The Normal Synovium

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1 Introduction

The synovium is the soft tissue that lines diarthrodial joints, ensheaths tendons and forms the lining of bursae. The term is inclusive of a continuous layer of cells (intima) and the underlying tissue (subintima). Specialised macrophages and fibroblasts form the intima; the subintima contains resident fibroblasts, infiltrating cells and blood vessels in a scaffolding of extracellular matrix. Between the intimal surfaces is a small amount of fluid, usually rich in hyaluronan (hyaluronic acid). Taken together, this structure provides a non-adherent surface between tissue elements. The synovium is of ectodermal origin and bereft of basal lamina unlike other serosal surfaces that also have non-adherent properties. Recent evidence suggests that a mesenchymal cadherin, cadherin 11 (a member of the cadherin family which are usually calcium dependant integral membrane proteins responsible for tissue architecture and morphogenesis) is expressed on synovial fibroblasts and may be critical in the development of the synovial lining by facilitation of cellular organization, compaction, and matrix development to form a recognizable synovial lining. (Firestein, 2007; D. M. Lee et al., 2007)

Despite the obvious importance of the absolute need to have extensive background knowledge of the normal synovium to appreciate synovial changes in disease states such as rheumatoid arthritis (RA) and the spondyloarthritidies (SpA), our knowledge of the normal synovial membrane architecture is surprisingly inadequate. The normal synovium is relatively acellular; it has a distinct 1-2 cell thick intimal lining and a sublining. The sublining is relatively acellular as well containing scattered blood vessels, adipocytes and fibroblasts with very few lymphocytes or macrophages. The intima is 20-40 μm thick in cross section in contrast to the areolar subintima, which can be up to 5 mm thick.

There can be considerable variations in the synovial membrane. At several sites, there is no distinct membrane, especially where subintima consists of fat pad or fibrous tissue. There can be considerable variation in the intima as well with some sites having absence of intimal cells. (Smith, 2011) Bursae have minimal or no hyluronan-rich fluid.(Canoso, Stack, & Brandt, 1983) Ganglia, despite containing hyaluronan-rich fluid are devoid of a typical intima; they do not occur at sites of shear and hence are not really synovial tissue. In contrast to the appearance of the synovium in health, in disease the synovium may lose recognisable lining structure and may only be definable by its relation to a joint; in inflammatory arthritis such as rheumatoid arthritis, the synovial thickness increases. The intimal layer becomes several layers thick owing to increased CD68+ macrophages; the subintima thickness increases because of cellular infiltration with T and B lymphocytes, macrophages and plasma cells, stromal oedema and blood vessel proliferation.

2 Structure

Structurally, the normal synovium can be divided into three types, with the subintimal structure forming the basis for these synovial subtypes: fibrous, areolar and adipose. (Key, 1932) (Figure 1 – 3). Of these, the areolar form (Figure 1) of synovium is the most specialised. It may have projections or villi, but more commonly it is crimped into folds that may disappear when stretched. A 2-3 cell deep (Singh, Arayssi, Duray, & Schumacher, 2004; Smith et al., 2003) layer forms the surface; below are capillaries, and further below a plexus of small arterioles and venules (Davies, 1950; Wilkinson & Edwards, 1989) associated with mast cells. Lymphatic vessels- albeit infrequent in the fibrous form of synovium- are present in all types of synovial tissue. (Xu et al., 2003) These vessels are usually found in the deep subintimal layers
of the normal synovium and in the presence of inflammatory arthritis such as RA, are found to be numerous and widespread. The synovium also has nerve fibres, usually present in association with blood vessels. (Mapp, 1995) The connective tissue matrix in the normal synovium consists of a fine fibrillar matrix with a few type I collagen fibres in the intima; what lies below is a layer relatively rich in type I collagen that forms the physical membrane. The loose connective tissue that lies deepest allows free movement of the synovial membrane. (Ghadially, 1978)

Adipose synovium (Figure 2) usually lies in relation to fat pads, but can be seen within villi; it has a complete intimal layer and a superficial network of capillaries. Villi usually have a central arteriole and...
Fibrous synovium (Figure 3) is easier to describe than define; it consists of fibrous tissue such as ligament or tendon on which lies an intermittent layer of cells. In annular pads found in finger joints, fibrous synovium may be indistinguishable. (Smith, 2011)

