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# Paper-based bioactive scaffolds for stem cell-mediated bone tissue engineering

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ABSTRACT

Bioactive, functional scaffolds are required to improve the regenerative potential of stem cells for tissue reconstruction and functional recovery of damaged tissues. Here, we report a paper-based bioactive scaffold platform for stem cell culture and transplantation for bone reconstruction. The paper scaffolds are surface-engineered by an initiated chemical vapor deposition process for serial coating of a water-repellent and cell-adhesive polymer film, which ensures the long-term stability in cell culture medium and induces efficient cell attachment. The prepared paper scaffolds are compatible with general stem cell culture and manipulation techniques. An optimal paper type is found to provide structural, physical, and mechanical cues to enhance the osteogenic differentiation of human adipose-derived stem cells (hADSCs). A bioactive paper scaffold significantly enhances *in vivo* bone regeneration of hADSCs in a critical-sized calvarial bone defect. Stacking the paper scaffolds with osteogenically differentiated hADSCs and human endothelial cells resulted in vascularized bone formation *in vivo*. Our study suggests that paper possesses great potential as a bioactive, functional, and cost-effective scaffold platform for stem cell-mediated bone tissue engineering. To the best of our knowledge, this is the first study reporting the feasibility of a paper material for stem cell application to repair tissue defects.



## 1. Introduction

In order to achieve tissue reconstruction and functional recovery of damaged tissues, stem cell-based tissue engineering requires three-dimensional (3D) functional scaffolds to improve the regenerative potential of stem cells. Such functional 3D scaffolds can be constructed via the systematic control and optimization of the biochemical, biophysical, and mechanical characteristics of the scaffolds. Biophysical and mechanical cues including elasticity, stiffness, and topography can regulate stem cell phenotype and functions such as self-renewal, proliferation, and differentiation of stem cells [1–7]. Thus, 3D scaffolds with well-defined, tunable structural features and mechanical properties are crucial for improving the therapeutic and regenerative efficacy of stem cells in the context of tissue engineering. Significant efforts have been made in developing such 3D scaffolds for tissue engineering. For example, electrospinning, lithography, microfabrication, and self-assembly techniques have been widely explored to fabricate 3D scaffolds appropriate for specific tissue applications [8–12]. However, polymer types applicable to each technique are limited and achieving the desired properties for tissue engineering via the exquisite manipulation of structural and mechanical features of the scaffold is extremely challenging using these conventional methods. In addition, recently developed 3D scaffolds often require complicated fabrication processes and expensive instruments, impeding the manufacturing and the mass production of the scaffolds [13,14].

In this regard, paper would be an attractive alternative as a 3D scaffold platform to control the behaviors and improve the regenerative potential of stem cells. Paper is basically a bundle of cellulose microfibers and inherently forms a microfibrous porous 3D architecture. Given that there are a large number of different types of commercially available paper products (e.g., filter paper and weighing paper), paper also offers great diversity in surface

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topography, internal microstructure, and mechanical properties for 3D scaffold fabrication. In particular, paper produced from natural sources is biocompatible and, because it can be supplied in large quantities using well-established fabrication processes with extremely low costs, also cost-effective [15]. Hence, paper may provide a great advantage of establishing an alternative culture platform and tissue-engineering scaffold for therapeutic application of stem cells.

Actually, paper has already been used within biomedical engineering to fabricate microfluidic chips, electronic devices, and biosensors [16–18]. Recent studies have highlighted the applicability of paper materials as a cell culture platform. Morphological and physiological characteristics of 3D tumor-like tissue could be recapitulated by culturing tumor cells on paper and stacking the cell-seeded sheets to mimic 3D tumor spheroids *in vitro* and 3D tumor mass *in vivo* [19,20]. Mosadegh et al. also reported a 3D paper scaffold system to model cardiac ischemia by constructing tissue-like structures using multilayered paper seeded with cardiac fibroblasts suspended in hydrogel [21]. Although these previous reports demonstrate the feasibility of the paper-based platform as a model system to mimic tissue-specific morphology, physiology, and function, paper materials have yet to be tested for therapeutic and regenerative applications.

