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ISOLATION AND CULTIVATION OF MOUSE MESENCHYMAL STEM CELLS ON POLYPROPYLENIC SCAFFOLD

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Abstract: The bone marrow represents an important source of adult mesenchymal stem cells with differentiation potential in various types of cells an tissues. The mesenchymal stem cells issued from this level dispose of a multilinear differentiation capacity. The mesenchymal stem cells were isolated on the basis of their ability to adhere to the culture dishes after 48 h. After several successive passages in order to obtain an homogenous culture; these cells were cultivated on prolypopylenic scaffold and their spontaneous diffrentiation on osteogenic line was followed. The differentiated cells were identified by means of special colorations that lead to the calcium coloration from the extracellular matri. Culture of mesenchymal stem cells on polypropylene scaffolds could offer a new solution to grafting using autologous cells from the patient.

INTRODUCTION

In addition to hematopoetic stem cells; bone marrow contains stem precursor cells for several mesenchymal cell types; such as osteoblasts; chondrocytes; adipocytes and myoblasts (2;5). The multipotential of mesenchymal stem cells; their easy isolation and culture; as well as their high ex vivo expensive potential make these cells an attractive therapeutic tool capable of playing a role in a wide range of clinical applications in the context of both cell and gene therapy strategies (4;8).

Mesenchymal stem cells (MSC) derived from adult bone marrow are possible cells source for tissue engineering (9). Often the use of these cells for regenerative therapy requires some support; three-dimensional scaffold to drive cellular growth and differentiation.

An ongoing increasing number of biomaterials have been proposed as scaffolds for tissue regeneration; with the aim of reproducing the milieu where the complex interaction between cells and their matrix occurs (1;5;6).

Polypropylene (PP) macroporous mesh is universally accepted for use in the repair of large abdominal wall defects. The use of prosthetic biomaterial (mesh) to buttress the defect of abdominal walls; creating a tension-free repair; has been shown to be superior to primary repair alone (3;7;10).

In this study; we report our experience with adult mouse MSC cultivation in polypropylene scaffold.

MATERIAL AND METHODS

Cell preparation and culture methods

Experiments were carried out on male CD1 mice. Six-week-old CD1 mice were sacrificed by cervical dislocation. Their femurus were carefully cleaned of adherent soft

tissue; epiphyses were removed under sterile conditions; and the marrow was harvested by inserting a syringe needle (20-gauge) into one end of the bone and flushing with DMEM (Gibco) supplemented with 20% FCS (HyClone) and 100μg/ml penicillin- streptomycin (Gibco) 2mM L-glutamine. After being drawn and expelled from the same syringe three times to disperse the marrow. The resultant suspension was inoculated in plastic Petri dishes. After 24 h; the nonaderent cells were removed by replacing the medium. The cells were cultured in a monolayer at 37˚C and 5% CO_2 for 5 days after explantation. When primary cultures became nearly confluent; the cells were detached with 0;025% trypsin containing 0;02% EDTA for 10 minutes at 37˚C. The action of trypsin was stopped by adding FBS. Cells resulting from this replating were designated first passage cells. The confluent culture was re-inoculated every 7 days at the initial density of $1;5x10^3$ cell/cm^2. Nutrient medium was replaced every 3 days.

Cell seeding and differentiation on polypropylene scaffold

Polypropylene (PP) macroporous mesh were cut; under sterile conditions into 3cm/3cm pieces. MSC (seven passages) were suspended at a concentration of $3x10^3$cell/ml. After 4 h at 37°C the medium was replaced with fresh medium and the scaffolds were maintained in a humified atmosphere at 37°C with 5% CO_2. The medium was changed every 3-4 days (24 days).

Alizarin red staining

Paraformaldehyde (4%) fixed MSC seeded scaffold were incubated 30 min at room temperature in a solution containing 1% alizarin red and 1% ammonium hydroxide; after that rinsed twice with distilled water and allowed to dry completely.

RESULTS AND DISCUSION

It is generally accepted (9) that mesenchymal stem cells can be recognized as the adherent cells derived from bone marrow capable of extensive proliferation with fibroblastic profile and with the ability to spontaneity differentiation into osteocyte; condrocyte; adipocyte. After seven passages; an almost homogeneous population of fibroblastic-like cells was observed throughout the flask with some round and floating cells.

Osteogenic differentiation of MSC growth on polypropylene scaffolds was evaluated with alizarin red staining. This histological staining is based on the capacity of alizarin red to specifically stain matrix containing calcium and its positive appearance considered an expression of bone matrix deposition.

