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Use of bone marrow derived stem cells in a fracture non-union

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ABSTRACT

This is an attempt of using *in vitro* cultured mesenchymal stem cells (MSCs) from bone marrow in joining of a fracture non-union. Bone marrow cells were obtained and differentially centrifuged for MSCs that were grown *in vitro* in mesenchymal stem cell basal medium aseptically, for 10 d. The cell mass was injected around the fracture non-union. Healthy conditions of development of tissue regeneration at the trauma site and due bone joining were recorded. It is concluded that *in vitro* cultured MSCs had a blithesome effect on the fracture non-union.

1. Introduction

Stem cells from bone marrow, adipose tissue, blood and a few more sources are popular in regenerative medicine, as those have the self-renewal capacity and the potentiality for acting as progenitor cells. Bone marrow cells particularly, have the stroma with osteogenetic progenitor cells - mesenchymal stem cells (MSCs, 0.05%), apart from the hematopoietic component (hematopoietic stem cells or HSCs, 1% to 2%).

MSCs are popular for the capability of chondrogenic differentiation too and those could be expanded in culture through many generations. MSC therapy in combination with osteoconductive scaffold or osteoinductive protein as tools in regenerating bone fracture is reported from preclinical models of fracture healing[1,2]. Till date, there has been no standardized clinical translation of decades of preclinical investigations with animals on MSC therapy; nevertheless, bone fracture has always been an acute morbid condition. A viable protocol with MSC, for the holistic 'bone healing' with restoration of vascularity of the injured zone and bone-gap filling, without any opportunistic infection at the surgical site, is limited[3].

Moreover, non-union in bone fractures nowadays has become a commonplace of orthopaedic treatment, *a priory* due to multiple unknown factors of which, polluted food could be a reason[4], and surgical site infection with multidrug resistant bacteria could be the other[5], as inveterately the surgeon prescribes antibiotics at the end of the surgical protocol. Secondly, at the fracture site, muscular and vascular tissues get sometimes heavily injured, limiting supply of blood for a long period, thereby limiting the supply of nutrients and influx of stem cells for the regeneration of the damaged tissue and progressive union of the bone fractures[6]. Eventually, a part of the tissue becomes dead and promotes cross infections.

This is a case report involving the use of *in vitro* cultured

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autologous bone marrow cells for bone-regeneration of a tibio-fibular non-union.

2. Case report

Fractures of both tibia and fibula of a 28-year-old male were fixed with an interlocking nailing, previously in some other hospital; he was unable to walk without any support and the fracture site was tender till 8 months. The patient reported us with multiple discharges from the injured site that was addressed with dressing.

There were bacterial infections at the site, which were controlled by broad-spectrum-third-generation cephalosporins, cefuroxime and cefixime along with clavulanic acid. On an X-ray examination, the inadequate callus formation at the fracture site was evident (Figure 1), hence the case was diagnosed as a non-union, which could be due to long-standing bacterial infection at the surgical site. Further, 'autologous stem cell therapy' with prior consent of the patient, following good manufacturing practice protocol and an approval through Institutional Ethical Committee was adopted.

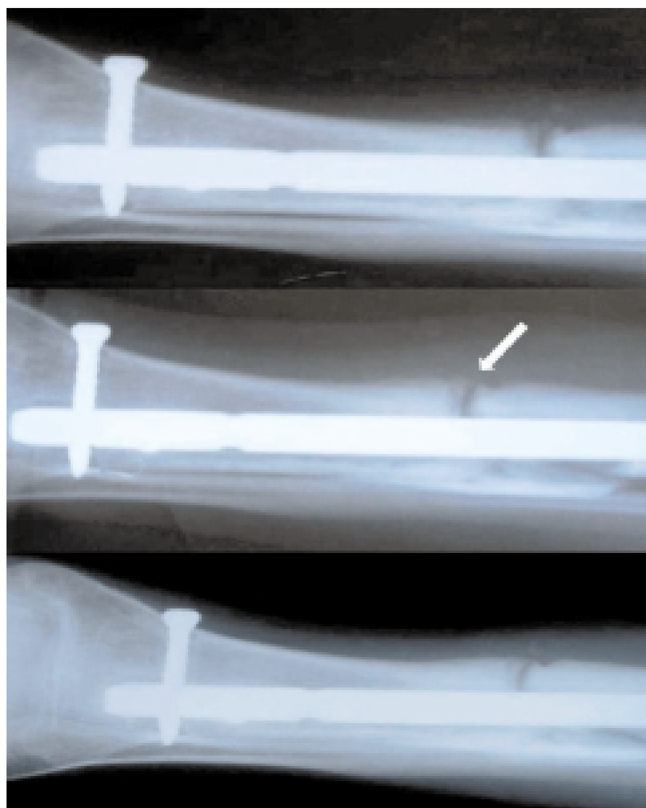

Figure 1. Prognosis of fracture non-union in X-ray plates following months of observation after injection of MSCs, after upper plate, 1 month; middle plate, 3 months; and lower plate, 4 months. The arrowrepresents callus formation and reduction of gap at the end of 3 months.