The synovial intima consists of synovial lining cells or synoviocytes that are of two microscopically, immunohistochemically and functionally differing types. This distinction was first proposed by Barland et al. ((Barland, Novikoff, & Hamerman, 1962) who reported the two distinct cell types on the basis of differences on electron microscopy. Type A synoviocytes are more numerous, have a prominent Golgi apparatus, numerous vacuoles, many filopodia, mitochondria, intracellular fibrils, micropinocytic like vesicles and manifest surface markers of the macrophage lineage. Type B synoviocytes contain large amounts of granular endoplasmic reticulum with fewer large vacuoles, micropinocytic vesicles and mitochondria; these cells show fibroblast lineage surface markers. (Barland, et al., 1962) Evidences from several elegant immunohistochemical animal studies and other lines of evidence point to intimal macrophages as being true macrophages, derived from bone marrow derived precursors (though it is not certain if differentiation occurs in situ or prior to arrival), whereas the intimal fibroblasts are locally derived. (Barland, et al., 1962; Bartok & Firestein, 2010; J. C. Edwards, 1994; J. C. Edwards & Willoughby, 1982; Henderson, Revell, & Edwards, 1988) Fibroblasts are the dominant cell type in the normal synovium, and in most disease states including RA, the increase in synovial intimal cells reflects a rise in intimal macrophages secondary to influx from the vascular compartment orchestrated by cytokines and cell adhesion molecules. (Henderson, et al., 1988; Smith, et al., 2003; Smith & Walker, 2010) Immunohistochemistry, as opposed to electron microscopy, is now the preferred method for cellular identification. Intimal macrophages show expression of surface markers such as CD68 (a macrophage marker related to
lysosomal glycoprotein (Holness & Simmons, 1993)), CD163 (a monocyte/macrophage lineage marker), CD45 (common haematopoietic antigen) and non-specific esterase (NSE) activity; intimal fibroblasts reveal intense activity of the enzyme uridine diphosphoglucose dehydrogenase (UDPGD, an enzyme involved in hyaluronan synthesis and a specific marker of this cell type) (Wilkinson, Pitsillides, Worrall, & Edwards, 1992) and prominent expression of vascular cell adhesion molecule 1 (VCAM-1, a cell adhesion molecule) and CD55 [complement decay-accelerating factor (DAF)].

2.1 Synovial Macrophages

Synovial macrophages (Figure 4) are present in both the intimal and subintimal layers in the normal synovium. They bear typical macrophage lineage markers and show strong CD68 and CD163 positivity but less so for CD14 (a membrane protein that normally functions as a pattern recognition receptor). A subset of intimal (but not subintimal) macrophages show strong FcγRIIIa expression; interestingly, this corresponds closely to sites of macrophage activation in rheumatoid disease: synovial, alveolar, serosal, scleral, salivary gland, lymphoid and bone marrow macrophages, and Kupffer cells. (Bhatia, Blades, Cambridge, & Edwards, 1998) Z39Ig, a recently described inducible cell surface receptor linked to the classic complement pathway, is also expressed by synovial macrophages. Expression of this receptor can occur during macrophage differentiation and induce activation of the transcription factor NF-κB and production of the matrix degrading enzyme matrix metalloproteinase 9 (MMP-9). (Kim et al., 2005; M.Y. Lee et al., 2006; Poulter & Janossy, 1985; Walker, 2002)

![Figure 4: Synovial macrophages (normal synovium, x200 magnification), stained for CD68](image)

Macrophages normally make up a minority of cells in the normal intima in contrast to that in inflammatory arthritis in which macrophage numbers increase dramatically (Figure 5). In RA synovium macrophages can account for up to 80% of the intimal layer, with the usual pattern being that of a superficial layer of macrophages with an intimal phenotype below which lie a layer of intimal fibroblasts. The subintima may have a zone of NSE-weak, strongly CD14+ FcγRI+ macrophages in association with venules.
Figure 5: Synovium in rheumatoid arthritis (x400 magnification). Note the thickened intimal layer containing mainly CD68+ macrophages (red; arrow) on the surface and weakly CD55+ synovial fibroblast cells (blue; block arrow) beneath.

