MESENCHYMAL STEM CELLS – A BIOLOGICAL ALTERNATIVE IN OSTEARTICULAR RECONSTRUCTION IN DOGS


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Abstract. Mesenchymal stem cells (MSC) posses the ability to differentiate into any type of somatic cells if properly stimulated. This study documented the effects of using mesenchymal stem cells loaded scaffolds in repairing large bone and cartilage defects in dogs.

The innovative approach to osteoarticular reconstruction in dogs by using auto- or allogenic MSC loaded scaffolds, opens up new possibilities for treating diverse conditions with bioreactive materials and cells.

The MSC were obtained from the humeral bone marrow aspirates. The multipotent cells underwent a specific cultivation and differentiation protocol, derived from the standard procedure. The protocol was altered to best fit the cells behaviour as it follows: the cultivation period needed to reach the 60-80% junction point, differed from dog to dog as well as the potency of the cell cultures, this determining the moment of their passage; the MSC were poured on the scaffolds and only then the differentiation medium was added, this allowing the cells to differentiate on the osteogenic and osteoinductive material, thus obtaining a better cell load and disposition of the bioactive scaffold.

INTRODUCTION

The work initiated by Friedenstein and collaborators, cited by Bruder, Scott P., Fox, Barbara S.(2), provided definitive evidence that bone marrow contains, in addition to the hematopoietic progenitors, a population of spindle-shaped clonogenic fibroblast precursor cells or fibroblast colony-forming units (CFU-F). These cells, which were defined in vivo as quiescent resting cells, after proper in vitro stimulation can enter the cell cycle and develop colonies that resemble small deposits of bone or cartilage. Since CFU-F exhibit a high ability for self-renewal and multipotentiality, it was speculated that these marrow stromal stem cells were the precursors of a number of different mesenchymal cell lineages. Thus, the concept that the marrow stromal moieity was part of a wider stromal mesenchymal system in adult organisms was developed (2).

Bone marrow has been shown to contain a population of rare cells capable of differentiating to the cells that form various tissues. These cells, referred to as mesenchymal stem cells (MSCs), are capable of forming bone when implanted ectopically in an appropriate scaffold (3).
The current standard for bone grafts is the autograft, in which tissue harvested from the patient, usually from the iliac crest, but possibly from the distal femur or the proximal tibia. The graft is then placed at the injury site. This tissue is ideal as a bone graft because it possesses all of the characteristics necessary for new bone growth, namely osteoconductivity, osteogenicity, and osteoinductivity.

Osteoconductivity refers to the situation in which the graft supports the attachment of new osteoblasts and osteoprogenitor cells. It provides an interconnected structure through which new cells can migrate and new vessels can form. Osteoinductivity refers to the ability of a graft to induce nondifferentiated stem cells or osteoprogenitor cells to differentiate into osteoblasts. Alternatives to autografts are allografts. Taken from donors or cadavers, allografts circumvent some of the shortcomings of autografts by eliminating donor site morbidity and issues of limited supply.

Allograft-based bone graft substitutes involve allograft bone used alone or in combination with other materials (eg, AlloGro, Opteform, Grafton, Orthoblast). Factor-based bone graft substitutes are natural and recombinant growth factors used alone or in combination with other materials such as transforming growth factor-beta [TGF-beta], platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and bone morphogenetic protein (BMP).

Cell-based bone graft substitutes use cells to generate new tissue alone or seeded onto a support matrix (eg, mesenchymal stem cells).

Ceramic-based bone graft substitutes include calcium phosphate, calcium sulfate, and bioglass used alone or in combination (eg, OsteoGraf, Norian SRS, ProOsteon, Osteoset).

With polymer-based bone graft substitutes, both degradable and nondegradable polymers are used alone and in combination with other materials (eg, Cortoss, open porosity polylactic acid polymer [OPLA], Immix).

For the repair of bone defects, a tissue engineering approach would be to combine cells capable of osteogenic (i.e. bone-forming) activity with an appropriate scaffolding material to stimulate bone regeneration and repair.

A study demonstrated that allogeneic, stem cells, derived from adult bone marrow, in combination with a calcium phosphate ceramic regenerated a large, long bone defect. It was the first study to demonstrate that the use of these allogeneic stem cells, i.e. cells obtained from an unrelated donor, without the use of immunosuppressive therapy, do not provoke an adverse immune response in bone repair. Cells were implanted for up to 4 months in the canine. In addition, these stem cells accelerated the repair of the defect and formed equivalent amounts of bone tissue as autologous stem cells did. So, this study demonstrated the feasibility of an allogeneic stem cell approach as an "off-the-shelf" tissue engineering therapy.

MATERIALS AND METHODS

This study was performed on 7 medium-sized healthy dogs, with ages ranging from 1 to 3 years of age, of which 4 were males and one a female. Each one was examined radiographically and with TC scan. Vital function levels were taken before each bone marrow sampling.

