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A novel strategy of spine defect repair with a degradable bioactive scaffold preloaded with adipose-derived stromal cells

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Abstract

BACKGROUND CONTEXT: Although the use of mesenchymal stem cells (MSC) with scaffolds for bone repair has been considered an effective method, the interactions between implanted materials and bone tissues have not been fully elucidated. At some specific sites, such as the vertebral body (VB) of the spine, the process of bone repair with implanted biomaterials is rarely reported. Recently, adipose tissue was found to be an alternative source of MSC besides bone marrow. However, the strategy of using adipose-derived stromal (ADS) cells with bioactive scaffold for the repair of spinal bone defects has seldom been studied.

PURPOSE: To use a sintered poly(lactide-co-glycolide) acid (PLGA) microspheres scaffold seeded with induced rat ADS cells to repair a bone defect of the VB in a rat model.

STUDY DESIGN: Basic science and laboratory study.

METHODS: A sintered porous microspheres scaffold was manufactured by PLGA. ADS cells were isolated from Fischer 344 rats and then induced by osteogenic medium with growth and differentiation factor 5 (GDF5) in vitro. Before implantation, cells were cultured with inductive media for 2 weeks as a monolayer situation and 1 more week on a PLGA scaffold as a three-dimensional structure. These assembled bioactive scaffolds then were implanted in lumbar VB bone defects in Fischer 344 rats. The ex vivo differentiation of the cells was confirmed by von Kossa staining and real-time polymerase chain reaction. The performance of cells on the scaffold was detected by scanning electron microscopy and (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. In vivo bone formation was quantitatively measured by computed tomography study. And the effect of tissue repair was also evaluated by histological studies.

RESULTS: Proliferation and differentiation of cells were confirmed before in vivo implantation. Quantification of bone formation in vivo through serial three-dimensional computed tomography images revealed that the VB implanted with GDF5-induced cells demonstrated more bone formation than the control groups. Besides the bone formation period that occurred between 2 and 4 weeks in all groups, a second bone formation period was found to occur only in the groups that received cells with previous induction in vitro. This second period of significant bone formation happened simultaneously with collapsing of the scaffolds. It was then demonstrated histologically that vascularization early in the process and cooperation between host bone and implanted cells accompanied by collapse of the scaffold may be the factors that influence bone formation. This study

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not only provides a therapeutic strategy of using biomaterial for bone repair in the spine, but also may lead to a technological method for studying the relationship between implanted stem cells and host tissue.

CONCLUSIONS: Adipose-derived stromal cells maintained in culture on a scaffold and treated with osteogenic induction with growth factor ex vivo could be used to enhance bone repair in vivo.

Keywords: Poly(lactide-co-glycolide) acid; Microspheres; Vertebral body; Bone defect; Growth and differentiation factor 5; Bone repair

Introduction

The use of bioengineering techniques to manufacture biomaterials for the repair of bone tissue has been an attractive proposition that has been explored for many years. Although novel materials with potential for bone repair have been developed and remarkable progress has been made, as of yet the relationship and interaction between the implantations and host bone tissue during in vivo applications have not been fully understood [1]. Possible reasons for this include the failure of in vitro test environments to adequately reproduce the in vivo conditions, the level of precision necessary for the treatment of very specific local bone disease, and differences in the performance of the biomaterials in vitro compared with in vivo. Because of the differences between specific situations in vivo and ex vivo, it is necessary to consider the interaction between the biomaterials and bone specifically at the in vivo location of interest to address critical issues in optimizing the therapeutic strategy, which will likely be crucial for effective bone repair.

Bony defects of the vertebral body (VB) are a common residual effect of the reduction of a spine fracture or the removal of a spine tumor. Besides the general requirements for bone formation in vivo, the challenge facing attempts to develop effective bioengineering techniques for the repair of VB is the need to promote bone repair in an environment where there is a deficiency of bone marrow with a huge loss of bone volume. Moreover, the scaffold needs to be strong enough to support the compression force exerted by body weight. Implantation of stem cells can partially compensate the insufficiency of bone marrow for repair of tissue. One of the strategies is to use stem cells that have previously been seeded onto a scaffold in vitro and then induced for the in vivo tissue repair. Induction in vitro provides a controlled situation, which will maximize the cell differentiation induced by the osteoinductive factors. Moreover, the extracellular matrix created by the cells while in culture in vitro will provide a more compatible environment for the survival, proliferation, and differentiation of the cells after implantation. Compared with the implantation of scaffold, cells, and growth factors, respectively, the implantation of a scaffold carrying induced cells will be more equivalent to transplantation of an organ-like structure into the host.

In previous work, a scaffold with a sintered microsphere structure was developed by using poly (lactide-co-glycolide) (PLGA) [2–4]. This kind of PLGA scaffold is porous, degradable with the strength at the similar level as cancellous bone [4]. The compatibility of this PLGA scaffold for the proliferation and differentiation of adipose derived stromal (ADS) cells was confirmed in vitro [5]. The combination of this kind of PLGA scaffold with ADS cells that have been osteo-induced with growth and differentiation factor 5 (GDF5) will be a theoretically suitable design for bone repair in a VB defect. We therefore tested the possibility of using such a construct, for the repair of bone defects in VB.

Materials and methods

Fabrication of scaffold

The PLGA (50:50, MW=72.3kD) (Lakeshore, Birmingham, AL, USA) scaffold was fabricated as previously described [2,3,5]. Briefly, PLGA was dissolved in methylene chloride at 1:5 weight/volume then added drop wise to a 1% poly (vinyl alcohol) (Fisher Scientific, Pittsburgh, PA, USA) solution with continuous stirring at 250 rpm overnight. The resultant microspheres of PLGA were collected, washed five times in distilled water, and air dried in a vacuum overnight. Microspheres of between 500 and 700 μM in diameter were collected and placed in a metal mold (4 mm diameter and 2.5 mm high). The sintered PLGA scaffold was then formed by maintaining the mold at 80°C for 3 hours, followed by air cooling to room temperature. After retrieval from the mold, the scaffolds were immersed in 70% alcohol for 5 minutes, washed three times in sterile water, and desiccated by air drying under an ultraviolet light in a laminar flow hood for 2 hours.

The scaffold was then given a plasma treatment using methods described previously [6,7]. Briefly, the scaffolds were placed in the chamber of a Plasma Cleaner (South Bay Technology Inc., CA, USA). Then the chamber was evacuated to 10 Pa before oxygen was injected; the pressure of the chamber was maintained at 20 Pa. The scaffolds were treated in the chamber for 5 minutes with glow discharge of plasma created by a power source maintained at 50 W and a frequency of 13.56 MHz.

Cell preparation

The adipose tissue was harvested from the inguinal cavity of an 8-week-old Fischer 344 male rat and digested