SURVIVABILITY AND FUNCTIONALITY OF BONE MARROW STROMAL CELLS FOLLOWING IMPLANTATION IN TENDON REGENERATION IN RABBIT MODEL

James CH Goh, Hongwei Ouyang, Eng Hin Lee

Department of Orthopedic Surgery, National University of Singapore, SINGAPORE

Introduction

It has been well documented that bone marrow stromal cells (MSCs) are progenitors of various structural and connective tissues, including bone, cartilage, and fat. Recent studies reported that MSCs could suppress lymphocyte proliferation in vitro and allogeneic MSCs implanted by systemic infusion could “home” to bone marrow of non-MHC matched baboons. These results have positive implication on the application of allogeneic MSCs for on-site tissue repair. Although autologous MSCs exhibited the potential for tendon repair, there is no report tracing the behaviors of the exogenous MSCs after implantation in tendon site. Furthermore the possibility of allogeneic MSCs for tendon repair has not yet been evaluated. As such, the purpose of this study is to investigate the fate and function of allogeneic MSCs after local delivery to tendon sites for repair and regeneration.

Methods:

This study was carried out sequentially: firstly, the in-vivo fate of allogeneic MSCs at patella tendon window-wound site was determined with the use of three trace markers (CFDA dye, LacZ and GFP genes), and subsequently the effect of MSCs on the quality of tendon repair was evaluated using a knitted PLGA scaffold delivery system in a rabbit Achilles segment defect model.

For the survivability study, MSCs were labeled with CFDA (a fluorescent dye), or transfected with LacZ or GFP marker genes before implantation into a 5 x 5 mm² window wound site of rabbit patella tendons. The specimens were harvested at 2, 4 and 8 weeks post-operatively. Immediately after retrieval of the implant, the viability of the labeled seeded cells was assessed using a confocal microscope and X-gal staining. The samples were duplicated at each time point for each method.

For the functionality of the MSCs, the animal model used was that of an adult female New Zealand White rabbits with a 1-cm gap defect of Achilles tendon created. In Group I, 19 legs were treated with bone marrow stromal cells seeded into a knitted-PLGA scaffold with fibrin-glue. In Group II, 19 legs were repaired with the knitted scaffold alone, and in Group III, 6 legs were used as normal control. These tendon-implant constructs of Group I and II were evaluated post-operatively at 2, 4, and 12 weeks using macroscopic, histological and immunohistochemical techniques. Furthermore, specimens from Group I (n=7), Group II (n=7) and Group III (n=6) were harvested for biomechanical testing at 12 weeks after surgery.

Result and discussion:

In the survivability study, X-gal staining exhibited positive staining at 2 and 4 weeks but not at 8 weeks after implantation. CFDF or GFP marked cells could be traced as long as 8 weeks after implantation. The cell morphology was changed from rounded shape at 2 weeks to spindle shape at 4 and 8 weeks. These results showed that the allogeneic bone marrow stromal cells could survive for at least 8 weeks and changed into tenocyte-like cells in the tendon wound site of the rabbit model. Thus, indicating that bone marrow stromal cells have good potential for gene delivery in tendon regeneration.

In the functionality study of using MSCs for Achilles tendon repair, it was shown histologically that at 2 and 4 weeks, Group I specimens exhibited earlier tissue formation and remodeling as compared to Group II. For instance, in an in-vitro study, Bartholomew et al (2002) found that MSCs could suppress lymphocyte proliferation². In another in-vivo study, allogeneic MSCs have been shown to have the potential to “home” to bone marrow in a baboon model³. Even human MSCs were able to engraft and demonstrate site-specific differentiation in fetal sheep with immunologic competence⁴. Osiris therapeutic company has used allogeneic baboon MSCs for bone gap repair and medial meniscus regeneration⁵. The mechanism for such a response is not clearly understood. However, it has been shown that human MSCs expressed class I human leukocyte antigen but not class II, which may limit immune recognition⁶. The as-yet-unknown mechanism warrants further research into the transplant immunology of MSCs. Future study in this area will provide fundamental understanding in the use of allogeneic MSCs in tissue engineering.

Acknowledgement

This research was supported by a grant from the National Medical Research Council, Singapore. We would also like to thank Dr Mo Xue Mei, Dr Wang Zhuo, Ms Chong SueWee and Ms Julee Chan for their assistance.

Reference: