INTRODUCTION
Stem cell-based tissue engineering has provided an alternative strategy to treat cartilage lesions and synovium-derived mesenchymal stem cells (SMSCs) are considered as a promising cell source for cartilage repair. Compared with other sources, such as bone marrow, adipose tissue, periosteum and skeletal muscle, SMSCs are more attractive because they display greater proliferative ability and chondrogenic potential. SMSCs are routinely isolated from synovium by relying solely on their adherence to the plastic surface of tissue culture flask. However, the isolated cell population is heterogeneous that always contains non-MSCs, such as macrophages, adipocytes, fibroblasts, mast cells and endothelial cells. Most of these non-MSCs have no chondrogenic potential and may interfere with chondrogenesis of SMSCs. CD105 has been proven playing an important role in the control of chondrogenic differentiation of MSCs, and CD105 may be a relatively specific marker that can be used for antibody-based cell sorting. Alginic acid and chitosan have been widely used as a delivery matrix for cartilage tissue engineering. The objective of this study was to explore whether the chitosan-alginate composite 3-D porous scaffold can provide a suitable environment for chondrogenic differentiation and proliferation of CD105-positive (CD105⁺) enriched SMSCs.

METHODS
Composite scaffold of chitosan and alginate was fabricated by freeze drying process. SMSCs were isolated from rat joint synovium and CD105⁺ cells were enriched using magnetic activated cell sorting (MACS). Sorted cells were subsequently seeded onto the 3-D porous scaffolds and cultured in chondrogenic culture medium in presence of TGF-β3 and BMP-2 for 2 weeks in vitro. As a control, the same amount of MACS-sorted cells and non-sorted cells was cultured in pellet. The microstructures of the porous scaffold, cell morphology and deposition of extracellular matrix were observed by SEM. The amount of total DNA was measured using Hoechst 33258 dye. GAG was quantified using dimethylmethylene blue (DMMB) dye-binding assay. The expression of mRNA for chondrogenesis-related genes was detected by semi-quantitative RT-PCR. In addition, cell cycle of the cells was also assessed. Statistical analyses were performed by SPSS 13.0 statistical software, and p values of less than 0.05 were considered statistically significant.

RESULTS
No obvious differences in morphology were noted between non-sorted and MACS-sorted cells. Most cells displayed positive expression of CD105 with immunofluorescence staining in MACS-sorted cell population, while a few positive cells were observed in non-sorted cell population (Figure 1). After 2 weeks in culture, SEM results showed that cells attached and proliferated well on scaffolds, and secreted extracellular matrix were also observed (Figure 2). Sorted cells cultured in scaffolds differentiated into a chondrogenic phenotype, as evidenced by the mRNA expression of type II collagen (col2α1), aggrecan and Sox9 (Figure 3). From day 7 to day 14, the percentage of MACS-sorted cells in 3-D scaffold culture group in the S and G2/M phases increased and the percentage of cells in the G0/G1 phase decreased. From day 7 to day 14, the percentage of MACS-sorted cells in 3-D scaffold culture group in the S and G2/M phases increased and the percentage of cells in the G0/G1 phase decreased (Figure 5).

DISCUSSION
In the present study, the results suggest that CD105⁺ SMSCs could be effectively isolated and enriched by MACS techniques, and the chitosan-alginate composite 3-D porous scaffold could maintain chondrogenic phenotype of MACS-sorted cells in the defined chondrogenic culture medium in presence of TGF-β3 and BMP-2, as indicated by the high expression level of chondrogenesis-related genes. Furthermore, compared with the conventional pellet culture, 3-D porous scaffold culture would promote cell proliferation and extracellular matrix (ECM) secretion. The results demonstrate that the chitosan-alginate composite 3-D porous scaffold has good biocompatibility with the MACS-sorted cells and could provide a suitable microenvironment for supporting chondrogenic differentiation and proliferation of cells. The evidence also supports that CD105⁺ enriched SMSCs could be a potential cell source for cartilage tissue engineering.