Controlled-release of GDF-5 from fibrous scaffold for enhanced hASCs-based bone regeneration

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ABSTRACT SUMMARY

Human adipose-derived stem cells (hASCs) are considered to have multipotency and easily induced to differentiate into osteoblast and growth and differentiation factor-5 (GDF-5) is an appealing candidate for potentiating osteogenic differentiation. 1,25-dihydroxyvitamin D3 also plays an important role in osteogenic induction, so it was expected that controlled-release of GDF-5 from micro-fibrous poly(L-lactic acid) (PLLA) scaffold might enhance osteogenic differentiation of hASCs through synergistic effect between GDF-5 and 1,25-dihydroxyvitamin D3. GDF-5 and 1,25-dihydroxyvitamin D3 loaded group achieved the best result in alkaline phosphatase activity, osteogenic marker gene expression and ECM mineralization. This study suggested that controlled-release of GDF-5 with 1,25-dihydroxyvitamin D3 might potentially enhance osteogenic differentiation of hASCs and that the local delivery of osteo-inductive factor might be important for stem cell-based bone regeneration.

INTRODUCTION

The human adipose-derived stem cells possess at least tri-lineage potentials; bone, cartilage, and fat. However, some kinds of adult stem cell have problems with low harvesting efficiency and pain from harvesting. As hASCs can be easily obtained in large quantities with minimal discomfort during harvesting from a donor, they might be promising cells for tissue engineering.

It was hypothesized that biocompatible and biodegradable scaffolds can serve hASCs with osteogenic factors released from the scaffolds. Micro fibers were prepared by wet-spinning method and then globular shaped micro-fibrous scaffolds were fabricated with poly (L-lactic acid) (PLLA) micro fibers including factors, such as GDF-5 and 1,25-dihydroxyvitamin D3. Growth and differentiation factor-5 (GDF-5), known as BMP-14, has gained interest recently. The ability of GDF-5 to regulate and promote osteoblastic and osteogenic properties has made it an appealing candidate for potentiating osteogenic differentiation. More specifically, as working with ASCs, GDF-5 appears to be as effective as BMP-2 for potentiating osteogenic differentiation in vitro. The mechanism of action, however, is not as clear. 1,25-dihydroxyvitamin D3 plays a physical role in bone formation and maturation. Importance and requirement of 1,25-dihydroxyvitamin D3 in bone biology starts from the very beginning, affecting proliferation and differentiation of early members of osteoblastic lineage in addition to enhancing matrix mineralization for mature bone formation.

This study represents an efficacy of GDF-5 released from micro-fibrous PLLA scaffold on enhancing osteogenic differentiation of hASCs with 1,25-dihydroxyvitamin D3 synergistically.

EXPERIMENTAL METHODS

PLLA was dissolved in methylene chloride/acetone mixture (9:1) at a concentration of 7w/v%. To spin osteogenic factor unloaded micro fibers, pH 7.4 phosphate-buffered saline (PBS) was incorporated into PLLA solution at a concentration of 1v/v%. 1,25-dihydroxyvitaminD3 was dissolved in acetone at a concentration of 0.01w/v%. GDF-5 was dissolved in PBS at a concentration of 1w/v%. Collected fibers were freeze-dried to evaporate remained solvent and we made globular shaped micro-fibrous scaffolds (diameter 5mm, weight 10mg) out of the freeze-dried micro fibers.
Fluorescein isothiocyanate-bovine serum albumin (FITC-BSA) as a model drug was used instead of GDF-5 during in vitro release study to evaluate a release behavior of protein drug from polymeric drug carriers.

Cell proliferation was measured by Cell Counting Kit-8 after incubation for 1, 3, 7, 14 days. Alkaline phosphatase (ALPase) is located on the cell membrane of osteoblast and its activity has been shown to correlate well with osteoblastic activity. To evaluate calcium deposition, after fixed cell-scaffold being embedded in paraffin blocks, sections of 4 μm thickness were sequentially recovered and then von Kossa and H&E staining were performed.

RESULTS AND DISCUSSION

In this study, micro fibers having an ability of controlled-release of osteogenic factors were prepared. Figure 1 shows a release profile of FITC-BSA, as a model drug. In vitro controlled-release of GDF-5 lasted for more than 2 weeks, this release profile appropriated to the timing of cellular events with GDF-5 during fracture healing.

With osteogenic factor unloaded group, cell viability and proliferation were highly improved, whereas with other two groups, growth inhibition was observed. Because in osteoblasts and ASCs, 1,25-dihydroxyvitamin D3 is generally associated with inhibiting cellular proliferation and inducing differentiation.

With GDF-5 & 1,25-dihydroxyvitamin D3 loaded group, ALPase activity was significantly higher than other groups at 21 days. Calcium deposition occurred remarkably under an existence of 1,25-dihydroxyvitamin D3 and was also the best in GDF-5 & 1,25-dihydroxyvitamin D3 loaded group (Figure 3).

REFERENCES
