progenitor cells. The aim of this study is to determine the useful of the hAMSC and the hAEC on regenerating human joint cartilage in an in vitro model.

Methods: HAM was used as support for the culture of hAMSCs or hAECs. Focal injuries in human joint cartilage biopsies were done. Later, a pellet of cells (hAMSCs or hAECs, depending on the repair model developed) was implanted into the focal defects of cartilage. HAM, with the cells grown on it, was placed in direct contact with the cartilage surface to be repaired. These implants were cultured in DMEM+10% FBS for 8 weeks. The repair tissues were analyzed by histological and histochemistry analysis considering the ICRS macroscopic evaluation of cartilage repair.

Results: hAMSCs and hAECs cultured on HAM and transplanted onto focal injuries of cartilage penetrated into the nearby surface of the chondral defect. The Hematoxylin and Eosin staining showed that HAM or hAECs pellet filled the chondral defect. There was a good integration between the repair tissue and native cartilage. Type II collagen and aggrecan stainings of repair tissue were slightly positive on the extracellular matrix, and positive inside the cytoplasm of the cells. The safranin O staining expressed the presence of proteoglycans. Finally, type I collagen stainings were weak or totally negative (Figure 1A). We realized an ICRS macroscopic evaluation of cartilage repair to compare both kinds of progenitor cells in the in vitro model. hAMSCs displayed better degree of defect repair, greatest integration to border zone and, in general, higher repair assessment (Figure 1B).

Conclusions: We get reduce the area of the defect with quality integration. The morphology of the repair tissue showed a fibrocartilaginous appearance and a high cellularity. hAMSCs showed better results considering the ICRS macroscopic evaluation of cartilage repair.

479 THE EFFECT OF HYDROXYAPATITE MICROPARTICLES ON THE DIFFERENTIATION OF MESENCHYAL STEM CELLS SEEDED INTO COLLAGEN GELS

F. Laydi1,2, G. Cauchois1,2, J-F. Stoltz3, N. de Isla1,2, 1Nancy Université, Faculty of Med., Vandoeuvre-lès-Nancy, France; 2CMU UMR 7561, Vandoeuvre-lès-Nancy, France

Purpose: In vitro differentiation of mesenchymal stem cells (MSC) for osteo-articular tissue engineering involves specific differentiation factors like cytokines and growth factors. Moreover, scaffolds are often used, principally hydrogels, to culture MSC. In this work we studied the influence of the hydrogel composition on MSC differentiation without using specific differentiation factors.

Methods: Hydrogels were prepared by mixing rat tail type I collagen (Coll), hydroxyapatite (Hap) microparticles at different sizes (150μm-large, 80μm-medium and 30μm-small) and bone marrow MSCs to obtain a final concentration of 3 mg/ml coll; 1 mg/ml, 2 mg/ml or 3 mg/ml of Hap and 5.10^5 cell/mL. pH was adjusted to 7 and the mixture was distributed on each well of 96-well culture plates. Gels without Hap were used as controls. The behavior of MSC over 21 days of culture was studied: viability by confocal microscopy, gel contraction, cell metabolism, cell proliferation and transcription factors specific of differentiation pathways (Sox-9 for chondrogenesis, Runx-2 for osteogenesis, PPAR-γ for adipogenesis) by PCR.

Results: Images obtained by confocal microscopy showed that type I collagen/hydroxyapatite hydrogels are biocompatible with the MSC. For all the conditions essayed, there were few dead cells, all over the culture period. Moreover, we observed that the behavior of the cells follows an evolution in two phases: the first, until 14 days in culture supports the cellular proliferation and the contraction of the gel while the second period, from day 14 to day 21, involves a cellular commitment of the cells in differentiation pathways. While Hap concentration or particle size inside gels did not influence cell viability or proliferation, its presence influences cell differentiation. The gene expression analysis shows a significant increase of the chondrogenic transcription factor Sox-9 for the cells culture on gels containing Hap. Moreover, differences were found according to particle size or concentration.

Conclusions: The results suggest that MSC differentiation can be modulated by hydrogels composition. That will be useful for tissue engineering because to avoid the use of cytokines or growth factors could facilitate therapeutic applications.

Therapy – Non-Pharmacologic

480 EFFECTS OF ORAL ADMINISTRATION OF HYAL-JOINT® FOR THE TREATMENT OF KNEE OSTEOARTHRITIS WITH SYNOVIAL EFFUSION: A DOUBLE-BLIND PLACEBO-CONTROLLED STUDY

I. Möller1, D. Martinez-Puig2, C. Chetrit2, 1Inst. Poal de Reumatologia, Barcelona, Spain; 2BIOIBERICA S.A., Palafolls, Spain

Purpose: To evaluate the clinical efficacy of (Hyal-Joint®) in patients with moderate to severe osteoarthritis of the knee coursing with persistent knee pain and joint effusion.

Methods: 70 adults with moderate to severe knee OA (grades II-III of K-L criteria) and presenting lateral or longitudinal synovial effusion (>4 mm at the suprapatellar recess) were recruited between March and August 2010 in a prospective, double-blind, placebo-controlled trial with a parallel design. Patients were randomly assigned to receive either Hyal-Joint® (Bioribeca, Palafolls, Spain; n = 35) or Placebo (PCT; n = 35) at a dose of 80 mg/d during a period of 3 months. The primary outcome was the course of synovial effusion assessed monthly in the suprapatellar recess using ultrasonography (US) equipment with a high frequency linear array. The maximal synovial thickness and effusion depth were measured in mm using the longitudinal scale according to a standard published protocol. The secondary outcome was the pain intensity assessed weekly using the Huskisson’s visual analogue scale (VAS).

Results: At baseline the mean synovial effusion was similar between treatment groups (6.85 and 6.16 mm for Hyal-Joint® and placebo groups respectively). Synovial effusion was progressively reduced in both groups during the experimental period, reaching a lower value in the Hyal-Joint® group (2.72 mm) in comparison with the placebo group (3.15 mm) at 3 months. Pain intensity was also similar between groups at baseline (6.4 and 6.5 cm for for Hyal-Joint® and placebo groups respectively). Pain perception was progressively reduced from baseline until the end of the trial in both groups. From week 9 until week 12 pain reduction was more pronounced in group Hyal-Joint® group in comparison with placebo. In the Hyal-Joint® group, at week 9, pain intensity was 50% reduced from basal values, while the reduction was only of 32% for the placebo group. Differences between treatment groups in both pain intensity and synovial effusion were clinically relevant and tended to reach statistical significance.

Conclusions: Our results suggest that the use of Hyal-Joint® for management of the knee osteoarthritis courses with synovial effusion is safe and well tolerated, tending to show clinical benefits in pain remission and helping to control synovial inflammation.