

MESENCHYMAL STEM CELLS AND GROWTH FACTORS FOR CARTILAGE REPAIR



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The future directions in cartilage repair are moving towards the possibility to perform one step surgery: these could include the use of stem cells and growth factors. The use of autologous mesenchymal stem cells and growth factors represents an improvement on the currently available techniques as this avoids the first surgery for cartilage biopsy and subsequent chondrocyte cell cultivation.

Mesenchymal stem cells (MSC) have a high proliferation and multi-lineage differentiation potential. Once MSC are cultured in the appropriate microenvironment, they can differentiate to chondrocytes and form cartilage; onset of chondrogenesis requires a chemically defined serum free medium supplemented with dexamethasone, ascorbic acid and growth factors such as TGF- β . Johnstone et al. [*Exp Cell Res.* 1998; 238: 265-72] cultured MSC as pellets at the bottom of a tube for 2 weeks in a specific serum free cocktail medium; under these conditions cells organize a cartilaginous matrix by secreting proteoglycans and type II collagen and cells appear as real chondrocytes embedded in their own matrix lacunae.

Wakitani et al. [*Osteoarthritis Cartilage.* 2002; 10: 199-206] used autologous culture of expanded bone marrow for repair of cartilage defects in osteoarthritic knees; they chose 24 patients with knee OA who underwent a high tibial osteotomy; patients were divided into cell transplanted group and cell free group. After 16 months follow-up, they concluded that MSC were capable of regenerating a repair tissue for large chondral defects.

Researches are currently exploring the possibility of manipulating stem cells in the laboratory to differentiate into chondrocytes and which can then be integrated into a synthetic scaffold for later implantation. Platelet rich plasma contains 3-6 times platelets of normal blood and growth factors (platelet rich in growth factors or PRGF) in these platelets there is a high density of alpha granules, which contain proteins. Furthermore, PRGF contains different growth factors.

Cugat et al. used PRGF to treat chondral defect in athletes and obtained good results, according to their experiences for other connective tissue repair, they showed that PRGF in physiological concentration is effective for the recovery of connective tissue furthermore local treatment is safe and does not alter the systemic concentrations of these proteins. Other studies presented the following positive results: PRGF can increase total GAG, collagen II synthesis and decrease degradation; induce chondrogenesis of MSC and promote chondrocyte proliferation, differentiation and adhesion. Barry et al. [*Exp Cell Res.* 2001; 268: 189-200] demonstrated that MSC cultured with TGF- β produced significantly more proteoglycans and collagen II. This study defined the phenotype of the differentiated cell and to understand in greater detail the sequential process of cartilage matrix assembly.

Stevens et al. [*J Orthop Res.* 2004; 22: 1114-9] have shown that addition of fibroblast growth factor-2 (bFGF) at the early stage of an in vitro culture of MSC with transforming growth factor-beta1 (TGF- β 1) significantly enhances proliferation which increased neo-cartilage formation.

Anitua et al. [*Rheumatology (Oxford)* 2007; 46: 1769-72] demonstrated that PRGF induced secretion of hyaluronic acid that may provide a homeostatic environment for tissue repair inside the joint.

In our institution we use bone marrow concentrated (BMC) for MSC and PRP in treating chondral defects: our technique consists of harvesting 40-60 mL of bone marrow aspirate from the iliac crest with aspiration kit and a centrifugations system (Harvest Smart PreP2 System, Harvest Technologies, Plymouth, MA; Extracell, Regen Lab, Mollens VD; Marrowstim, Biomet) following the method recommended by the manufacturer in order to have BMC and from these we will be able to increase concentration of BMC four to six times the baseline value.

At the same time we collect 30–60 mL of peripheral blood to prepare PRP, we separate plasma from the red blood cells by centrifugation (Harvest Smart PreP2 System, Harvest Technologies, Plymouth, MA; Extracell Glue, Regen Lab, Mollens VD). The PRP are then placed into a second centrifugation step to concentrate platelet to optimize the plasma volume and platelets efficiency number. Using autologous thrombin we activate the platelet and produce a sticky clot material that we paste it into the defect; finally we use to cover the treated defect with a collagenic membrane Chondro-gide (Geistlich Wolhusen, CH). 20 patients have been treated with this technique from 2006 and preliminary clinical results are encouraging: mean IKDC subjective score improved from 48.3 pre-op to 68.6 post-op at 20 months follow-up while mean Tegner score of 5.75 was similar to pre-injury level of 6.50 and final Lysholm was 92.6. Second look arthroscopy and biopsy were done in two patients after an average of one year with patients' consent, the implant sites were filled with good cartilage like materials and firm to palpation, biopsy showed hyaline like tissue with good integration to the surrounding tissue.

Conclusion

In conclusion, we agree with Nishimoto et al. [*Wound Repair Regen.* 2007; 15:156-62] in their observation that simultaneous concentration of platelets (PRP) and bone marrow cells (BMC), acting as a sources of growth factors and "working cells", can play important roles in future regenerative medicine. These studies show less morbidities and complications inherent to cartilage surgical techniques by lessening surgical procedures translating to lower cost for the patient. However medium term prospective randomised studies are suggested to confirm these preliminary results.