Therefore, versatile paper materials warrant testing as tunable scaffold platforms for stem cell and tissue engineering. For this purpose, it would be pivotal to develop a method for engineering the surface of paper scaffolds to permit cell culture and *in vivo* use. Meanwhile, it is critically important to maintain the inherent 3D microfibrous structures of the paper materials. Because paper can be easily damaged by heat or organic chemicals applied for surface engineering, it is extremely challenging to modify the surface of paper scaffolds without damaging the surface 3D microstructure. To achieve a systematic tailoring of the surface of the paper scaffold, an innocuous solvent-free method was adapted in this study. A solvent-free, vapor-phase polymer-coating method, termed initiated chemical vapor deposition (iCVD), was employed to provide favorable biochemical surface properties (e.g., adhesiveness and water resistance). The iCVD method allows for the conformal coating of functional polymer films onto various surfaces, including nanopatterned substrates, fabrics, and membranes, without damaging them [22,23]. In the iCVD process, free radical polymerization of monomers occurs in the gas phase, which minimizes undesirable side reactions that can destroy various functional groups in the polymer chain [24–27]. In addition, the coating process can be performed at mild substrate temperatures (15–40°C), enabling the conformal deposition of polymer film without damage and distortion of the substrate. Hence, the original microstructures and topographies of the paper scaffolds can be retained, while biochemical factors can be modified independently on the surfaces of paper scaffolds [28,29].

Here, for the first time we demonstrate the feasibility of surface-engineered paper scaffolds for stem cell culture and engineering for tissue reconstruction. Three types of commercially available paper materials (weighing paper [WP], chromatography paper [CP], and wiping tissue [WT]) were tested for adhesion, proliferation, differentiation, and gene transfection of human adipose-derived stem cells (hADSCs). Given that those paper materials possess totally different surface geometries, internal porous structures, and mechanical properties, stem cell behaviors and fate may be modulated in different ways by the distinct types of paper scaffold. A paper scaffold made of WP was found to significantly enhance osteogenic differentiation and *in vivo* bone regeneration of hADSCs. Stacking the papers with osteogenically differentiated hADSCs and human endothelial cells led to vascularized bone formation *in vivo*. Our study strongly suggests that this material possesses great potential as a bioactive, functional scaffold platform for stem cell-mediated bone tissue engineering.

## 2. Materials and methods

### 2.1. Preparation of paper scaffolds

Paper scaffolds were prepared from three types of commercial paper materials: weighing paper (WP), chromatography paper (CP), and wiping tissue (WT), which are paper types commonly available in the laboratory. The paper was used as received without any cleaning or pretreatment before iCVD. 1H,1H,2H,2H-perfluorodecyl acrylate (PFDA) monomer (97%), glycidyl methacrylate (GMA) monomer (97%), and the *tert*-butyl peroxide (TBPO) initiator (98%) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. The polymerized PFDA (pPFDA) and polymerized GMA (pGMA) films were deposited onto the paper scaffolds in an iCVD reactor (Daeki Hi-Tech Co., Daejeon, Korea). To deposit pPFDA film on the paper surface, PFDA monomer was heated to 70 °C and the vaporized PFDA was fed to the reactor through needle valves at a flow rate of 1.1 sccm. The vaporized TBPO was fed at room temperature into the reactor via metering valves at a flow rate of 0.8 sccm. The PFDA and TBPO were fed into the reactor concurrently with a reactor pressure of 100 mTorr. The paper scaffold was placed on a stage cooled by a recirculating chiller to keep the scaffold temperature at 40 °C, and the filament temperature was maintained at 200 °C. After the deposition of pPFDA film, GMA monomer was vaporized and fed into the reactor at a flow rate of 1.9 sccm. The flow rate of TBPO was maintained at 0.8 sccm. The deposition of pGMA film was initiated by increasing the filament temperature to 200 °C. The cooled stage temperature was 25 °C during the pGMA deposition. The growth rate of film deposition was monitored *in situ* using a He–Ne laser (JDS Uniphase, Milpitas, CA, USA) during the deposition process.

