INTRODUCTION:
Tissue engineering has proved to be one of the most promising therapies for articular cartilage defects. Difficulty in in-vitro cell expansion and low cell quantity was encountered both in chondrocytes and bone marrow derived stem cells. Adipose derived stem cells (ADSCs) are an alternative cell source with several advantages including easy harvest, high proliferation rate, and multilineage potentials. For the approach of cartilage tissue engineering, a proper three dimensional (3D) microenvironment is necessary to promote the differentiation of stem cell into chondroinductive for transplantation. Hyaluronic acid (HA) has been reported that it enhances mesenchymal cell both cell proliferation and chondrogenic differentiation. We hypothesized that HA modified PLGA scaffold could provide a suitable microenvironment for ADSCs than PLGA in cartilage tissue engineering. To test our hypothesis, we developed a novel HA modified biodegradable PLGA (PLGA/PEI/HA) scaffolds for the application of cartilage tissue engineering. The cytocompatibility of HA modified PLGA and unmodified PLGA was compared by testing the cell adhesion and proliferation of ADSCs in these two scaffolds.

MATERIALS AND METHODS:
Human adipose derived stem cells (ADSCs) isolation & characterization: Adipose tissue was obtained from orthopaedic patients undergoing surgery. Adipogenesis, osteogenesis, and chondrogenesis were induced to determine the minimal potential of the ADSCs. HA modified porous poly DL-lactic-co-glycolic acid (PLGA/PEI/HA) scaffold and unmodified porous poly DL-lactic-co-glycolic acid (PLGA) scaffold preparation: The scaffolds were prepared as disks with 12mm in diameter, 1mm in thickness, and pore size within 300-400 μm. The porous PLGA/PEI/HA scaffolds were prepared by blending pure PLGA with pre-conjugated amine-terminated PLGA-PEI di-block copolymer (PLGA/PEI). HA was chemically conjugated to the surface exposed amine groups on the PLGA/PEI scaffolds. Unmodified porous PLGA (PLGA) scaffolds were prepared by blending pure PLGA with the same amount of no PEI conjugated PLGA polymer. Morphology of the scaffolds was observed by scanning electron microscopy (SEM) (Fig. 2A, B). Cytocompatibility: 1. Cell adhesion on scaffold: The PLGA/PEI/HA and PLGA scaffolds were seeded with 1ml of cell suspension at a density of 10^5 cells/ml. The unattached cells were collected from the medium in culture dish 5, 15, 30, 60 min after seeding. The unattached cell numbers were counted by using a hemocytometer. 2. Cell proliferation on scaffold: The PLGA/PEI/HA and PLGA scaffolds were seeded with 1ml of ADSCs scaffold (10^5) cells/ml suspension. The bioactivity was determined by MTS assay.

RESULTS:
Characterization of human ADSCs: Human ADSCs incubated in monolayer showed a spindle-shaped morphology. (Fig. 1A) ADSCs were induced adipogenesis after 14 days of induction (Fig. 1B; Oil-Red-stained). Chondrogenesis (Fig 1C; Alcian blue stained) and osteogenesis (Fig. 1D; von Kossa stained) were also induced. These characteristics indicated that these ADSCs are multipotent stem cells. Scaffold structure: Both PLGA and PLGA/PEI/HA scaffolds showed to posses an open macroporous and interconnected structure, indicating they are suitable for three-dimensional culture of ADSCs, and these characteristics did not change by HA modification. In vitro cytocompatibility: 1. Cell adhesion: The number of ADSCs adhered to PLGA was significantly higher than that on PLGA/PEI/HA at the first 5min of experiment. However, no significant difference was found between these 2 groups after 15-60min of the experiment. 2. Cell proliferation: MTS assay was used to measure the relative living cells on scaffold. There was no significant difference of living cells grow in both scaffold after 1 and 3days after seeding. However, cells grown in PLGA/PEI/HA scaffold were significantly more than that on PLGA after 5 day of culture.

DISCUSSION:
For stem cell based cartilage tissue engineering, a 3D scaffold to accommodate enough cells and provide a chondroinductive microenvironment is required. In this study, we have successfully isolated and characterized the ADSCs from human subjects. ADSCs are capable to differentiate into adipogenic, osteogenic, and chondrogenic lineage. We also have developed a novel HA modified PLGA scaffold, and the in vitro cytocompatibility of unmodified PLGA (PLGA) and HA modified scaffold (PLGA/PEI/HA) was compared. Our results indicated that PLGA/PEI/HA scaffold enhanced cell proliferation of ADSCs. The results suggest that HA modified PLGA scaffold could provide a more suitable microenvironment for the usage of ADSCs in cartilage tissue engineering.

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