The bone marrow was obtained from the proximal humeral epiphysis. From each dog a quantity of 4 ml of bone marrow aspirate was taken onto 1 ml of anticoagulant (Heparine).

All the samples were processed according to the standard protocol, using DMEM cultivation medium and the following differentiation mediums:
- For the osteoblastic line – Dexamethasone enriched medium;
- For the chondroblastic line - TGF β1 enriched medium.

The differentiation process was carried out directly on the scaffolds. A number of 4-6 million cells/osteogenic scaffold, meaning about 50,000 cells/cm², and a number of 1-3 million cells/ chondrogenic scaffold, meaning about 20,000 cells/cm² was decided to be loaded onto the scaffolds. The bone marrow samples were obtained and processed as follows:
- in one day – samples were taken from dogs 1, 2, 3, 4;
- on another day 09 – samples were taken from dogs 1, 5, 7, 8, a single sample/dog;

The samples were processed on the same day they were obtained, thus leading to a gap of 1 day between the sample/cultivation batches.

Standard procedure in MSC cultivation and differentiation:

- **day 0** – the cultivation begins;
- **day 2-3** – oxigenation and medium refreshing;
- **day 10-21** – reaching the semiconfluence point (60-80% are closely attached to each other) ==>tripsinization ➞ PASAGE I;
- **day 21-42** – cultivation, tripsinization and PASAGE II;
- **day 42- 63** – cultivation and passage onto differentiation mediums;
- standard culture medium (DMEM= FCS and FGF);
- **osteoblastic differentiation media contain** Dexamethasone, interval needed – about 3 weeks;
- **chondroblastic differentiation media contains** TGF β1, idem.

- The differentiation process is carried out in vitro, on well plates, and directly on scaffolds.

In the lab, the samples were transferred into 15 ml tubes, then PBS was added. The supernatant was discarded, onto the remaining sediment being added standard culture media (DMEM= FCS10% + 2µg FGF).

The cells were placed into cell culture plates, approximatively 1 million cells/cm². The plates were incubated at 37 °C, for 2 weeks, untill reaching the semiconfluence point (junction point). The culture evolution was followed microscopically.

When the cell cultures reached the semiconfluence point, the culture was washed with PBS for removing FCS.

Then, the warm trypsin was added (7.5 ml/large plate) and contact maintained for maximum 2 minutes. The loose cell were passed onto the cold FCS medium. The mix was centrifugated at 1,500 rpm, for 10 minutes, then passed again onto the ice. The supernatant is discarded and the cells are resuspensioned with PBS. From the cell solution, 1 ml is taken and Tripan coloured. The cells are placed again onto plates in concentrations of 3,000-5,000 cells/square centimeter.

On the 25.05.2006 other samples were collected from dogs 1, 3, 5, 7, 8, double samples being taken from dog 1 and 8.

On the 13.06.2006, samples 1,3,5,7 were trypsinized.
This process corresponds to PASAGE I.
On the 20.06.2006, samples 1,3,5,7 made it to PASAGE II.
As a support for the cells were used matrixes and membranes. A number of 4-6 million cells/osteoblastic scaffold was used, mening around 50,000 cells/cm², for the chondroblastic scaffolds being used a number of 1-3 millions of cells/scaffold, meaning around 20,000 cells/cm².
Three marked well plates were used, each having 3 horizontal lines consisting of 4 wells, which corresponded to PASAGE I, and below these, other 3 lines corresponding to PASAGE II, as shown below.

Table 1.

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The Indexes mbr = membrane; mx = matrix; prolif = proliferation witness; The Indicatives C = chondroblastic differentiation media; O = osteoblastic differentiation media; M = proliferation witness (contains FGF).

Over the scaffolds differentiation mediums were added (about 1,5-2 ml), and in the M well=witness normal FGF medium.

RESULTS AND DISCUSSIONS

Ceramic materials are considered to be the ideal carriers for undifferentiated MSC, favouring their spatialization through a bone similar mineral composition. Their 3D structure varies with the technology used for producing them, especially regarding the presence of structural microcanaliculi which allow free passage for cytokines and cells.

Direct on scaffold differentiation allows a better dispersion of the cells in the carrier structure, thus skipping in vivo the period of time needed for matrix colonisation. This allows for immediate synthesis to begin without the issue of matrix colonisation level/matrix resorption. A study demonstrated that allogeneic, stem cells, derived from adult bone marrow, in combination with a calcium phosphate ceramic regenerated a large, long bone defect. It was the first study to demonstrate that the use of these allogeneic stem cells, i.e. cells obtained from an unrelated donor, without the use of immunosuppressive therapy, do not provoke an adverse immune response in bone repair (5).

CONCLUSIONS

The in vitro cultivation and differentiation techniques of multipotent embrionar cells allow new approaches in the osteoarticular reconstruction field.
The use of scaffolds as an in vitro differentiation support for stem cells, eliminates the inconvenient of uneven cell distribution in the scaffold mass.

BIBLIOGRAPHY