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Editorial

The hepatic extracellular matrix.

I. Components and distribution in normal liver

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Abstract. The unique nature of the hepatic extracellular matrix (ECM) is predicated by the special configuration of the space of Disse. Whereas other epithelial organs have two basement membranes (BM) and a substantial ECM interposed between endothelial and epithelial cells, the liver lobule has no BM and only an attenuated ECM, consisting mostly of fibronectin, some collagen type I, and minor quantities of types III, IV, V, and VI. This configuration, together with the abundant fenestrations and gaps of the sinusoidal endothelial cells, seems ideally suited to facilitate the rapid bidirectional exchange of macromolecules normally taking place between plasma and hepatocytes. During organogenesis, the liver anlage is vascularized by continuous capillaries with BM, but by day 13.5 of development (in the rat) the vessels in the immediate proximity of hepatocytes become fenestrated, lacking specialized junctions and BM, suggesting that the hepatocytes produce signals capable of modulating the endothelial phenotype. In regeneration, hepatocyte proliferation precedes vascular proliferation resulting in the formation of hepatocyte clusters that, temporarily, lack sinusoids. Eventually, vascular proliferation follows and the normal hepatocyte-vascular relationships are restored. During this period laminin synthesis by Ito cells is prominent. As soon as hepatocytes become stable, secretion of the sinusoid phenotype-maintaining factors resumes and laminin synthesis and secretion terminates. The interplay between extracellular matrix and liver cells is essential for normal homeostasis and its modification results in derranged hepatic function.

Key words: Basement membrane – Cirrhosis – Collagen – Fibrosis – Matrix

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Introduction

Although the extracellular matrix (ECM) (Rojkind and Ponce-Noyola 1983; Martinez-Hernandez 1984; Cunningham 1987a, b; Schuppan 1990) is only a small component of the liver (Rojkind and Martinez-Palomo 1976; Seyer et al. 1977; Seyer 1980; Rojkind et al. 1983), it has a crucial role, providing a structural framework and maintaining the hepatocyte differentiated state. This role of the hepatic ECM has been dramatically demonstrated in cell culture, where the hepatocyte phenotype is dependent on the nature of the ECM upon which it is cultured (Rojkind et al. 1980; Reid and Jefferson 1984; Bissell et al. 1987; Schuetz et al. 1988). The ECM modulates repair in many tissues (Kurkinen et al. 1980; Martinez-Hernandez 1985a), including the liver (Abe et al. 1984; Martinez-Hernandez 1985a). Therefore, defining the ECM distribution in the normal liver, its phenotypic expression in various reparative states, and the cells responsible for its synthesis in vivo is an important step in understanding its role in homeostasis and repair.

In these editorials we review the contributions of light and electron microscpic immunohistochemistry to our understanding of physiopathology of the hepatic ECM. In the first part we review those components relevant to the liver, and describe the localization of individual ECM components in normal adult liver, ontogenesis, and post-natal development. In the second part we review, the hepatic response to injury: regeneration and cirrhosis. Comparing and contrasting the data from these studies provides new insights into the role of the ECM in hepatic function, ontogenesis and repair.

Morphology and distribution of extracellular matrix components

The ECM of the various organs is composed of the same groups of molecules, and in many cases, the same individual components. Different concentrations, ratios, and associations of these components result in an ECM

tailored to the needs and functions specific to the organ. In this respect, the ECM components can be viewed as basic building blocks, used to generate the matrix best suited to the needs of the cells in a particular organ, to maintain homeostasis, support differentiation at the appropriate developmental stage, and to adapt to new stimuli. The liver, like any other organ, has a specialized matrix. To understand the organization of this matrix, it is helpful to have a notion of the main components and general patterns of the ECM.

Collagens

The collagens are a heterogeneous family of macromolecules that form the major structural scaffold of all ECM. Of the 13 characterized collagen types only the following have been found in the hepatic ECM.

