INTRODUCTION
Increasing experimental interest has emerged for the use of bone marrow stromal cells (BMSc) to enhance bone healing and spine fusion in various clinical situations. Hyaluronan (HY) is an almost ubiquitous component of extracellular matrices. It produces that, in part, early in embryogenesis mesenchymal cells migrate, proliferate and differentiate. A low-molecular weight HY fully expresses the osteogenic potential of mesenchymal cells through the subsequent proliferation and differentiation of osteoprogenitor cells using proper conditions. The present study investigated whether HY at the different concentrations has an effect on proliferation on an in vitro system of porcine BMSc culture and evaluated how porcine BMSc responded to HY, dexamethasone (Dex), recombinant human bone morphogenetic protein-2 (rhBMP-2) on the in vitro system of osteogenesis during an early stage.

MATERIALS AND METHODS
Porcine BMSc was isolated from iliac bone chips of 3-month-old Danish Landrace pigs and cultured in Dulbecco’s modified Eagle’s medium (DMEM) and 10% FBS until confluent (10-14 days). After 2 days and 7 days of cell subculture with 800,000 Da HY (sodium hyaluronate, LifeCore® Biomedical, MN) at the concentration of 0, 0.5, 1.0, 2.0, and 4.0 mg/mL, cellular proliferation was determined by H-thymidine incorporation into DNA. Further, BMSc were subcultured with 12 combinations of HY (0, 1.0, 4.0 mg/mL), Dex (-, +), and rhBMP-2 (0, 10 ng/mL, Genetics Institute, Inc., MA) for 2 days and 7 days. DNA incorporation of H-thymidine, alkaline phosphatase (ALP) activity and Pro-collagen type I C-terminal propeptide (PICP) were measured. Analysis of variance (ANOVA) with repeated measurements was used for statistical analysis.

RESULTS
After time intervals of subculture on Day 2 and Day 7, cellular proliferation in the presence of HY was accelerated when compared to that in the absence of HY. Cellular proliferation at the high concentration (4 mg/mL) of HY was higher than that at other lower concentrations (0.5, 1.0, 2.0 mg/mL). No difference was found among 0.5, 1.0, 2.0 mg/mL. When BMSc subcultured with HY, Dex, and/or rhBMP-2 on Day 2 and Day 7, HY, rhBMP-2, Dex-HY interaction and rhBMP-2-HY interaction significantly stimulated cellular proliferation, respectively. Specifically, cellular proliferation was significantly increased in the presence of 4.0 mg/mL HY, and in combination with 4.0 mg/mL HY and Dex, 4.0 mg/mL HY and rhBMP-2, 1.0 mg/mL HY and Dex compared to the cell culture in DMEM and 10% FBS. When BMSc subcultured with HY, Dex, and/or rhBMP-2, ALP activity on Day 7 was significantly increased when compared to Day 2. Dex-rhBMP-2 interaction significantly elevated ALP activity on day 2. Specifically, it was significantly increased in the presence of Dex and rhBMP-2, 4 mg/mL HY alone and in combination with Dex, and 1 mg/mL HY in combination with Dex and rhBMP-2 compared to cell culture in DMEM and 10% FBS on Day 7 (Fig. 1). When BMSc subcultured with HY, Dex, and/or rhBMP-2, PICP did not show a significant difference on Day 7 when compared to Day 2. It was significantly decreased in the presence of HY, Dex-HY interaction and rhBMP-2-HY interaction (Fig. 2).

DISCUSSION
These results suggested that hyaluronan stimulated bone marrow stromal cell proliferation, differentiation and enzyme secretion such as alkaline phosphatase during the early stage. More importantly, hyaluronan interacted with dexamethasone and rhBMP-2 to generate direct and specific cellular effect, which would be important in bone repair.

Keywords: Cell-material interaction, Stem cells, Growth factor, Cell therapy

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REFERENCES