0.9, respectively. The scores of cartilage damage in the BMP-M group were less severe than PBS group. The histological severity of OA in BMP-M group was significantly lower than PBS and BMP-S group according to the modified Mankin's histological grading. Remarkable inflammation or hyperplasia of synovium was not observed in any group.

**Conclusions:** Local administration of therapeutic agents to joints is reasonable in OA which is not systemic disease. The osmotic pumps were previously used for the maintenance of effective concentration of therapeutic agents in the joint of animal experiments. Biodegradable GHM was reported to achieve controlled release of growth factors in the joint had protective effects on OA development in an animal model. This method might be a useful conservative treatment for human OA in the future.

**Fig. 1. In vivo release kinematics of BMP-7.**

**Fig. 2. Gross morphological evaluation (score).**

**231 A SHORT TIME WINDOW TO PROFIT FROM IL-4 PLUS IL-10 ADDITION TO PROTECT CARTILAGE FROM BLOOD-INDUCED DAMAGE IN VITRO**

**M.E. van Meegeren1,2, N.W. Jansen1,2, G. Roosendaal2, S.C. Mastbergen1,2, J.A. Verhaar2, M.J. Hoogduijn4, G.J. van Osch1,2. Rheumatology Service. Hosp. Clinico San Carlos de Madrid., Madrid, Spain;2Dept. of Orthopaedics, Erasmus MC, Rotterdam, Netherlands;3Dept. of Internal Med., Erasmus MC, Rotterdam, Netherlands;4Dept. of Otorhinolaryngology, Erasmus MC, Rotterdam, Netherlands**

**Purpose:** Exposure of joint cartilage to blood can occur after joint trauma, during or after major joint surgery, or due to hemophilia. This ultimately leads to joint damage, having both the inflammatory characteristics of rheumatoid arthritis and the degenerative characteristics of osteoarthritis. It has been reported that interleukin (IL)-10 limits blood-induced cartilage damage in vitro and that IL-4 has some cartilage protective properties. Our aim was to study whether the combination of IL-4 and IL-10 has an additive effect on prevention of blood-induced cartilage damage when administered during (prevention) and after the onset (treatment) of blood-exposure.

**Methods:** Human full thickness articular cartilage explants were cultured for 4 days in the presence or absence of 50% v/v homologous blood, mimicking a joint bleed. Either IL-4 or IL-10 alone was added (0, 10, 30, or 100 ng/ml) during blood exposure (n=9 donors). Additionally, a combination of IL-4 and IL-10 was added during blood exposure (prevention), as well as 2, 4, 8, 24, or 48 hours after start of blood-exposure (treatment) (n=5 donors). After 4 days of culture the medium was refreshed and cartilage was cultured for an additional 12 days in the absence of additives to determine long-term effects of short-term blood-exposure and treatment. This mimics the in vivo situation where blood is cleared from a joint within approximately 4 days and where IL-4 and IL-10 have short half live times. Cartilage matrix turnover, in terms of proteoglycan synthesis, release, and content, was determined at day 16.

**Results:** Cartilage cultured in the presence of blood decreased proteoglycan synthesis rate, increased proteoglycan release, and decreased proteoglycan content as measured after 16 days of culture (all p <0.05). This blood-induced damage to the cartilage matrix was limited both by IL-10 and IL-4 alone in a dose-dependent way. At 10 ng/ml, IL-10 and IL-4 improved proteoglycan synthesis rate with 41% and 125%, respectively; proteoglycan release decreased with 42% and 59%, and proteoglycan content increased both with 19% (all p <0.05 compared to blood-exposure).

Most importantly, the combination of IL-4 and IL-10 (10 ng/ml) was more protective against damage caused by blood than IL-10 alone. The decrease of proteoglycan synthesis rate due to exposure to blood was further improved with 157%, and proteoglycan release decreased with 59% (both p <0.05 compared to IL-10 alone).

When IL-4 plus IL-10 were added after the onset of blood-exposure, it appeared that it had to be administered within 8 hours after start of the bleeding to still be able to protect against blood-induced cartilage damage. At later time points addition was ineffective.

**Conclusions:** Besides IL-10, as shown previously, also IL-4 protects against blood-induced cartilage damage. This study demonstrates that the combination of IL-4 and IL-10 is clearly more effective than the individual components. Importantly, the protective effect is only evident when IL-4 and IL-10 are added within 8 hours after start of blood-exposure. This implies that treatment should be started within this time span to prevent blood-induced cartilage damage as a result of trauma or surgery.

**232 ENCAPSULATION IN ALGINATE BEADS RETAINS LONG-TERM IMMUNOMODULATORY PROPERTIES OF BONE MARROW MESENCHYMAL STEM CELLS**

**E. Villafuertes1,2, G.M. van Buul2,3, N. Kops2, B. Fernandez-Gutierrez1, J.A. Verhaar2, M.J. Hoogduijn4, G.J. van Osch1,2. Rheumatology Service. Hosp. Clinico San Carlos de Madrid, Madrid, Spain;2Dept. of Orthopaedics, Erasmus MC, Rotterdam, Netherlands;3Dept. of Radiology, Erasmus MC, Rotterdam, Netherlands;4Dept. of Internal Med., Erasmus MC, Rotterdam, Netherlands;5Dept. of Otorhinolaryngology, Erasmus MC, Rotterdam, Netherlands**

**Purpose:** Mesenchymal stem cells (MSCs) are a source of pluripotent cells. They are able to self renew, differentiate into cells of mesoderm lineage and modulate the immune response. Here we focus on the immunomodulatory properties of MSCs for therapeutic use in osteoarthritis. We studied the possibility to encapsulate MSCs in alginate beads in order to achieve long-term immunomodulatory activity of MSCs and to place them in the jointcavity. We evaluated the expression of immunomodulatory factors by MSC upon stimulation with inflammatory factors when encapsulated in alginate compared to monolayer. Furthermore we studied the survival and differentiation capacity of MSCs after long-term encapsulation in alginate.

**Methods:** Human bone marrow MSCs of two different donors were encapsulated in 1.2% alginate or seeded in monolayer and after preconditioning for 48 hours they were stimulated by cytokines (50 ng/ml IFNγ, 50 ng/ml TNFα) for 24 hours. Each experiment was performed in triplicate. We studied the gene expression of MSCs compared to unstimulated control cultures. To evaluate the behavior after long-term encapsulation the above experiment was repeated after 30 days of preculture in alginate. We also measured the amount of DNA in a time course experiment and evaluated MSCs multi-lineage differentiation capacities after 1 and 30 days of encapsulation in alginate beads. Mixed model ANOVA was used to calculate statistical significance. A p-value <0.05 was considered statistically significant.

**Results:** Stimulation with IFNγ and TNFα increased the expression of TNFα, IL1β, IL-6 (Fig1A), IDO (Fig1B) and COX2 in MSCs, whereas VEGF was downregulated (p <0.05). TGFβ1 and TIMP2 didn’t reach significance but showed a trend toward downregulation after cytokine treatment. The response of MSCs in alginate was similar to MSCs in monolayer culture. After 30 days in alginate the amount of DNA/alginate bead was unchanged, suggesting MSCs survive but do not proliferate in alginate. The cells still responded to inflammatory cytokines by increasing the expression of TNFα, IL1β, IL-6 (Fig1A), IDO (Fig 1B) and COX2 and