We also quantified the expression of proliferating cell nuclear antigen (PCNA), ALP, and Noggin genes, which showed increases of these genes in response to the combinatorial delivery of adenoviral vectors and was consistent with the changes in PDGF and BMP7 expression. In addition, when each adenoviral vector (PDGF or BMP7) is localized to a particular compartment, the expression of downstream genes regulated is significantly upregulated compared to the other compartment. This means that PCNA expression (downstream of PDGF) was significantly upregulated in the PDGF compartment with the presence of either BMP7 or empty vector in the adjacent compartment. Conversely, ALP and Noggin expression (downstream of BMP7) was significantly upregulated in the BMP7 compartment with the presence of either PDGF or empty vector in the adjacent compartment. This demonstrates that the gene vectors in each compartment have the greatest effect in their respective compartment. Furthermore, use of a 3D-printed scaffold in this experiment demonstrates that the CVD coating technology can be successfully scaled-up from 2D applications.

In summary, this multigene delivery strategy using CVD coating and adenovirus has several key advantages over other methods for gene therapy or protein delivery. Our system produces much more prolonged and sustained levels of protein than recombinant protein delivery. This approach provides a distinct advantage because recombinant proteins would need to be delivered every 24–48 h to achieve a similar level, requiring many more office visits for clinical applications. Additionally, recombinant protein is not localized to the regeneration region and can escape into the surrounding tissues. The CVD method would

reduce the number of office visits required for clinical application and protein would be expressed by the cells in the area of regeneration. In addition, compared to other gene therapy methods involving adenovirus, the CVD method localizes the adenovirus onto theoretically any scaffold material allowing for a variety of applications. Because the viruses are immobilized on the scaffold material, this method does not require the scaffold to be seeded with a patient's cells prior to implantation. Native cells can infiltrate the scaffold and, once there, be transduced by the immobilized vectors. This would reduce the time, cost, and regulatory burden for clinical applications of these scaffolds as a patient's cells would not need to be harvested, expanded in culture, transduced with virus, and seeded onto a scaffold before the scaffold could be implanted in a patient. Furthermore, because the viruses are immobilized, they cannot spread into the surrounding tissues and infect cells outside the area to be regenerated. This is particularly helpful for regenerating tissues in a compartmentalized manner and creating delineation between regions. Future work aims to regenerate multiple tissue types simultaneously using this method with different adenoviral vectors in each region.

Experimental Section

Materials

PLGA (M_w : 31–52 kDa), 75:25 ratio of lactic acid to glycolic acid monomers was purchased from Evonik Industries. PCL (M_w : 43–50 kDa) was purchased from Polysciences, Inc. (Warrington, PA, USA). 6AL-4V titanium discs were obtained from the University of Michigan Engineering department.

Polymer and Titanium Disc Fabrication

Selective laser sintering was used to fabricate 5 mm dia. $\times$ 1 mm discs using PCL and 4% hydroxyapatite. The PLGA discs were prepared on two-layer silicone molds, prepared following instructions of SYLGARD 182 Silicone Elastomer Kit and poured into aluminum-lined glass petri dishes to create a lower 10 mm $\times$ 90 mm diameter mold of uniform thickness. The upper mold layer is 1 mm thick with 5 mm diameter holes created with a biopsy punch. PLGA crystals were melted into the mold at 90–130 °C and flattened, resulting in PLGA discs 1 mm $\times$ 5 mm in diameter. Titanium discs were manufactured from 0.040" $\times$ 2.0" $\times$ 2.0" 6AL-4V titanium sheets by slicing 1 mm $\times$ 5 mm diameter discs using a Computerized Numerical Control (CNC) machine.

CVD Polymerization

PCL, PLGA, and titanium discs were coated with a layer of amine-reactive polymer using a custom-built CVD system previously reported.^[3] During the coating process, discs were fixed inside the deposition chamber at 15 °C. The starting material (synthesis is described elsewhere^[3]) was sublimated at 120 °C, which was then pyrolyzed at 540 °C to form into a stream of reactive di-radical vapor. The stream of reactive di-radicals then entered the deposition chamber where they deposited and polymerized simultaneously on the discs. The whole process operated at a pressure of 0.1 mbar using argon as carrier gas.

Chemical Characterization via XPS

XPS data were recorded with an Axis Ultra X-ray photoelectron spectrometer (Kratos Analyticals, UK) outfitted with a monochromatized Al K α X-ray source at 150 kW. Survey and high-resolution XPS spectra were taken at 160 and 20 eV, respectively. All spectra were calibrated with respect to the nonfunctionalized aliphatic carbon with a binding energy of 285 eV. The averaged experimental composition obtained on the three types of polymer-coated materials is listed in Figure 1b and compared to the theoretical composition of the polymer.

