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The Characteristics of Mineral Trioxide Aggregate/Polycaprolactone 3-dimensional Scaffold with Osteogenesis Properties for Tissue Regeneration

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Abstract

Introduction: The aim of this study was to investigate whether the mineral trioxide aggregate/polycaprolactone (MTA/PCL) hybrid 3-dimensional (3D) scaffold supplies a suitable microenvironment for the osteogenic differentiation of human dental pulp cells (hDPCs) and to further consider the effect of the MTA/PCL composite on the biological performance of hybrid scaffolds. **Methods:** MTA was suspended in absolute alcohol and dropped slowly into PCL that was generated with the printable MTA-matrix. Then, the MTA/PCL composite was prepared into highly uniform scaffolds with controlled macropore sizes and structure using a 3D printing technique. Mechanical properties and the apatite precipitation of the scaffolds were evaluated as well as the cell response to the scaffolds by culturing hDPCs. **Results:** The results showed that the MTA/PCL 3D scaffold had uniform, 450-μm, high-porosity (70%) macropores and a compressive strength of 4.5 MPa. In addition, the MTA/PCL scaffold could effectively promote the adhesion, proliferation, and differentiation of hDPCs. **Conclusions:** The 3D-printed MTA/PCL scaffolds not only exhibited excellent physical and chemical properties but also enhanced osteogenesis differentiation. All of the results support the premise that this MTA/PCL porous scaffold would be a useful biomaterial for application in bone tissue engineering. *(J Endod 2017;■:1–7)*

Key Words

Human dental pulp cell, mineral trioxide aggregate, osteogenesis, polycaprolactone, scaffold

Significance

MTA/PCL composite was prepared into highly uniform scaffolds with controlled macropore sizes and structure by a 3D printing technique. The 3D-printed MTA/PCL scaffolds not only exhibited excellent physical and chemical properties but also enhanced osteogenesis differentiation.

Mineral trioxide aggregate (MTA) has been used in endodontic treatments because of its excellent biocompatibility, sealing ability, and ability to promote pulp tissue regeneration (1–3). MTA is a calcium silicate (CS)-based cement that contains calcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, gypsum, bismuth oxide, and other mineral oxides that has been reported to be less cytotoxic than other root-end fillings or endodontic materials both *in vivo* and *in vitro* (2, 4). Recently, several researchers showed CS-based materials can assist in hard tissue formation and regeneration because of Si ion release and apatite formation abilities (5, 6). In addition, the bioactivity of CS-based materials has led to their use in constructing scaffolds with various cells for tissue regeneration (6, 7). MTA aggregate presents good osteoconduction and reduces inflammation in human dental pulp cells (hDPCs). The CS-based materials promote hard tissue regeneration and have the ability to stimulate odontogenic and osteogenic differentiation in various types of cells (5, 8–10). However, the poor fracture toughness and wear resistance of MTA limit its implant performance and life span in a load-bearing environment (9).

Polycaprolactone (PCL) has already been approved for several medical and drug delivery devices and now is extensively used for tissue regeneration because of its cost-effectiveness, durability, excellent biocompatibility, and biodegradability (11). In the human body, PCL breaks down over a period of time with no side effects; it generally takes 6 months to 2 years to degrade *in vivo* depending on its molecular weight (12). Therefore, several scaffolds that combine ceramic powder with PCL have been shown to exhibit good properties and biological activity in hard tissue engineering (13–16). Over the last year, a 3-dimensional (3D) printing method was developed to fabricate more ideal structural scaffolds with better control of pore morphology, pore size, and porosity (17–19). Thus, 3D printing can now be used to fabricate various versatile, solid free-form structures providing unprecedented flexibility in both material and geometry, thus creating a potential way to produce customized scaffolds for growing irregular tissues (20). With extrusion-based 3D printing, PCL-contained composites can be

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melted and extruded in a computer-controlled pattern to construct scaffolds layer by layer (21).

The aim of this study was to investigate whether an MTA/PCL hybrid 3D scaffold supplies a suitable microenvironment for the osteogenic differentiation of hDPCs and to further consider the effect of the MTA/PCL composite on the biological performance of hybrid scaffolds.

Materials and Methods

Preparation of Scaffolds

An MTA/PCL matrix was produced using the thermal pressing method (19). First, reagent-grade PCL (Mw = 43,000–50,000; Polysciences, Warrington, PA) was placed in a 150°C oven for 2 hours. Then, MTA (ProRoot MTA; Dentsply, Tulsa, OK) powder was suspended in absolute alcohol and dropped into PCL. The composite was placed in a 100°C oven, and it been the printable materials (MTA/PCL = 6/4). The concept for this device was based on a precision 3-axis positioning system (BioScaffolder 3.1; GeSiM, Grosserkmannsdorf, Germany). The composite pastes were loaded into a syringe and dispensed through a steel nozzle at 95°C by applying a pressure of 500 kPa; a 500-μm line with a line height of 300 μm and a total of 7 lines were printed in parallel with a gap diameter of 500 μm between the composites lines. The MTA/PCL scaffold was plotted layer by layer up to 16 layers through the extrusion of the paste as a fiber.

