faster (1.12±0.1 days doubling time) in monolayers than SSEA-4 expressing cells (1.29±0.1 days) ($p<0.05$).

Conclusions. Our observations indicate that the stem cell surface antigen SSEA-4 can be used to select for chondroprogenitors with enhanced chondrogenic differentiation capacity in cultured human chondrocytes. Future research will be focussed on the cellular characterisation of purified SSEA-4-positive cells to confirm their superior chondrogenic potential in vivo.

Keywords. cartilage, tissue engineering, surface marker

(8.O13) THE ROLE OF CELLULAR COMMUNICATION IN BONE MARROW DERIVED STROMAL CELL CHONDROGENIC DIFFERENTIATION

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Bone marrow-derived stromal cells (BMSCs) are envisioned as regenerative cells for numerous tissues, including cartilage. Success of BMSC-based therapies, however, relies on a number of methodological improvements, among which is better understanding and control of their differentiation pathways. We investigated here the role of cellular communication (through paracrine signaling and/or cell-cell contact) in the chondrogenic potential of BMSCs.

Bovine BMSCs (n=3 donors) were encapsulated in alginate beads as dispersed cells at 3, 7, and 14 millions cells/ml and as micro-aggregates at 7 millions cells/ml thus creating different paracrine signaling and cell-cell contact conditions. BMSCs were cultured for 21 days under hypoxia (2%O2) and TGFb3 stimulation (10ng/ml). At d0 and d21, cell phenotype was characterized by RT-qPCR (type I and II collagens, sox9, aggrecan, TGFb); produced matrix by histology (Alcian blue staining) and biochemical assays (glycosaminoglycan (GAG) and DNA content); cell morphology by histology (phalloidin staining); and cell viability by live/dead staining.

In all conditions, BMSCs stayed viable and DNA content remained constant up to 21 days. Major chondrogenic markers (type II collagen, aggrecan, sox9) were clearly up-regulated at day 21, with a higher up-regulation for dispersed cells (Figure). Matrix production (GAG/DNA content) increased in time but without significant differences between groups (Figure). Histological analysis is under progress. This study showed that, under TGFb stimulation and in the range of cell concentrations used here, endogenous paracrine signaling does not significantly affect BMSC chondrogenic differentiation, as all dispersed conditions led to the same outcomes. Cell-cell contact (micro-aggregates) has a negative effect on chondrogenic marker expression that is not reflected at the matrix level. Endogenous paracrine signaling and cell-cell contact, however, may have a greater impact on BMSC chondrogenic differentiation under other stimulants such as mechanical loading which may rely on endogenously produced factor or cell-cell communication for amplification of their effects.

Keywords. Bone marrow-derived stromal cells; chondrogenic differentiation; paracrine signaling; cell-cell contact

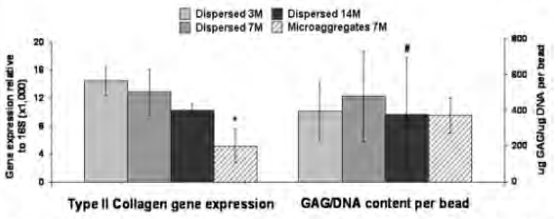

Fig. Type II collagen gene expression and GAG/DNA content at day 21

Values are means ±SD; n=3 donors; *p<0.05 compared to other groups; # data were extrapolated from standard curves. The assay is being repeated to interpolate the data.

(8.O14) PERIOSTEAL FLAP SUBSTITUTE FOR AUTOLOGOUS CHONDROCYTE IMPLANTATION

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Autologous chondrocyte implantation (ACI) is one of the options available to treat osteoarthritis. In this procedure, a periosteal flap is harvested and secured over the defect site to hold the implanted chondrocytes in place. However, the use of the graft is often associated with graft hypertrophy and an increase in subchondral bone density. Hence, a synthetic substitute is highly desirable. In this study, we have a developed a PVA-based membrane to address the problems associated with the use of the periosteal flap. The membrane displayed good mechanical properties, with a Young's modulus of about 1MPa – above the minimum required for hyaline cartilage. Modification of the membrane to present the integrin-binding peptide, RGD, improved initial cell attachment by up to 4-fold, pointing towards improved chondrocyte survival in vivo. In vitro culture of bone marrow-derived human mesenchymal stem cells (hMSCs) revealed that the cells remained attached and viable on the membranes for up to 2 months. Gene expression studies for bone markers, namely collagen type I, RunX2 and bone sialoprotein (BSP), of hMSCs cultured on the membranes showed lower expression as compared to hMSCs cultured on tissue culture plastic, thus lowering the risk of graft hypertrophy. In vivo implantation of the membrane material showed good biocompatibility. These findings demonstrated that the RGD-modified PVA membranes are a potential substitute for the periosteal flap used in ACI, as well as other applications in which the periosteum is required.

Keywords. Biomaterials, Membrane, Cartilage

(8.O15) COMPRESSIVE BIOMECHANICAL PROPERTIES OF A NEW BIO-COLLAGEN SCAFFOLD FOR CARTILAGE TISSUE ENGINEERING

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Introduction. Defects of cartilage in nose and ear are frequent problems caused by trauma or cancer. The need for biomaterials for reconstruction of auricle or nasal septum therefore is enormous. A newly developed bio-collagen scaffold from decellularised porcine cartilage