A small number of antigen presenting dendritic cells are also present in the normal synovium; these significantly increase in numbers in the diseased synovium, though in this state identification is difficult because of increased overlap of identifying markers. (Poulter & Janossy, 1985; Wilkinson, Worrall, Sinclair, & Edwards, 1990) Available evidence indicates that both intimal and subintimal macrophages derive from the bone marrow via circulating monocytes, many of which probably arrive via subintimal venules and migrate to the intima; this migration is supported by several mediators including CD11/18, Very Late Antigen-4 (CD49d/CD29), Very Late Antigen-5 (CD49e/CD29), and Vascular Cell Adhesion Molecule-1 (CD106). (J. C. Edwards, 1994; J. C. Edwards & Willoughby, 1982; Shang, Lang, & Issekutz, 1998; Smith, 2011)

2.2 Synovial Fibroblasts

Synovial fibroblasts (Figure 6) are thought to derive from division within synovium although the exact site remains uncertain. While it is possible that intimal fibroblasts might have their origin from a different lineage as opposed to subintimal fibroblasts, the current view supports a single origin that can take on an intimal phenotype in response to local stimuli (J. C. Edwards, 2000); the alternative hypothesis relates to mesenchymal origin for these cells. (Bartok & Firestein, 2010) Rates of cell division within the intima are very low, even in disease, as shown by thymidine labelled studies. (Mohr, Beneke, & Mohing, 1975) Following arthroplasty or synovectomy intimal cells reappear and express CD55, UDPGD, and VCAM-1; these may arise from intimal rests but it is probable that they are replaced from the subintima.

Synovial fibroblasts are adapted to hyaluronan production, with UDPGD activity being a specific marker of this cell type. This enzyme converts UDP-glucose into UDP-glucoronate, which is one of the two substrates required by hyaluronan synthase for assembly of the hyaluronan polymer. (Wilkinson, et al., 1992) Disease states led to reduced UDPGD activity in synovial fibroblasts. CD55 expression by synovial fibroblasts is used to distinguish these from synovial macrophages. When cell suspensions gen-
Eroded from inflamed synovium are grown in tissue culture, cells showing fibroblast characteristics and ramifying processes with production of high levels of metalloproteinases are seen. (Krane, Goldring, & Dayer, 1982)

Figure 6: Synovial fibroblasts (arrow) in the normal synovium (x200 magnification) stained for CD55.

Synovial fibroblasts express several adhesion molecules including VCAM-1, intercellular adhesion molecule (ICAM)-1, CD44, β1 and β3 integrins. (Connolly, Veale, & Fearon, 2011; J. C. Edwards, 1995; Smith, et al., 2003) The expression of VCAM-1 is intriguing and unusual as it is absent in most other fibroblast populations, though CD44 and β1 integrins can be expressed in low levels on normal fibroblasts. (J. C. Edwards, 1995; Smith, et al., 2003) VCAM-1 in this context may be important in cellular trafficking; its ligand α4β1 integrin is present on mononuclear leukocytes but not granulocytes. VCAM-1 on synovial fibroblasts may allow transmigration of polymorphs into synovial fluid and potentially trap macrophages and lymphocytes within the synovial membrane in disease states such as RA. Disaggregated and cultured synovial fibroblasts lose VCAM-1 and DAF expression but readily reacquire these markers following cytokine stimulation. In culture, fibroblasts can be induced to express complement receptor-2 (CR2, CD21), though this receptor is not usually expressed on synovial fibroblasts in the normal synovium. (Leigh, Cambridge, & Edwards, 1996) This receptor, along with DAF and VCAM-1 is involved in B lymphocyte survival as is a bone marrow stromal cell marker, BST-1, reported to be expressed on fibroblasts in rheumatoid (but not normal) intima. (B. O. Lee et al., 1996) Synovial fibroblasts can be induced to express several other molecules including the chemokine SDF-1 and bone morphogenetic proteins and their receptors (Fowler et al., 1998; Marinova-Mutafchieva, Taylor, Funa, Maini, & Zvaifler, 2000; Seki, Selby, Haupl, & Winchester, 1998) under various conditions. In addition, lubricin, a glycoprotein found in synovium and the superficial zone of articular cartilage, (Jay, Britt, & Cha, 2000) derives from the same gene as megakaryocyte stimulating factor. A defect of this gene leads to the Camptodactyly- Arthropathy- Coxa vara- Pericarditis (CACP) syndrome. (Marcelino et al., 1999)