Collagen type I. Collagen type I ($\alpha1(I)_2$ $\alpha2(I)$) is the most ubiquitous of this heterogeneous family, forming the major structural buttress of the ECM (Linsenmayer 1981; Amenta et al. 1986, 1988). It is the major collagen of bone, tendon, skin, and mature scars. Collagen type I forms thick, banded fibres that by electron microscopy have a 67 nm periodicity and is the major constituent of collagen bundles. Electron microscopic immunohistochemistry indicates that collagen bundles are heterogeneous (Amenta et al. 1986, 1988; Karkavelas et al. 1988). Collagen types III, V, VI, fibronectin (FN), and several proteoglycans are often found on the surface of collagen type I fibres (Boselli et al. 1981; Martinez-Hernandez et al. 1981a; Amenta et al. 1986, 1988; Martinez-Hernandez 1987; Karkavelas et al. 1988).

Collagen type III. Type III $\alpha1(III)_3$ is a ubiquitous collagen, particularly abundant in embryonic and pliable tissues, such as blood vessels, uterus, alveolar septa, and the gastrointestinal tract (Fleischmajer et al. 1981; Konomi et al. 1981; D'Ardenne et al. 1983; Keene et al. 1987). The early embryonic expression of collagen type III is recapitulated in wound healing (Kurkinen et al. 1980; Williams et al. 1984). Some controversy exists concerning the morphology of collagen type III fibres, some investigators describe a thin, banded fibre (Keene et al. 1987), while others describe tortuous, beaded fibres, (Amenta et al. 1986, 1988) closely associated with thick, banded collagen type I fibres. Retention of the aminopropeptide in the collagen type III molecule (Fleischmajer et al. 1985; Fleischmajer 1986) may limit the diameter of this fibres and may be responsible for the beaded morphology observed by electron microscopy (Amenta et al. 1986). The tortuous nature of these fibres may, in part, contribute to tissue pliability (Amenta et al. 1988).

Collagen type V. Collagen type V (3 α chains are known) is a widely distributed protein, present as a minor component in the interstitium of most organs (Miller et al. 1982; Bentz et al. 1983; Konomi et al. 1984; Bronckers et al. 1986; Schuppan et al. 1986; Chanoki et al. 1988). Collagen type V forms thin filaments, often found in association with fibres of other collagen types (Amenta et al. 1986). For this reason, it is thought to act as a connector among collagen types. Although some authors reported the localization of collagen type V in basement membranes BM; (Madri and Furthmayr 1979; Laurie et al. 1984; Grant et al. 1985), the majority of ultrastructural studies (Mark and Ocalan 1982; Martinez-Hernandez et al. 1982a; Miller et al. 1982; Linsenmayer et al. 1983; Konomi et al. 1984; Amenta et al. 1986, 1988; Bronckers et al. 1986; Schuppan et al. 1986; Chanoki et al. 1988) have failed to demonstrate collagen type V in these structures and to date, no type V has been isolated from any BM. Therefore, collagen type V should not be considered a BM component, but as minor component of most ECM, forming thin, delicate filaments in the interstitial space.

Collagen type VI. Collagen type VI was first identified in chick aortic intima; therefore, named intima collagen (Jander et al. 1983; Bruns 1984, 1986; Hessle and Engvall 1984; Mark et al. 1984; Trueb and Bornstein 1984; Ayad et al. 1985; Gibson and Cleary 1985; Zimmermann et al. 1986; Peltonen et al. 1990). By electron microscopy rotary shadowing, the molecule has a dumbell shape, with the globular, non-collagenous domains at each end of a 105 nm helical domain (Furthmayr et al. 1983; Engel et al. 1985). Monomers overlap to form anti-parallel dimers, which aggregate to form tetramers, stabilized by large numbers of disulphide bonds (Engel et al. 1985). Recent studies have demonstrated a ubiquitous distribution of collagen type VI (Amenta et al. 1986, 1988) in most connective tissues. Collagen type VI forms fine filaments in close association with other collagen types and has been proposed as a connecting protein (Amenta et al. 1986, 1988; Karkavelas et al. 1988). In human placenta, neurofibroma, murine lung, liver, kidney, and uterus, collagen type VI is present as 6–10 nm filaments. The association of collagen type VI with other collagen types demonstrates the heterogeneity of collagen bundles (Amenta et al. 1986, 1988; Karkavelas et al. 1988).