### 2.2. Paper scaffold characterization

Fourier transform infrared spectroscopy (FT-IR, IFS 66V/S, BRUKER, Billerica, MA, USA) spectra were obtained in normal absorbance mode and averaged over 64 scans with an optical resolution of 0.085 $cm^{-1}$. The chemical composition of the paper surfaces with polymer films was analyzed by X-ray photoelectron spectrometry (XPS, MultiLab 2000, Thermo, Waltham, MA, USA). The spectra were recorded using an Al Kα radiation X-ray source (200 W, 14 kV, KE = 1486.6 eV) under a vacuum base pressure of $5 \times 10^{-10}$ Torr. The XPS spectra were collected in the range of 0–1100 eV with a resolution of 1.0 eV at a pass energy of 50 eV. The atomic ratios of the polymer film-coated paper surfaces were calculated from the areas of C1*s*, O1*s*, and F1*s* peaks in the XPS survey scans multiplied by the appropriate sensitivity factors. Scanning electron microscopy (SEM, Magellan400, FEI Company, Hillsboro, OR, USA) was used to monitor the surface morphology of the polymer film-coated paper scaffolds. Water contact angle (WCA, Phoenix 150, Surface Electro Optics, Suwon, Korea) measurements were performed with a 5-μL deionized water droplet at room temperature. The Young's modulus and tensile strength of the paper scaffolds were measured at room temperature using a universal testing machine (UTM, INSTRON 5583, Instron Corporation, Canton, MA, USA) with a crosshead speed of 3 mm/min. The width of the sample was 10 mm, and the length between the jaws was 100 mm.

### 2.3. Human ADSC culture on the paper scaffolds

The hADSCs derived from human adipose tissue (Invitrogen, Carlsbad, CA, USA) were seeded onto the paper scaffolds (1.5 cm × 1.5 cm) at a seeding density of $2.5 \times 10^4$ cells/$cm^2$ and cultured in Dulbecco's modified Eagle medium (DMEM, Gibco, Gaithersburg, MD, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco), sodium bicarbonate (3.7 g/L), penicillin (100 U/mL), and streptomycin (100 μg/mL) in 5% $CO_2$ at 37 °C. To prevent cell adhesion to the portions of the plate not well covered by the paper scaffold, ultra-low attachment plates (Corning, Cambridge, MA, USA) were used for cell seeding. After 1 day of culture, the attachment of viable cells was examined by calcein-AM staining (Invitrogen). To examine proliferation and long-term viability of hADSCs, the cells were seeded onto the paper scaffolds ($1.0 \times 10^4$ cells/$cm^2$) and stained by calcein-AM at days 1, 3, 7, and 14 in culture. The hADSCs were subcultured between paper scaffolds every four days, and the passaged cells were stained by calcein-AM. Cell viability ($n = 4$) was quantified using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma–Aldrich). Briefly, hADSCs were seeded on the paper scaffolds at a density of $2.5 \times 10^4$ cells/$cm^2$. One day after cell seeding, MTT solution (5 mg/mL) was added to cell culture medium and incubated for 4 h at 37 °C. The optical density of each sample was measured at 560 nm using a microplate reader (Tecan, Mannedorf, Switzerland). To examine adhesion of hADSCs on the 3D paper scaffolds, hADSCs were seeded onto the paper scaffolds (1.5 cm × 1.5 cm) at a density of $1.0 \times 10^4$ cells/$cm^2$ and cultured for one day. The cells on the scaffolds were fixed with 10% formalin and dehydrated by graded series of ethanol (50%, 70%, 80%, 90%, and 100%) for 15 min each. Then, the dehydrated scaffolds were coated with platinum (Pt) for 120 s. The Pt-coated specimens were observed by field emission SEM (JEOL-7001F, JEOL Ltd., Tokyo, Japan).