Glucose Effect of Pre-differentiation Culture on Chondrogenesis of Human Mesenchymal Stem Cells

INTRODUCTION
Human Mesenchymal Stem Cells (hMSCs) have been considered a promising cell source with the capability of quantity expansion and multi-differentiation potential for tissue engineering and regeneration medicine. However, their differentiation capacity tends to decrease with the number of cell passages. For clinical applications, it is necessary to optimize the culture condition in order to get quality cells.

Glucose is the major energy source in a culture medium for cells and a high concentration of glucose in the medium has shown its regulatory effects on MSC proliferation and differentiation [1, 2]. Previous studies focused on the glucose effects during cell differentiation while fewer studies have investigated the role of glucose in maintaining the differentiation capacity of hMSC before differentiation.

For chondrogenesis (CG), the supplement of transforming growth factor-β1 (TGF-β1) or TGF-β3 is critical for hMSCs to differentiate into chondrocytes. The TGF-β signaling induction involves the activation of Smad2/3 and the downstream Smad7. A recent report showed that the high glucose concentration up-regulates the TGF-β signaling to induce cell hypertrophy in mouse embryonic fibroblasts and rat kidney epithelial cells [3]. We therefore hypothesize that hMSCs maintained in a high glucose medium before chondrogenic differentiation up-regulate the expression of the molecules involved in the TGF-β signaling pathway to enhance CG thereafter.

METHODS
Cell Culture
Human MSCs were isolated from bone marrow following the protocol approved by the University of Wisconsin IRB and then cultured in low-glucose DMEM (1 mg/ml) containing 10% FBS. At passage 2, cells were divided into and maintained in three culture media: one with low-glucose DMEM (LG), another with median-glucose DMEM (2.75 mg/ml, MG), and the other with high-glucose DMEM (4.5 mg/ml, HG).

Glucose Effects on TGF-β Signaling Regulation
Total RNA was isolated from hMSCs in low- and high-glucose DMEM before and 2 hrs after the fresh medium change. Molecules of the TGF-β signaling pathway were analyzed using RT-PCR. In addition, the cultured medium was collected for 15 mins, 30 mins, 1 hr, 2 hrs, and 4 hrs after the fresh medium change to measure the concentration of soluble TGF-β1 in the medium using ELISA.

Chondrogenesis of hMSCs
For chondrogenesis, hMSCs maintained in low- and high-glucose DMEM during chondrogenesis. The cells were maintained in the CG medium and the expression level of phosphorylated Smad3 was assessed using Western blot.

RESULTS
Human MSCs maintained in low-glucose culture before chondrogenesis showed the up-regulated expression of collagen types II and IX, compared to the cells in high-glucose culture (Fig. 1). Though the chondrogenic potential decreased with the cell passage number, low-glucose-maintained cells continued to show the potential of up-regulated chondrogenesis up to the cell passage number of nine.

The ELISA result showed that a trace of soluble TGF-β1 was detected in the cultured media collected 15 mins, 30 mins, 1 hr, 2 hrs, and 4 hrs after the fresh culture medium change. There was no significant difference of the soluble TGF-β1 concentrations between these groups at different collection times (data not shown). Gene analysis results showed that the expression levels of TGF-β receptor 1 and receptor 2 were similar between the low-glucose- and high-glucose-maintained cells before and after fresh medium change (Fig. 2). The gene expression level of TGF-β1 was up-regulated after fresh medium change, but there was no difference between the cells maintained in the low- and high-glucose culture. This finding was consistent with that from the ELISA analysis, which also showed no noticeable difference of the soluble ligand concentration.

Finally, in order to assess the activation level of TGF-β signaling of hMSCs maintained in low-, median- and high-glucose during chondrogenesis, cells were made into cell pellets, and the expression level of activated Smad3 was assessed. The result showed that the highest level of the phosphorylated Smad3 was expressed in the low-glucose maintained hMSCs (Fig.3), and lowest level expressed by the high-glucose maintained cells.

DISCUSSION
Our result shows that hMSCs maintained in a low-glucose medium before chondrogenesis can be more effectively induced into chondrocytes by highly activating Smad3, compared to those in a high-glucose medium. The finding suggests that the low-glucose medium may have the potential to prime hMSCs for chondrogenesis, since the activation of Smad3 is a key step to activate Sox9 and subsequent chondrogenesis of hMSCs [4]. The relationship between glucose regulation and Smad3 activation is also shown in a previous study by Wu et al. using mouse embryonic fibroblasts and rat kidney epithelial cells [3]. Together, we demonstrate that hMSCs maintained in a low-glucose culture medium before differentiation, are primed for enhanced chondrogenesis.

REFERENCE