In addition to lubricin, synovial fibroblasts synthesize several normal matrix components including laminin, collagens, fibronectin, proteoglycans among other proteins. In inflammatory arthritis, trans-
formed fibroblasts have enormous capacity to produce cytokines, adhesion molecules and metalloproteinases. (Bartok & Firestein, 2010)

The presence of both clusterin (a glycoprotein involved in recycling and apoptosis) and podoplanin (a membrane glycoprotein with diverse functions) has recently been reported in normal synovial fibroblasts. Of interest, podoplanin (which in context of neoplasia is associated with poor prognosis and metastatic disease) has been shown to be highly expressed in RA synovial fibroblasts, known to have migratory and invasive potential. (Boland, Folpe, Hornick, & Grogg, 2009; Ekwall et al., 2011)

The synovium is also a source of mesenchymal stem cells. These cells have the ability to self-renew and differentiate; they compare favourably to bone marrow derived MSCs in terms of their ability to differentiate into bone, cartilage and adipose tissue. (Arufe, De la Fuente, Fuentes-Boquete, De Toro, & Blanco, 2009; Arufe, De la Fuente, Fuentes, de Toro, & Blanco, 2010; De Bari, Dell'Accio, Tylzanowski, & Luyten, 2001; Sakaguchi, Sekiya, Yagishita, & Muneta, 2005)

2.3 Other Sub-intimal Cell Populations

Other cells including CD3+ T cells, (including CD4+ and CD8+ cells), with some having a memory T cell phenotype, B cells and plasma cells can be found within the normal synovial tissue. (Singh, et al., 2004; Smith, 2004) It is possible that some of these cells may simply be trafficking through the normal synovium, but their role, if any, in the homeostasis of synovial tissue remains unknown. (Smith, 2011)

2.4 Inflammatory Cytokine Production within the Normal Synovium

Inflammatory cytokine production, including that of interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF-α) can be seen within the normal synovium, (Smith, et al., 2003) it is far less than that seen in inflammatory states such as RA, and in normal tissue is outweighed by the amount of anti-inflammatory cytokine production, at least in the case of IL-1 receptor antagonist (the naturally occurring inhibitor of IL-1). Similarly, receptor activator of nuclear factor kappa-B ligand (RANKL, an essential factor for the development of osteoclasts) seen in normal synovial tissue is low (Smith, et al., 2003) and easily outweighed by osteoprotegerin (Figure 7), its naturally occurring decoy receptor, thus effectively suppressing osteoclast function.

2.5 The Intimal Matrix

The intimal matrix has an amorphous or fine fibrillar ultrastructure, containing collagens III, IV, V and VI with minimal type I collagen. Despite the presence of several basement membrane components including laminin, fibronectin and chondroitin-6-sulfate-rich proteoglycan in the intimal matrix, the basement membrane is conspicuous by its absence. (Ashhurst, Bland, & Levick, 1991; Revell, al-Saffar, Fish, & Osei, 1995) This may be due to the absence of entactin, which normally links other components in basement membrane together. Intimal microfibrils include fibrillin-1 microfibrils that form a basketwork around cells and collagen VI microfibrils that form a uniform mesh. Hyaluronan is prominent mainly in the intimal and superficial subintimal layers of the normal synovium but fades in the deeper sublining layer possibly indicating diffusion of hyaluronan (HA) from the surface towards clearing lymphatics.

