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Comparison of autogenic and allogenic bone marrow derived mesenchymal stem cells for repair of segmental bone defects in rabbits

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ABSTRACT

Autogenic and allogenic bone marrow derived mesenchymal stem cells (BM-MSCs) were compared for repair of bone gap defect in rabbits. BM-MSCs were isolated from bone marrow aspirates and cultured *in vitro* for allogenic and autogenic transplantation. A 5 mm segmental defect was created in mid-diaphysis of the radius bone. The defect was filled with hydroxyapatite alone, hydroxyapatite with autogeneic BM-MSCs and hydroxyapatite with allogenic BM-MSCs in groups A, B and C, respectively. On an average 3.45×10^6 cells were implanted at each defect site.

Complete bridging of bone gap with newly formed bone was faster in both treatment groups as compared to control group. Histologically, increased osteogenesis, early and better reorganization of cancellous bone and more bone marrow formation were discernible in treatment groups as compared to control group. It was concluded that *in vitro* culture expanded allogenic and autogenic BM-MSCs induce similar, but faster and better healing as compared to control.

1. Introduction

Comminuted fractures of long bones involving varying amount of bone loss are frequently encountered in veterinary practice. Management of such fractures requires not only proper fixation but also maintaining the structural integrity at fracture site by preserving the loose bone pieces. In the cases of bone loss the gold standard method of bone replacement for the treatment of bone gap defects or non-union is the autologous bone graft, where a piece of bone is taken from another body site, and transplanted into the defect (Salgado et al., 2004). Though the success rate of this procedure is quite high, the number of cases in which it can be used are small, due to the limited amount of available tissue, and increased risk of donor site morbidity (Rose and Oreffo, 2002; Spitzer et al., 2002). The second most common treatment is allografting, using tissue from another animal of the same species after processing to reduce antigenicity. This treatment, however leads to a lower rate of graft incorporation with the host tissue (Salgado et al., 2004) and involves the risk of immune rejection and pathogen transmission in the recipient (Herberts et al., 2011). Alternative techniques to bone grafting encompass the use of ceramic or metal implants enriched with osteoinductive cells.

It is estimated that one in 100,000 cells in the bone marrow is a mesenchymal stem cell (MSCs). MSCs have been shown to differentiate along several lineages including bone (Pittenger et al., 1999; Jaiswal et al., 1997; Kadiyala et al., 1997), cartilage (Pittenger et al., 1999; Mackay et al., 1998; Johnstone et al., 1998) and fat (Pittenger et al., 1999). MSCs are the most commonly used seed cells, having the potential for *in vitro* expansion and osteogenic differentiation (Patel et al., 2008; Barry and Murphy, 2004; Chao et al., 2007; Deans and Moseley, 2000). Autologous MSCs are the optimal type of seed cell; both animal experiments and clinical trials indicate that bone constructed using autologous MSCs has strong osteogenic ability (Lucarelli et al., 2004; Quarto et al., 2001). Ease of availability and capability of allogenic BM-MSCs to avoid immune rejection (Ryan et al., 2005) have made these cells an attractive alternative to autogenic marrow-derived cells (MDCs) for reconstructive surgery.

Allogeneic mesenchymal stem cells loaded on hydroxyapatite–tricalcium phosphate implants enhanced the repair of the canine femur without the use of immunosuppressive therapy (Arinzeh et al., 2003). Planka et al. (2008) reported that the transplantation of both autogenous and allogeneic MSCs into a defect of the growth plate appears as an effective method of surgical treatment of physeal cartilage injury. However, comparative evaluation of autogenic and allogenic BM-MSCs with hydroxyapatite granules for diaphyseal bone defect repair in rabbits is not well documented.

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The purpose of this study was to compare the therapeutic efficacy of autogenic and allogenic bone-marrow-derived mesenchymal stem cell transplantation with hydroxyapatite for the repair of bone segmental defect model in rabbits.

2. Materials and methods

2.1. Animals

Fifty-four clinically healthy, six to seven months old New Zealand white rabbits of either sex, weighing 1.4–2.2 kg were used. The Institute Animal Ethics Committee of the Indian Veterinary Research Institute, Izatnagar, India approved the study. Animals were housed in rabbit cages individually under uniform feeding and management conditions, water was provided *ad libitum*. The animals were acclimatized to approaching and handling for a period of 15 days before start of the study.

