immediate novel clinical applications. Validation and appropriate regulation of these new biological treatments are critical.

Autologous chondrocyte transplantation (ACT) is a promising approach for joint surface defect repair. The procedure involves autologous chondrocyte cell expansion, known to result in phenotypic de-differentiation. This may be associated with a reduction of cartilage forming ability *in vivo*, raising the need for proper quality controls for expanded chondrocyte populations. We have linked an *in vivo* cartilage-forming assay to molecular markers. This set of molecular markers can represent a quality control for chondrocyte expansion and transplantation, and a tool for developing procedures aimed at preserving/restoring the phenotypic stability of expanded chondrocytes. However, it is still to be established if the ACT procedure is the first line treatment for articular cartilage defects. Apart from the phenotypic instability, there are other disadvantages associated with this procedure. One of them is the cartilage biopsy needed to obtain chondrocytes. Therefore, we have set out to identify precursor cell populations capable of contributing to joint tissue repair. Our data indicate that besides bone marrow stromal cells, periosteal- and synovium-derived fibroblast-like cells (FLCs) are potential progenitors for joint repair. Both human periosteal- and synovium-derived FLCs can be obtained and expanded reproducibly and consistently, independent of the age of the donor. These cell populations are phenotypically stable over at least 10–15 passages. In addition, they are and maintain a multi-lineage potential, i.e. they are chondrogenic, osteogenic, myogenic and adipogenic grown under appropriate culture conditions. We have also linked these precursor cell populations to specific markers. These cell-based technologies provide important tools to optimise the repair and engineering of joints and joint associated tissues.

Education – Specialist training – Friday 15 June, 16.00-17.30/Small Hall

SP0090 3D SPHEROIDS FOR THE STUDY OF ENDOTHELIAL CELL DIFFERENTIATION AND ANGIOGENESIS

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Endothelial cells (EC) seeded in suspension culture rapidly undergo apoptosis. Addition of survival factors does not prevent apoptosis of suspended EC. We have developed a three dimensional model of organoid endothelial spheroids for the study of differentiated endothelial functions, such as survival factor activities, organotypic differentiation, and sprouting angiogenesis. The spheroids differentiate over time to establish a surface monolayer of EC and a centre of unorganised EC that subsequently undergo apoptosis. Surface EC establish firm cell-cell contacts and can be induced to express differentiation antigens that are downregulated in monolayer EC. In contrast, the unorganised cells in the centre of the spheroids will rapidly undergo apoptosis if they are not rescued by exogenous survival factors, such as VEGF, Ang-1, and FGF-2. In addition to using EC spheroids as a cellular differentiation model, we developed a novel collagen gel based three dimensional *in vitro* angiogenesis assay based on the use of EC spheroids as a cellular delivery device. Gel embedded EC spheroids act as focal starting points for the

sprouting of luminized capillary-like structures that can be induced to form complex anastomosing networks. Formation of capillary anastomoses is associated with directional sprouting of outgrowing capillaries towards each other. To analyse, if directional sprouting is dependent on cytokine gradients or tensile forces exerted through the extracellular matrix, we designed a "tension trimmer" that enables the application defined tensional forces on the extracellular matrix. Based on this assay, we present causal evidence that tensional forces on a fibrillar extracellular matrix, such as type I collagen but not fibrin, is sufficient to guide directional outgrowth of endothelial cells. Lastly, we have further developed the spheroidal cell culture system to establish organised coculture spheroids of endothelial cells and smooth muscles in order to study phenomena of vessel maturation as they are associated with the recruitment of mural cells. Coculture spheroids of EC and SMC differentiate spontaneously in a calcium-dependent manner to organise into a core of SMC and a surface layer of EC, thus, mimicking the physiological assembly of blood vessels with surface lining EC and underlying mural cells. Coculture of EC with SMC induces a mature, quiescent EC phenotype as evidenced by 1) a significant increase in the number of junctional complexes of the EC surface layer, 2) a downregulation of PDGF expression by cocultured EC, and 3) an increased resistance of EC to undergo apoptosis. Furthermore, EC cocultured with SMC become refractory to stimulation with VEGF (lack of CD34 expression upon VEGF stimulation; inability to form capillary-like sprouts in a VEGF-dependent manner in a three dimensional in gel angiogenesis assay). Taken together, the data establish the three dimensional EC spheroid model as a powerful tool for the study of endothelial cell differentiation and angiogenesis.

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SP0091 E-SELECTIN DELETION CONSTRUCTS ALLOW THE STUDY OF DIFFERENTIAL BINDING OF MONONUCLEAR AND POLYMORPHONUCLEAR LEUKOCYTES TO E-SELECTIN IN INFLAMMATION

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Endothelial cell activation is an important step in the inflammatory cascade which leads to the extravasation of leukocytes into the site of inflammation. The rolling of leukocytes is the beginning of this cascade and is mediated by the selectins. We have analysed the binding of both polymorphonuclear granulocytes and mononuclear cells to E-selectin presented exclusively by activated vascular endothelial cells. Four different monoclonal antibodies inhibited this adhesion differentially, and deletion constructs of E-selectin expressed on endothelial cells were also functionally distinguishable by their interaction with granulocytes and lymphocytes, respectively. Different binding mechanisms for these leukocyte subsets to endothelial E-selectin are proposed and signalling mechanisms via E-selectin, in particular Ca^{2+} signals into the leukocyte will be discussed.