2.6 The Vascular Net

Just beneath the synovial surface lies a rich microvascular network. The synovial vascular supply stems from multiple feeding small vessels which communicate and branch freely in the deeper layers of the
Figure 7: Normal synovium (x200 magnification) stained with an antibody against osteoprotegerin (arrow)

synovium; as they become more superficial they form multiple branches to contribute to the capillary network. These capillaries become less prominent with age; some capillaries are fenestrated and fenestrae tend to face the tissue surface. (Suter & Majno, 1964) Apart from fenestration of superficial capillary endothelial cells, there is little evidence of specialization in synovial endothelium. 50 to 100 µm beneath the surface, small venules are prominent. An anastomosing quadrilateral array is formed 200 µm beneath the surface with larger venules, arterioles and lymphatics (Figure 10). (Xu, et al., 2003)

In disease states such as RA in which increased turnover of hyaluronic acid and leukocyte trafficking is seen, vessels with lymphatic staining (can be immunohistochemically stained with LYV-1 antibody) characteristics are prominent. It has been proposed that failure of lymphatic drainage of synovial fluid may be a cause of villous proliferation in RA synovial tissue. If correct, this may reflect overloading of existing lymphatic channels with HA-rich extracellular fluid and leukocytes rather than a lack of lymphatic channels. (Xu, et al., 2003)

Physiologically, synovial fluid flow is increased by heat and exercise and reduced by immobilization. In the presence of knee effusion, even modest elevations in intra-synovial pressures of up to 45mmHg in the context of daily activities were associated with synovial blood flow compromise and hypoxia; (James, Cleland, Rofe, & Leslie, 1990) in the context of RA, hypoxia is thought to be a key regulator of synovial angiogenesis and inflammation. (Konisti, Kiriakidis, & Paleolog, 2012)

2.7 The Nerve Supply

The synovium has a rich nerve supply, (Mapp, 1995; Pereira da Silva & Carmo-Fonseca, 1990) including from the sympathetic nervous system (Widenfalk, 1991) with most of the nerve supply being perivascularly located and some extending into the intimal layers. In RA synovium, reduced nerve supply is seen, especially in the more superficial intimal regions.
3 Function of the Normal Synovium

The functions of synovial tissue are thought to be self-evident, but precisely defining them is difficult. (J. C. W. Edwards, 1987) The synovial lining provides a deformable packing that enables movement between adjacent, relatively non-deformable tissues; in addition, areolar synovium may also have specialized viscoelastic properties for coping with the stretching, rolling, and folding it undergoes during joint movement. Furthermore the synovium maintains an intact non-adherent tissue surface, provides cartilage lubrication and nutrition and regulates synovial fluid volume and composition.

3.1 Maintenance of Tissue Surface

To allow continued movement, synovial surfaces must be non-adherent; hyaluronan production by intimal fibroblasts may play an important role in this regard. Plasminogen activator and DAF from intimal fibroblasts may inhibit fibrin formation and scarring. Synovial fluid retention requires the allowance by the intimal matrix for free exchange of crystalloids and proteins but inhibit rapid transit of the viscous hyaluronan solution. These functions are probably subserved by the intimal macrophages and fibroblasts. The vasculature is probably important in both intimal cell nutrition and recruitment of new cells. Blood monocytes will replenish macrophages while perivascular fibroblasts may provide the main pool of intimal fibroblast precursors.

3.2 Lubrication

Synovial fluid probably lubricates cartilage because of the presence of a glycoprotein, especially a glycoprotein known both as ‘lubricin’ and ‘superficial zone protein’ because of its localization to the surface of both synovium and cartilage. (Jay, et al., 2000) Hyaluronan is probably responsible for retaining a constant synovial fluid volume during exercise (Levick & McDonald, 1995) and in maintaining a film of lubricant on the cartilage surfaces but does not appear to contribute to the ability of synovial fluid to lubricate cartilage. This ability of hyaluronan to maintain a constant synovial fluid volume during exercise is probably important as a cushion for synovial tissue and as a reservoir of lubricant for cartilage. It is likely that the rate of hyaluronan synthesis and its export into the joint cavity are dependent on the mechanical stimulation of intimal fibroblasts and influenced by the effectiveness of the synovial fluid cushion.