Following local anesthetization, an aliquot of 10 mL of bone marrow was harvested from the posterior iliac crest of the patient with a single puncture by an 11 gauge Jamshidi needle. The collected sample was heparinised, in a 50 mL size falcon tube and was further diluted with an aliquot of 10 mL of Dulbecco's phosphate buffer saline (DPBS). Bone-marrow mononuclear cells (BMNCs) were isolated by differential centrifugation of the mixture with the lymphocyte separation medium (LSM), following the manufacturer's protocol (HiMedia, Mumbai).

The pellet was washed with DPBS; cells were propagated *in vitro* with 'mesenchymal stem cell basal medium' (Stem Cell Technologies, Canada), in a CO_2 incubator maintained at 37 ℃ and 5% CO_2. Cells were observed daily; medium was changed in alternate days up to the day 9 (Figure 2).

On the day 10, cells were collected from culture plate, manually dissociated by flushing. Those were washed two to three times with DPBS to remove traces of the medium, if any.

At an operation room, the patient was injected percutaneously with 1 mL of cultured stem cell mass (3×10^7 cells/mL) into the site of non-union at 3-4 points with an 18 gauge needle at several places around the non-union zone, under c-arm guidance. After 1 h of rest the patient was discharged and was instructed to report at monthly intervals. Significant soft callus formation was marked after one month and there was gradual progress in subsequent X-ray examination along with the reduction of the fracture gap (Figure 2). At the end of six months, the patient was able to walk without any support and had no pain. At length, the fracture gap was obliterated, evident in an X-ray film.

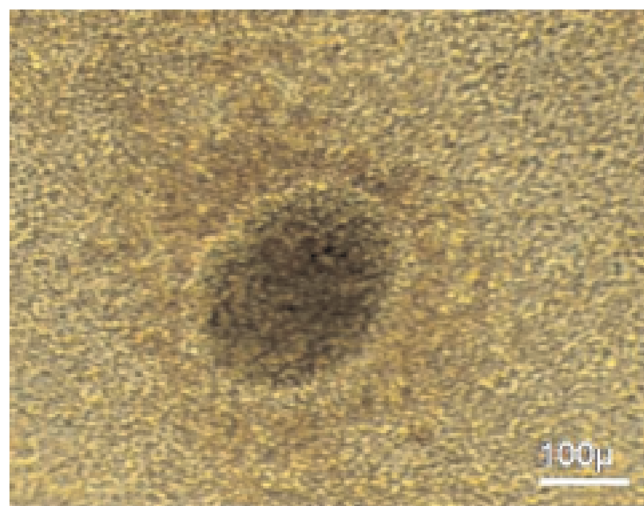

Figure 2. Confluent growth of bone marrow stem cells (MSCs) under a phase contrast microscope on the day 7 of culture.

3. Discussion

Use of MSCs in fracture healing and bone defects is a complex process, resulting in callus and bone tissue formation. It includes secretion of multiple factors in cascades of molecular events that often depend on nutritional status of patients, drugs administration, calcium balance, blood supply and stability of the fracture[7,8]. MSCs have precursors of osteoblasts and adipocytes, known as Westen-Bainton cells.

They regulate cellular growth and regeneration via secreting mechanisms during homeostasis and following an injury. The efficacy of MSCs in orthopaedics was known to be quite encouraging[8,9].

In this case study, the patient with the non-union since 8 months had obtained a significant clinical improvement in callus formation and tissue regeneration, following the injection of cultured autologous MSCs in a resource limited setting. Similar studies were also undertaken by animal models and humans[10].

At certain instances, culture method was modified by using different scaffolds to obtain a sizable number of cells for a quicker healing. The infused pluripotent cells, herein MSCs at the fracture site may have both direct and indirect role in the repair mechanism. It was known that in order to obtain a successful fracture healing, blood vessels, growth factors, and proliferative precursor cells need to act simultaneously to cause an inductive healing effect[10].

In addition to the cell proliferation, there was an enhancement of vasculature and a production of growth factors to convert the fibroblast into osteocytes, i.e., to trigger and harness the patient's own regenerative mechanism. Thus, cultured MSCs could be used in future similar problems of regeneration of body parts[11]. However, further work is needed for the development of appropriate protocol for treating non-union cases. Thus, the use of *in vitro* grown stem cells in an acute disease of bone non-union is epitomized herewith.

Conflict of interest

The authors declare they have no conflict of interests.

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