Adenovirus Immobilization to the Polymer

After CVD coating, discs were sterilized in 70% ethanol for 1 h, followed by virus immobilization and cell seeding. A 10 $\mu\text{g mL}^{-1}$ solution of Goat antiadenovirus (AbD Serotec; 0151- 9004) polyclonal antibody was prepared in phosphate-buffered saline (PBS). An antibody concentration of 10 $\mu\text{g mL}^{-1}$ was used because a previous study illustrated that this was the saturation point of the antibody on CVD coated materials.^[7] Ten discs were added to 12 mL of antibody solution and incubated overnight at 4 °C on a rotating mixer. Discs were rinsed 5 $\times$ 5 min with PBS. Adenoviruses containing GFP, PDGF, and BMP7 genes were prepared by University of Michigan Vector Core. Discs were incubated in 12 mL cold adenovirus solution (10^{11} or 10^{12} PN/10 discs) for 4 h at 4 °C followed by 5 $\times$ 5 min PBS rinses.

Verification of Antiadenovirus Antibody Binding

After incubation in antiadenovirus antibody, polymer-coated and as-received discs were incubated in PBS containing 10 $\mu\text{g mL}^{-1}$ AlexaFluor647 Rabbit anti-Goat IgG secondary antibody (Invitrogen, Inc.), 0.02% (v/v) Tween 20, and 0.1% (w/v) bovine serum albumin (Sigma-Aldrich, Inc.) for 1 h, followed by PBS rinses. Subsequently, fluorescence microscopy (Nikon E800) and ImageJ were used to quantify and compare the fluorescence signals obtained on polymer-coated and uncoated discs.

SEM Imaging and Quantification of Adenovirus

The viral particle distribution was determined by SEM. PCL, PLGA and Ti discs with immobilized Ad-GFP were fixed in 2.5% glutaraldehyde in PBS for 30 min. After two washes with distilled water and dehydration with ethanol, samples were desiccated under vacuum overnight. SEM images were captured using the AMRAY 1910 FE field-emission microscope and SEMTech Solutions SEM interface version V1.0.4.179. Samples were mounted for anisotropic magnetoresistance (AMR) using graphite conductive adhesive from Electron Microscopy Sciences and coated with gold using the Polaron SEM Coating System for 100–120 s. All images were taken at 10 000 $\times$ magnification. Viral particles were counted by two independent investigators using 10 SEM images of each material (Ti, PCL, PLGA) at each viral concentration (10^{11} , 10^{12}) resulting in a total of 60 images. Investigators counted bright particles $\approx$ 70–100 nm in diameter toward the number of adenovirus attached to the surface.^[20]

Protein Expression in hPDL Cells

After hPDLs were seeded on the CVD-coated PCL, PLGA, and Ti discs, cells transduced with Ad-GFP were examined for expression of green fluorescence protein at 72 h post-seeding. Images were captured using a Nikon SMZ18 fluorescence microscope at 15× to 80×. For PDGF-BB and BMP7, hPDL supernatants were harvested every 48 h over 14 d. Expression of PDGF-BB and BMP7 protein was detected by Quantikine ELISA assays.

Bioactivity of Proteins/Cell Proliferation

Release of PDGF-BB over 12 d from adenovirus-transduced hPDL cell supernatants was determined by ELISA (R&D Systems, Minneapolis, MN, USA) using the manufacturer's indications. Cell proliferation was measured at days 1, 3, 5, and 7 using an MTT assay (Life Technologies, Grand Island, NY, USA). Cells were grown in the presence of each group: 0.1% FBS, 2% FBS, 10% FBS medium, 2% FBS medium with 50 ng mL⁻¹ rhPDGF-BB, and 2% FBS medium with diluted, conditioned medium to determine the functional activity of the PDGF-BB.

Real-Time PCR Analysis

hPDL cells were plated at 5×10^5 cells per well in 12-well plates. Four groups were compared: i) growth media, ii) osteogenic media, iii) osteogenic media with 100 ng mL⁻¹ rhBMP7, or iv) osteogenic media with conditioned supernatant diluted to 100 ng mL⁻¹ BMP7. The medium was changed every 3 d for 21 d. Total RNA extraction was performed using an RNeasy kit (Qiagen, Valencia, CA, USA) and cDNA was synthesized (SuperScript II; Invitrogen, Carlsbad, CA, USA). TaqMan gene expression reagents were used for detection of COLIA1, BSP, OCN, OPN, ALP, COL5A1, COL3A1, and β -actin. An ABI PRISM 7700 sequence detector (Applied Biosystems) was used for messenger RNA (mRNA) expression calculations based on a relative standard curve and normalized to β -actin.

