Supporting Information

Development and characterization of waste equine bone-derived calcium phosphate cements with human alveolar bone-derived mesenchymal stem cells

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Thermogravimetric study

To evaluate the protein contents in the thermally treated equine bone powder, measurement procedure of mass change by pyrolysis was conducted, commonly implemented methods for xenogenous bone graft in Korea by guided from Food & Drug Administration (KFDA). The EB powder was prepared 500 mg (m1), and heat treated via electric furnace at 1000 C in normal atmosphere for 12 h. The cooled EB powder weighgt (m2) was measured. The ration of pyrolysis weight (R) was calcuated following equation:

$$\mathrm{R} = \frac{m_1 - m_2}{m_1} \times 100 \ \ (\%)$$

X-ray diffractometry (XRD)

The crystalline phases in the hydroxyapatite (HA) and tricalciumphosphate (TCP) were detected using an X-ray diffractometer (New D8 Advanced XRD, Bruker, Germany) with Cu-Kα radiation at 40 kV and 40 mA. The samples were scanned at 3° to 55° using steps of 2θ and a counting time of 60 s per step. The crystalline phase compositions were identified with reference to the standard JCPDS cards that were available in the system software.

Multipotent characterization

The isolated stem cells were cultured in 12 well culture plate at 37 ˚C and 100 % humidity with 5 % CO_2 in α-minimal essential medium (α-MEM) supplemented with 10 % fetal bovine serum (FBS, Hyclone, USA), 1 % antibiotic, and 1 % ascorbic acid (Sigma Aldrich, St. Louis, MO, USA). The medium was changed every 2 days. When the cells reached 90 % confluence, the differentiation potential of the isolated stem cells was cultured under conditions that were appropriate for osteogenic, neurogenic, adipogenic, and differentiation for 2 weeks. The four types of differentiation induction medium - osteogenic, neurogenic, adipogenic, angiogenic (PromoCell, Heidelberg,

Germany) - was changed every 2 days. After 2 weeks, the medium was removed, and wash two times briefly with PBS. For fixation of cells, the 0.5 % paraformaldehyde was treated for 30 min at 25 °C. For alizarin red S staining, the fixed cells were incubated with 0.5 % Alizarin red S (Sigma, St. Louis, MO) solution at room temperature 20 min, wash briefly twice with PBS. For nissle staining, the fixed cells were incubated with 0.5 % crysel violet (Sigma Aldrich, St. Louis, MO, USA) solution at room temperature. wash briefly twice with PBS. For oil red O staining, the fixed cells were incubated with 0.5 % Oil red O (Sigma-aldrich, St. Louis, MO) solution at room temperature. wash briefly twice with PBS. For alcian blue staining, the fixed cells were incubated with 0.5 % alcian blue (Sigma Aldrich, St. Louis, MO, USA) solution at room temperature. wash briefly twice with PBS.Measurement of protein content

Figure S1. Characterization of heatreatment of equine bone. (a) FT-IR results of equine bone before and after heat treattreatment. (b) TGA results of equine bone specimen. (c) Weight loss ratio ($m_2/m_1 \times 100$ (%)) of equine bone powder after thermal treatment at 1200 °C. (m_1: initial weight of equine bone powder, m_2: after thermal treatment at 1200 °C for 12 h weight of equine bone powder)

Fig. S2. XRD analysis of the TCP and HA.

Fig. S3. Characteristics of DPSCs and PDLSCs. (A) Cell viability results for the DPSCs and PDLSCs on the EB-CPCs. The cell viabilities of the hABMSCs were measured at an absorbance of 450 nm (error bars indicate standard deviations, n = 3). * indicates a significant difference ($p < 0.05$). (B) Alkaline phosphatase (ALP) activities of the DPSCs and PDLSCs on the EB-CPCs. Osteogenic media were used for 7 and 14 days (error bars indicate standard deviations, n = 3). * indicates significant difference ($p < 0.05$).

Figure S4. Evaluation of multilineage potential of hABMSCs, DPSCs, and PDLSCs. The cells were cultured for 1 and 14 days under the differentiating condition to each lineage. Calcium deposits in the culture plate were stained using Alizarin red O staining of each cells. Cresyl violoet staining for evaluation neurogenic differentiation was conducted to each cells. Oil red O staining for evaluation adipogenic differentiation was conducted to each cells. Each cells were stained with Alcian blue for evaluation chondrogenic differentiation.