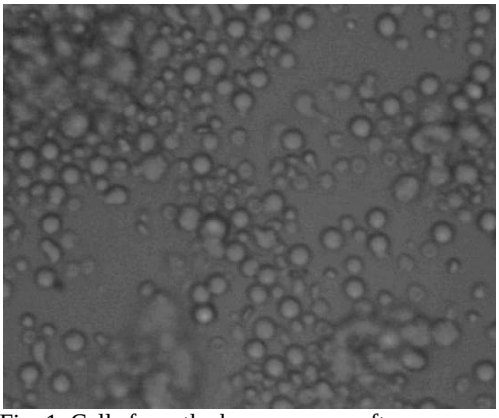

Fig. 1. Cells from the bone marrow after recovery

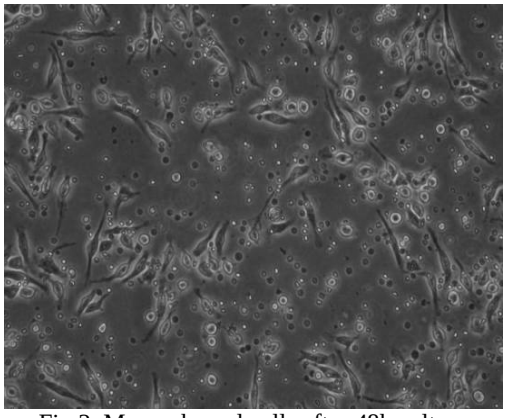

Fig.2. Mesenchymal cells after 48h culture

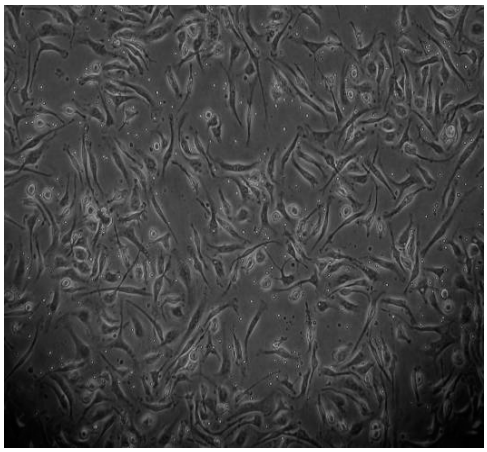

Fig 3. Mesenchymal stem cells passage 1(20x)

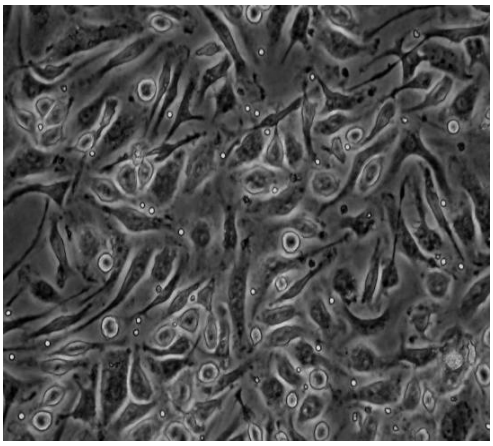

Fig.4 Mesenchymal stem cells passage 7(40x)