Joint effusions are created by two, probably interrelated mechanisms. (Pelletier, Martel-Pelletier, & Abramson, 2001) Mechanical irritation of the synovium by bone and cartilage leads to an effusion with reasonably normal composition (frictional force induced hyaluronan production retains plasma diacylosate in the joint cavity). In addition, a low grade inflammatory immune reaction to bone and cartilage products may contribute to an effusion. Recent proteomic evidence suggests that the increased vascular permeability of inflammation may be related not only to increase in inter endothelial gaps, but also to glycocalyx damage and aquaporin upregulation. (Shahrara, Volin, Connors, Haines, & Koch, 2002)

3.3 Chondrocyte Nutrition

The synovium provides the major route for chondrocytes nutrition. In a normal joint, a surprisingly large proportion of hyaline cartilage lies within 50µm of a synovial surface with only a small proportion of cartilage apposed to the other articular surface in any one position; the synovium packs most of the space between less congruent areas. In immature but not adult joints the incomplete subchondral plate may con-
tribute to nutrition and hence cartilage nutrition especially in areas that do not come into close contact with synovium must be by an indirect route. In this case, nutrition may occur by indirect routes through cartilage matrix and the apposed articular cartilage may be more important. Although synovial blood vessels potentially provide the most direct route for cartilage nutrition, there is little evidence of structural adaptation for this function. Diarthrodial joints have high levels of transforming growth factor β (TGF β) in cartilage and the synovium. TGF β is known to exist in two forms: a latent form that can be activated in vivo and vitro, and an active form that interacts with cell surface receptors. (Albro et al., 2012; Fava, Olsen, Keski-Oja, Moses, & Pincus, 1989). In the latent form, TGF β is linked non-covalently to a 70 kDa latency associated peptide; this complex is called the large latent complex. TGF β must undergo activation to be able to bind to receptors and induce a biological response. Recent in vivo experiments suggest that shearing of synovial fluid as a result of physiologic joint motion may play an important role in TGF β activation, which may be essential to maintain the biochemical content and structural integrity of healthy cartilage. (Albro, et al., 2012; Fava, et al., 1989; Miossec, Naviliat, Dupuy d'Angeac, Sany, & Banchereau, 1990)

4 The Synovium as a Site of Pathology in Inflammatory Arthritis

Synovitis or inflammation of the synovial membrane is a consequence of a multitude of immunological and inflammatory disorders, including RA, systemic lupus erythematosus and spondyloarthritis. The knowledge of normal synovium including its considerable normal variation is critical to understand relevant changes in synovial tissue architecture and immunopathology in disease states. Despite this variation, there are general consistencies across the broad spectrum of normal, which can be contrasted with that seen in the chronically inflamed synovial tissue. An example is the marked increase in synovial lining layer thickness, with a reverse of normal ratio of type A to type B synoviocytes, favoring type B cells in normal synovium and types A cells in RA. Other examples include changes in subintimal cell content, cytokine and chemokine production, vascular and lymphatic changes as well as production of metalloproteinases and stimulators of osteoclast formation. It is important to understand these synovial changes of chronic inflammation and contrast them with those seen in the normal synovium, to identify suitable therapeutic targets at various stages in the evolution of a chronic inflammatory arthritis.

The identification of TNF, IL-1, IL-6 and IL-17 as four likely therapeutic targets is an example of how such a strategy can lead to useful therapeutic interventions being introduced into the management of several chronic inflammatory arthritides including RA, psoriatic arthritis and ankylosing spondylitis. Recent work on a mouse model of arthritis has also raised the possibility of cadherin-11 expression on synovial fibroblasts as a potential therapeutic target in the treatment of RA.(D. M. Lee, et al., 2007)(Firestein, 2007) Inhibition of cadherin-11 interactions in this model interfered with both the synovial inflammation and the cartilage invasion by pannus, without any effect on bone erosion, which is predominantly dependent on osteoclast formation. Inflammation in this model could be substantially ameliorated by antibodies to cadherin-11 or a cadherin-Fc fusion protein. In further studies, cadherin 11 expression (Figure 8) has been found to promote invasive behavior of fibroblasts and is increased by IL-17 and tumour necrosis factor α (TNF α), cytokines very relevant in RA pathophysiology. (Kiener et al., 2009; Park et al., 2011; Vandooren et al., 2008)

There is still much to be learned about the immunological microenvironment of articular tissues, particularly the normal synovium.
5 Summary

In summary, the normal synovium consists of the intima and subintima, and is essential for normal articular homeostasis. There is a considerable morphological and immunological variation in the cytokine, cellular and vascular content in health and disease, which highlights the importance of having an understanding of the normal synovium prior to appreciating changes in pathological states such as the inflammatory arthritides. Knowledge of the architecture of the normal synovium is surprisingly limited.

References