Mechanical Testing and Characterization of Scaffolds

The compressive strength of scaffolds was measured on an EZ-Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 1 mm/min. The maximal compression load at failure was obtained from the recorded load-deflection curves. The phase composition of the cements was analyzed using X-ray diffractometry (XRD; Bruker D8 SSS, Karlsruhe, Germany) operated at a scanning speed of 1°/min. The specimens were coated with gold, and their morphologies were investigated using a scanning electron microscope (JSM-6700F; JEOL, Tokyo, Japan) under the lower secondary electron image mode at a 3-kV acceleration voltage.

In Vitro Soaking

The scaffolds were soaked in a simulated body fluid (SBF) solution at 37°C. The SBF solution was similar to human blood plasma and consisted of 7.9949 g NaCl, 0.2235 g KCl, 0.147 g K_2HPO_4, 0.3528 g $NaHCO_3$, 0.071 g Na_2SO_4, 0.2775 g $CaCl_2$, and 0.305 g $MgCl_2 \bullet 6H_2O$ in 1000 mL distilled H_2O. The pH was adjusted to 7.4 with hydrochloric acid and tris(hydroxymethyl)aminomethane. After immersion for various time periods, the scaffolds were removed from SBF, and the microstructure and strength were investigated. After immersion for different time points, the Ca and Si ion concentrations released from the specimens were analyzed using an inductively coupled plasma atomic emission spectrometer (OPT 1 MA 3000DV; Perkin-Elmer, Shelton, CT).

hDPC Isolation and Culture

The hDPCs were freshly derived from caries-free, intact premolars that had been extracted for orthodontic treatment purposes as described previously (22). The patient gave informed consent, and approval from the Ethics Committee of the Chung Shan Medicine University Hospital, Taichung, Taiwan, was obtained (CSMUH no. CS14117). After extraction, the tooth was split sagittally with a chisel. The pulp tissue was then immersed in phosphate-buffered saline (PBS; Caisson, North Logan, UT) solution and digested in 0.1% collagenase type I (Sigma-Aldrich, St Louis, MO) for 30 minutes. After being transferred to a new plate, the cell suspension was cultured in Dulbecco modified Eagle medium (DMEM, Caisson) and supplemented with 20% fetal bovine serum (GeneDireX, Las Vegas, NV) and 1% penicillin (10,000 U/mL)/streptomycin (10,000 mg/mL) (Caisson) in a humidified atmosphere with 5% CO_2 at 37°C; the medium was changed every 3 days. The odontogenic differentiation medium was DMEM supplemented with 10^{-8} mol/L dexamethasone (Sigma-Aldrich), 0.05 g/L L-Ascorbic acid (Sigma-Aldrich), and 2.16 g/L glycerol 2-phosphate disodium salt hydrate (Sigma-Aldrich).

Cell Adhesion and Proliferation

The scaffolds were sterilized by soaking in 75% ethanol followed by exposure to ultraviolet light for 1 hour before being cell cultured. After being directly cultured for various time periods, cell viability was evaluated using the PrestoBlue assay (Invitrogen, Carlsbad, CA), which is based on the detection of mitochondrial activity. Thirty microliters of PrestoBlue solution and 300 μL DMEM were added to each well followed by 30 minutes of incubation. After incubation, 100 μL of the solution in each well was transferred to a 96-well enzyme-linked immunosorbent assay plate. The plates were read in a multiwell spectrophotometer (TECAN Infinite Pro M200; Tecan, Männedorf, Switzerland) at 570 nm with a reference wavelength of 600 nm. Cells cultured on the tissue culture plate without cement were used as a control (Ctl).

Fluorescent Staining

After being cultured for 7 and 14 days, the cells were washed with cold PBS, fixed in 4% paraformaldehyde (Sigma-Aldrich) for 15 minutes, and then permeabilized with PBS containing 0.1% Triton X-100 (Sigma-Aldrich) at room temperature. The F-actin filaments were stained with phalloidin conjugated to Alexa Fluor 488 (Invitrogen) for 1 hour. The nuclei were stained with 300 nmol/L 4′, 6-diamidino-2-phenylindol (Invitrogen) for 30 minutes. After washing, the morphology was obtained using a Zeiss Axioskop2 microscope (Carl Zeiss, Thornwood, NY).

Osteogenic Differentiation

The secretion of alkaline phosphatases (ALPs) was determined on days 3 and 7 after cell seeding. Briefly, the cells were lysed by using 0.2 % NP-40 (Sigma Aldrich) and centrifuged at 6000 rpm for 15 minutes. ALP activity was determined using p-nitrophenyl phosphate (Sigma-Aldrich) as the substrate. Each sample was mixed with p-nitrophenyl phosphate in 1 mol/L diethanolamine buffer. The reaction was stopped by the addition of 5 N NaOH and was quantified by absorbance at 405 nm. Osteocalcin (OC) protein released from the hDPCs was cultured on MTA scaffolds for 7 and 14 days. An enzyme-linked immunosorbent assay kit (Invitrogen) was used to determine the protein content following the manufacturer's instructions. The concentration of OC proteins was measured by correlation with a standard curve. Analysis of blank disks was treated as a Ctl.

Calcium Deposition

The hDPCs cultured on the scaffold surfaces were stained with alizarin red S to examine mineralized nodule formation and calcium deposition [13]. Briefly, the cells were fixed with 4% paraformaldehyde (Sigma-Aldrich). After 15 minutes, the cells were incubated in 0.5% alizarin red S (Sigma-Aldrich) at pH 4.0 for 15 minutes at room temperature. To quantify the stained calcified nodules after staining, the samples were immersed with 1.5 mL 5% sodium dodecyl sulfate in

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