The salient features of the major collagen types, are summarized in Table 1.

Non-collagenous glycoproteins

The fibronectins (FN) represent a class of large molecular weight glycoproteins which exist in plasma and cellular forms (Linder et al. 1975, 1978; Engvall and Ruoslahti 1977; Timpl et al. 1979; Martinez-Hernandez et al. 1981a; Oh et al. 1981; Yamada 1981; Hynes and Yamada 1982; Ruoslahti et al. 1982; Hynes et al. 1984; Kornblihtt et al. 1984, 1985; Tamkun et al. 1984; Odermatt et al. 1985; Oldberg and Ruoslahti 1986; Paul et al. 1986). Plasma FN is also present in amniotic, cerebrospinal fluid, and urine. Cellular FN is ubiquitous in the ECM of most organs, coating collagen fibres and bundles of various diameters, as well as coating the cell surfaces of stromal cells (Stenman and Vaheri 1978;

Table 1. Diagram of the composition, associations, aggregate structure and distribution of ten collagen types

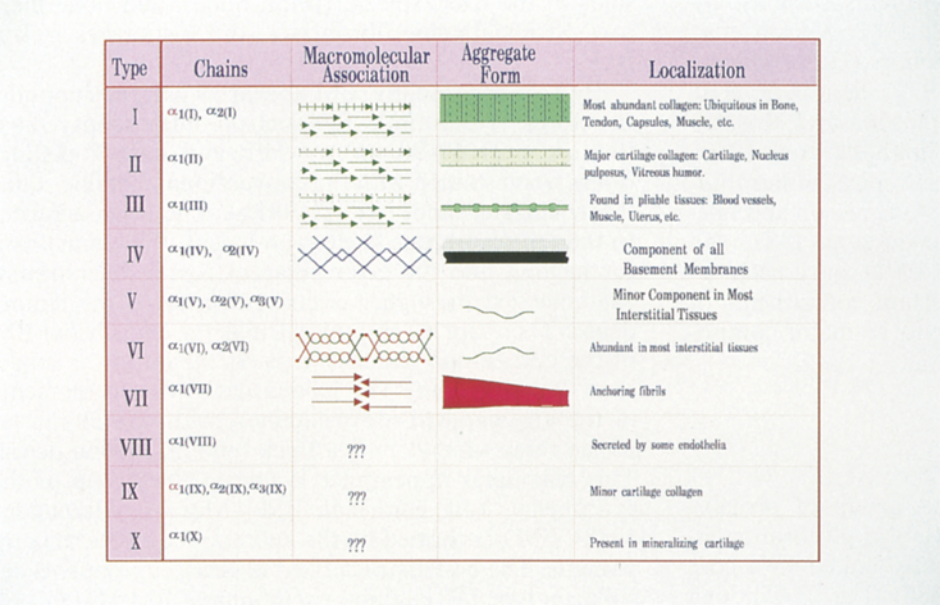
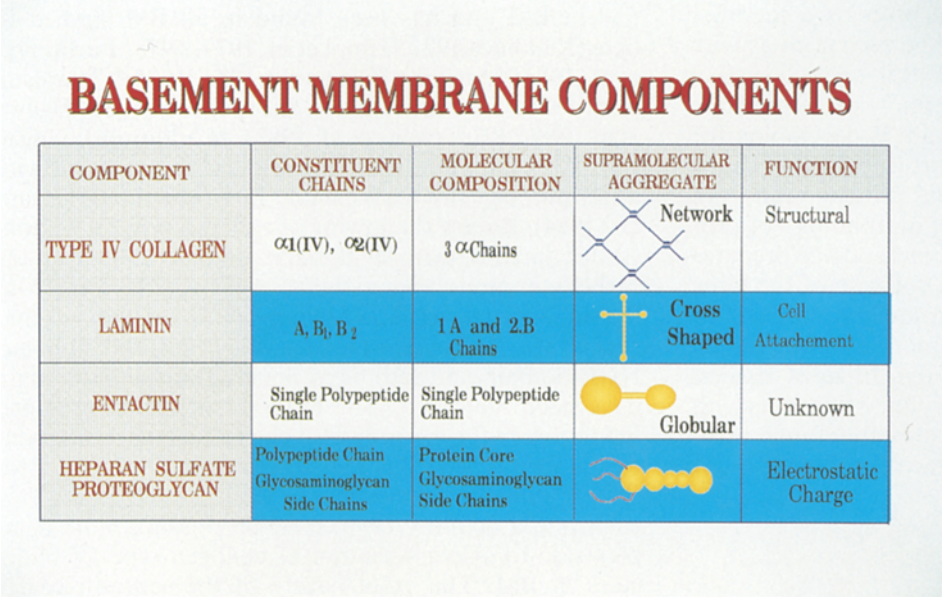