2.2. Experimental design

The rabbits were divided randomly into three groups viz. Group A (Hydroxyapatite with Dulbecco's Modified Eagle's Medium – low glucose (DMEM-LG)), Group B (Hydroxyapatite with culture expanded autogenic BM-MSCs) and Group C (Hydroxyapatite with culture expanded allogeneic BM-MSCs). Each treatment was awarded to 18 animals, out of which six animals were euthanized using over dose of thiopental at each interval i.e. on 30th, 60th and 90th days to evaluate extent of bone healing by radiography, angiography and histology.

2.3. Isolation and culture of BM-MSCs

The rabbits were anesthetized with 6 mg/kg xylazine administered intramuscularly, followed 10 min later by 60 mg/kg ketamine (Amarpal et al., 2010). The area over the left and right iliac crests was prepared aseptically by shaving, scrubbing with cetrimide and painting with povidone iodine. The bone marrow aspirate was collected from the lateral aspect of the iliac crest using an 18 gage bone marrow biopsy needle. Approximately 2.5 ml of bone marrow aspirate was collected in the syringe containing 2500 IU of heparin. The same procedure was repeated for the contra-lateral iliac crest to collect another 2.5 ml bone marrow aspirate in the same syringe. Thus, a 5 ml of bone aspirate was collected from each animal.

The marrow sample was washed with equal volume of Dulbecco's phosphate buffered saline (DPBS, Thermo Scientific HyClone, Chemicals Co., USA) (5 ml) and disaggregated, by passing it gently through a 21-gage intravenous catheter and a syringe, to create a single cell suspension. Marrow sample with 5 ml of DPBS was loaded onto 5 ml of Ficoll–Paque plus (GE Healthcare Bio-Sciences, Sweden). The mono-nucleated cells were collected from the interface by centrifugation at 805 g for 30 min, and diluted with two volumes of DPBS. The cells were washed with DPBS and centrifuged at 201 g. After centrifugation, 5 ml of RBC lysis buffer was added to the cells, mixed properly and again centrifuged at 201 g for 10 min. The cells were again washed with DPBS at the same speed of centrifugation. The cells were resuspended in DMEM-LG (Thermo Scientific HyClone, Chemicals Co., USA) containing 10% fetal bovine serum (FBS, Thermo Scientific HyClone, Chemicals Co., USA) and antibiotics (mixture of 100 units/ml of penicillin and 100 μg/ml of streptomycin) (Sigma–Aldrich, India). The cells were counted by Neubar's counting chamber and plated at an average of 2.2×10^5 cells/cm^2 in T-25 flasks and maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air in a CO_2 incubator. After 3 days of primary culture, the non-adherent cells were removed with medium and fresh medium was added to the flasks. The medium was then changed every 3rd day until 14th–18th day. MSCs grew as symmetrical colonies. Upon reaching 80–90% confluency, as assessed by visual inspection under inverted microscope (Fig. 1), the cells were passaged at lower densities into new culture flasks. To obtain the cells from the culture flasks, culture medium was removed, and cells were washed with 0.05% trypsin and 0.53 mM ethylenediaminetetraacetic acid (EDTA) for 5 min. Trypsin–EDTA activity was stopped by adding

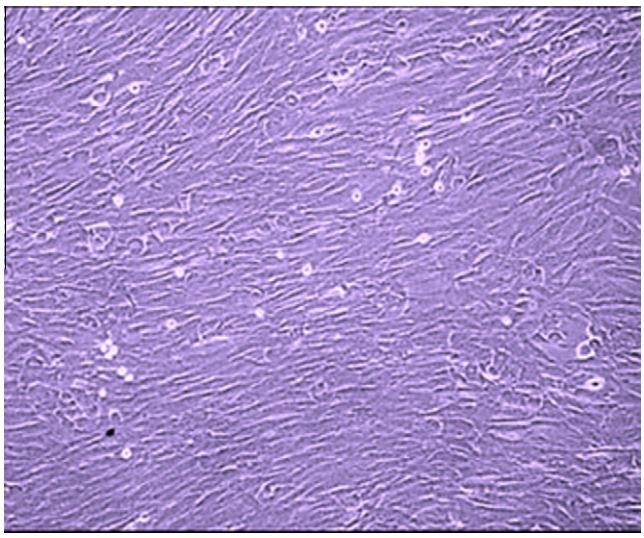

Fig. 1. Fully confluent monolayer of BM-MSCs before trypsinization of the cells for transplantation.

Fig. 2. (a) A 5 mm bone defect created in the central diaphysis of radius bone, (b) the defect filled with hydroxyapatite granules, and (c) application of BM-MSCs at the defect site.