Combinatorial Gene Delivery

A 20 μ L fibrin gel solution containing 4×10^5 hPDL cells was added to CVD-coated PCL scaffolds with Ad-BMP7 and CVD-coated PCL scaffolds with Ad-PDGFB immobilized. Each cell-seeded scaffold was directly adjacent to each other and bound with wire to ensure direct contact. Each pair of scaffolds was placed in a separate well of a 12-well plate and incubated for 72 h in growth media. Cells were then lysed for total RNA extraction and cDNA synthesis. Real-time PCR analysis was performed using primers for PDGFB, BMP7, Noggin, ALP, PCNA, and β -actin.

Dual Immunofluorescence Staining

4×10^5 hPDL cells were seeded using the same method as above. Each pair of scaffolds was placed in a separate well of a 12-well plate and incubated for 48 h in growth media. Scaffolds were then fixed in cold methanol at -20 °C for 6 min. After washing 3× with PBS, samples were preincubated for 1 h in 3% bovine serum albumin (BSA). The pair of scaffolds was incubated at 4 °C overnight with rabbit polyclonal anti-PDGF-BB (2 μ g mL⁻¹, Abcam, USA) and mouse monoclonal anti-BMP7 (1 μ g mL⁻¹, Abcam, USA), followed by 3 × 5 min

PBS rinses. Samples were incubated with anti-mouse TRITC-labeled (1:100) and anti-rabbit fluorescein isothiocyanate (FITC)-labeled (1:100) secondary antibodies for 1 h. Fluorescence images were taken under 1× and 100× magnification (Nikon SMZ18) and double-staining images were merged using ImageJ software.

Statistical Analysis

Data are expressed as the mean $\pm$ standard error of the mean or standard deviation. Statistical analysis was performed across two groups using unpaired *t*-test and multiple groups using analysis of variance with Tukey's test. A *p*-value of 0.05 or less was considered to be statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

a) A schematic showing the process for attaching adenovirus onto PCL, PLGA, and titanium discs. b) XPS analysis of PCL, PLGA, and titanium discs coated with polymer. The values in brackets are the theoretical values of the polymer coating. c) Mean fluorescence intensity per region of interest (ROI) of polymer-coated and noncoated discs following incubation in antiadenovirus antibody and Alexafluor647 (or FITC)-conjugated anti-Goat IgG secondary antibody ($n = 9$). ROI = 0.394 mm². d) SEM images of PCL, PLGA, and titanium discs with 10¹¹ and 10¹² PN of GFP-adenovirus immobilized onto the surfaces. Viral particles have been colorized to enhance visualization. e) Quantification of viral particles within ROI ($n = 10$). ROI = 101.4 μ m². For Figure 1c,e, ***: <0.0005.

Figure 2.

a) PDGF-BB protein expression from hPDL cells transduced with adenovirus-conjugated PCL, PLGA, and titanium discs. Ad-PDGF-BB was immobilized at 10^{11} or 10^{12} PN to each material and cells were added directly and incubated for 14 d in culture. Supernatants were harvested every 2–3 d and analyzed by ELISA. b) MTT assay to measure cell proliferation in response to PDGF-BB secreted from adenovirus-transduced hPDLs. Supernatant from each material in (a) was pooled and diluted to 50 ng mL^{-1} in Dulbecco's modified Eagle medium (DMEM). Cell proliferation rate measured over 7 d relative to 0.1% FBS, 2% FBS (vehicle), vehicle + 50 ng mL^{-1} rhPDGF-BB, and 10% FBS. For Figure 2a,b, ****, #####: <0.00001 ; ***, ###: <0.0005 ; **, #: <0.005 . For Figure 2b, *: experimental groups versus vehicle, negative control; #: experimental groups versus positive control.

Figure 3.

a) ELISA assays for BMP7 protein expression from hPDL cells transduced with adenovirus-conjugated PCL, PLGA, and titanium discs. Ad-BMP7 was immobilized at 10^{11} or 10^{12} PN to each material and cells were added directly and incubated for 14 d in culture. Supernatants were harvested every 2–3 d and analyzed by ELISA. ***: <0.0005 ; **: <0.005 ; *: <0.05 . b) Gene Expression from PDL cells. Real-time PCR results for gene expression from hPDL cells cultured with Ad-BMP7 transduced cell supernatant for 7, 14, 21 d. *, #: <0.05 . *: experimental groups versus vehicle, negative control; #: experimental groups versus rhBMP7.

Figure 4. a) Schematic diagram depicting the side-by-side placement of CVD-coated PCL scaffolds within a single-well prior to hPDL cell seeding. Green represents the Ad-PDGFB compartment and red represents the Ad-BMP7 compartment. b) After seeding cells in the Ad-PDGFB compartment and the Ad-BMP7 compartment, double immunofluorescence staining for PDGF-BB (green)/BMP7 (red) shows the localization of the dual delivery. c) Real-time PCR results for gene expression from combinatorially transduced hPDL cells. Ad-BMP7 and Ad-PDGFB were immobilized to one PCL scaffold each and hPDL cells were

seeded and incubated in a single-well format for 72 h prior to lysis for total RNA purification.

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