definition of occupational kneeling and squatting has varied between studies, but relative risks have often been two or higher.

It seems likely that the hazard arises from unusual mechanical stresses on the joint, and it is notable that the same occupational activities have also been linked with degenerative tears of the menisci. In addition, some occupations such as professional footballers carry a risk of acute meniscal injury which may later lead to osteoarthritis.

The challenge now is to translate these epidemiological observations into effective policies for the prevention of disease. In most developed countries, the requirement for heavy physical work has declined over recent decades as a consequence of increasing mechanisation. However, there are still many jobs that involve kneeling or squatting. Often, as for example in the construction industry, the work environment is difficult to control and the hazardous tasks cannot easily be eliminated or substituted. Knee pads may be used to reduce discomfort and protect against pre-patellar bursitis, but as yet we have no evidence that they reduce the risk of osteoarthritis.

Another approach might be to identify individuals who are at unusually high risk from kneeling because of the concomitant presence of other risk factors. For example, in a recent case-control study, the interaction of occupational kneeling with obesity was approximately multiplicative. It follows that the absolute reduction in risk from avoidance of obesity would be greater in workers who have to kneel in their jobs. Where the combination of occupational kneeling with other risk factors leads to exceptionally high risk, there may even be a case for selectively excluding such individuals from certain jobs.

Physiology of endothelial cell – Friday 15 June, 16.00-17.30/South Hall

SP0087 TISSUE ENGINEERING AND STEM CELL BIOLOGY: DEVELOPMENT OF BONES, JOINTS AND SYNOVIUM

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10.1136/annrheumdis-2001.34

Skeletal morphogenesis begins in structures called limb buds 4 1/2 weeks after gestation. The limb buds, comprised of mesodermal cells, form at discreet locations beneath a layer of ectoderm. The initial stimuli for limb development are still vague, but FGF-8 and possibly retinoic acid are important. By week 5 the limb primordium begins to bulge from the body wall. Despite the bud's simple structure it contains enough information to guide its development, for if transplanted to another site or cultured *in vitro* it will become a limb. At the apex of the bud is a thickened ectoderm (apical ectodermal ridge) and experiments have shown that continuous interactions between the AER and underlying mesoderm promote the initiation phase, outgrowth phase, and subsequent skeletal morphogenesis. The limb develops along three axis's; proximodistal (base of limb to digits); anteroposterior (digits 1 to 5); dorsoventral (top of hand/foot to palm/sole). Each is under the influence of "morphogens" made in discreet signalling centres.

Abstract SP0087 Table 1

Axis	Signalling Centre	Molecular signals
Proximodistal	Apical ectodermal ridge (AER)	FGF-2, 4, 8
Anteroposterior	Zone of polarising activity (ZPA)	Sonic hedgehog
Dorsoventral	Dorsal ectoderm	Wnt-7a (dorsal)
	Ventral ectoderm	En-1 (ventral)

Skeletal elements of the limbs first appear in a core of mesenchymal cells, then in cartilage (weeks 5 1/2 to 6 1/2), and finally in bone (6 to 8 weeks). The condensed cells that form the precartilaginous express bone morphogenetic proteins (BMP-2 and -4). As development continues they are restricted to cells that will become periosteum or perichondrium.

In contrast, BMP-6 is only expressed in maturing cartilage within limbs. Another signalling molecule, Indian hedgehog, found in the same location is thought to induce the BMP-6 expression.

Joint formation is a late feature in limb differentiation. Around week 6, between condensations in the precartilaginous, there develop transverse bands of tightly packed, flat mesenchymal cells, called interzones. At this time, growth/differentiation factor-5, a member of the BMP family, is expressed in the condensed cells. The interzone consists of two regions of high cell density surrounding a region of low cell density. The former will become articular cartilage, while the latter disappears through apoptosis leaving behind a cavity. At the periphery of each interzone are mesenchymal cells and blood vessels. These arise from the primordial perichondrium and will become the future synovium and joint capsule.

The synovium consists of an incomplete lining (the intima) sitting on a vascularized matrix. After birth two cell types can be identified. One (the type A synoviocyte) arises in the bone marrow, passes into the circulation and enters the joint as a monocyte, but during its progression into the intima acquires attributes of a tissue macrophage (including the CD68 phenotype). The other lining cell is a specialised fibroblast, similar to bone marrow stromal cells that supports hematopoiesis (type B – fibroblast-like synoviocyte). However, these only acquire VCAM-1 and the typical FLS phenotype after birth.

The shafts of long bones (diaphysis) are completely ossified at birth, but the ends of the bones (epiphysis) are still cartilaginous. Only later, when secondary centres of ossification develop does this segment gradually become bone. Even then, however, a layer of cartilage persists between the epiphysis and the growing end of the diaphysis. In man chondrocyte proliferation continues in this growth plate for about twenty years and not until epiphyseal fusion does bone growth cease.

SP0089 CELL-BASED TECHNOLOGIES IN JOINT REPAIR

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10.1136/annrheumdis-2001.35

Tissue engineering is a field of Biomedicine that is rapidly growing, and is critically driven by scientific advances in the areas of developmental and cell biology, material sciences and biochemistry/polymer chemistry. Regeneration of skeletal tissues, including joint surface repair, skin repair, and treatment of CNS disorders are among the most promising areas, and are closest to

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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10.1136/annrheumdis-2001.36

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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10.1136/annrheumdis-2001.37

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.