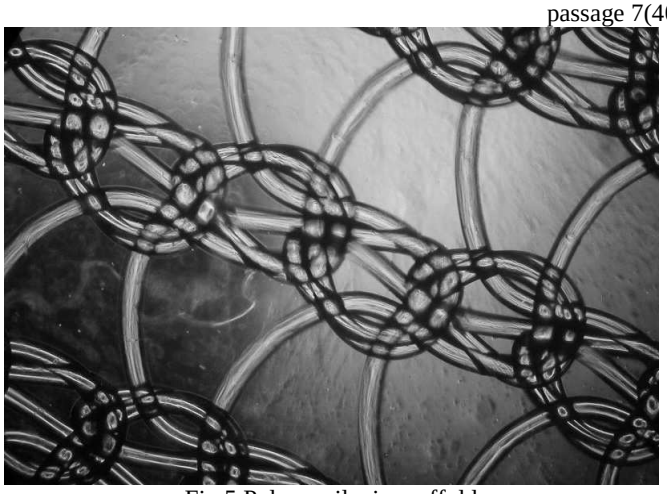

Fig.5 Polypropilenic scaffold

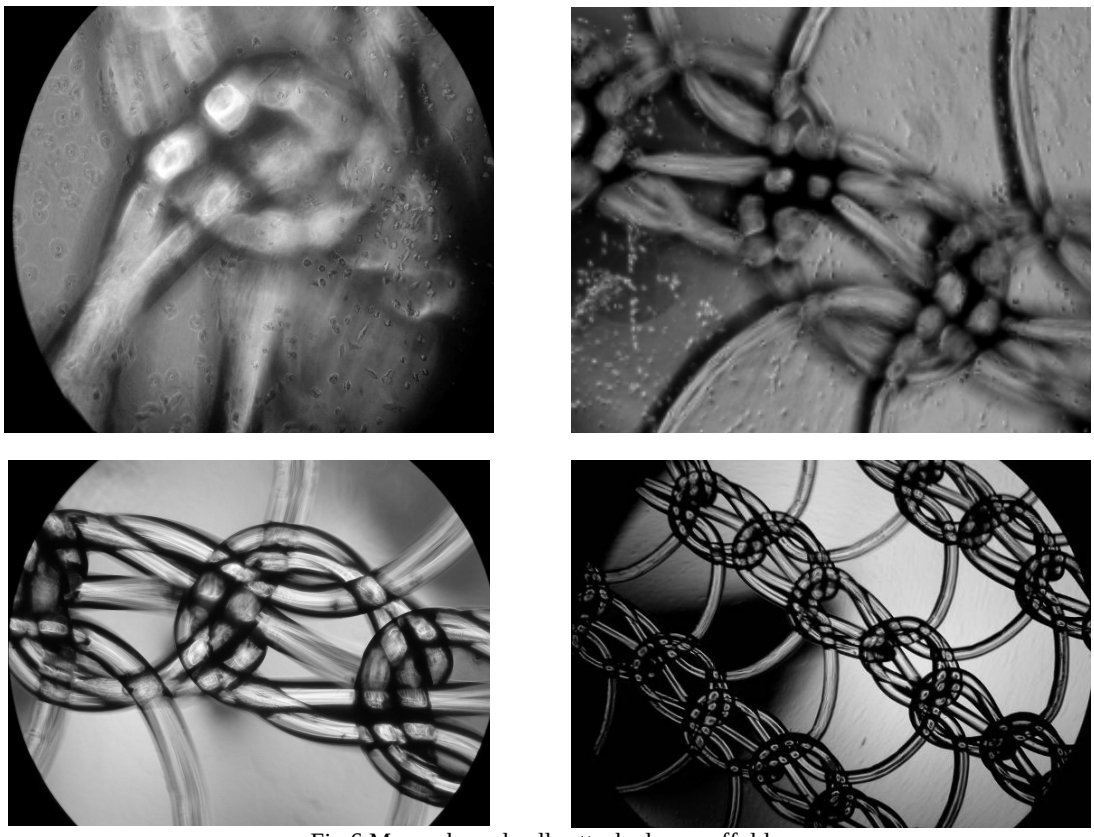

Fig.6.Mesenchymal cells attached on scaffold

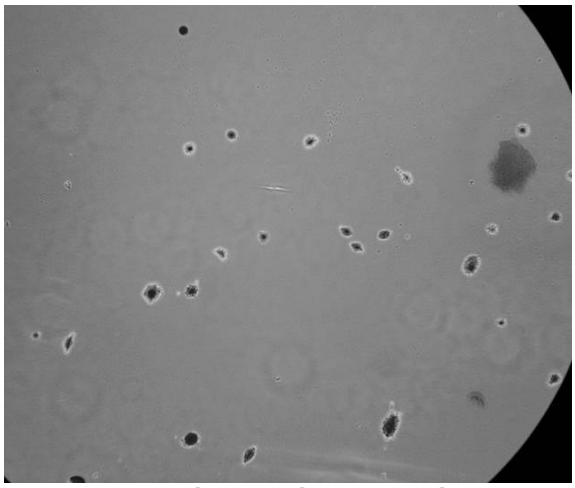

Fig.7. Alizarin Red staining results

CONCLUSIONS

- The present study shows that adult mouse bone marrow cells is suitable and feasible source of a great number of MSC and that the adult bone marrow derived MSC can be easily induced to differentiate into an osteogenic lineage.
- We have demonstrated that a commercial polypropilene scaffold is biocompatible with the MSC.

- The capacity of the bone marrow derived mesenchymal stem cells to differentiate towards an osteogenic lineage and to deposit calcium is easy demonstrated with alizarin red staining.
- We found that cultivation of MSC/scaffold constructs resulted in increased proliferation; differentiation and distribution of cells in scaffolds.
- Combinations of cells and three dimensional scaffolds can be used to replace damaged tissue.
- These results can be extended in human medicine. By using this scaffolds with analogus attached cells; predifferentiated or differentiated towards different cell lines; the treatment of some affections that imply large tissue defect becomes posible.

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