Table 2. Diagram of the composition, associations, and known functions of four basement membrane components



Ruoslahti et al. 1979, 1981; Wartiovaara et al. 1979; Hahn et al. 1980; Boselli et al. 1981; Amenta et al. 1983, 1986, 1988; D'Ardenne et al. 1983, 1984; Rosenkrans et al. 1983a, b; Fleischmajer and Timpl 1984; Gil and Martinez-Hernandez 1984; Miyakawa et al. 1985; Torikata et al. 1985; Rojkind and Kershenobich 1986). In most tissues, FN appears as granular aggregates or thin filaments often associated with collagen fibres or with the interstitial aspect of BM. The immediate proximity of FN to BM makes resolution of these structures by light microscopy almost impossible. Furthermore, since BM have prominent filtration properties, and FN is abundant in plasma, it is not uncommon to find some FN trapped in BM. Nevertheless, when tissues are perfused with physiological buffers prior to fixation (washing away substances trapped in BM), and FN localized by electron microscopy, it is found in the interstitium, but not in BM (Courtoy et al. 1980; Boselli et al. 1981; Martinez-Hernandez et al. 1981a; Amenta et al. 1983; Courtoy and Boyles 1983). By virtue of its multiple bind-

ing domains (Fig. 1) FN interacts with cells and a variety of molecules.

Several non-collagenous glycoproteins such as, osteonectin (SPARC) (Termine et al. 1981; Mason et al. 1986; Schulz et al. 1988), osteopontin (Oldberg et al. 1986), elastin (Damiano et al. 1979; Sandberg et al. 1981; Cleary and Gibson 1983; Gosline and Rosenbloom 1984; Sakai et al. 1986), fibrillin (Sakai et al. 1986), osteocalcin (Hauscha et al. 1975), and tenascin/hexabrachion (Chiquet and Fambrough 1984; Erickson and Iglesias 1984; Mason et al. 1986; Mackie et al. 1987; van Eyken et al. 1990; Ramadori et al. 1991), have been described and characterized as important components of the ECM; but they do not appear to be major components of the hepatic ECM.

Proteoglycans

Proteoglycans are a heterogeneous group of proteins containing a variable number (1–100) of glycosaminoglycan (GAGs) side chains, covalently linked to a core protein (Iozzo 1984; Ruoslahti 1988) (Fig. 2). Though the respective core proteins are the product of unique genes, the GAGs are often the same and in the past imparted their name to the proteoglycan. For example, the major proteoglycan of cartilage was called chondroitin sulphate proteoglycan. However, when the same GAG is present on different core proteins a modifier such as cell surface, or BM should be used such as with heparan sulphate proteoglycan (HSPG), i.e., cell surface versus BM-HSPG (Heinegard and Paulsson 1984; Stow et al. 1985a, b). A new nomenclature is developing for these molecules; for instance cell surface HSPG is called syndecan, BM-HSPG is perlecan. proteoglycans are widely distributed in all ECM. The morphology of proteoglycans is highly dependant on methods of preparation. Tissue dehydration results in collapse of the large hydrophilic domains, therefore the images obtained by electron microscopy represent a fraction of the actual space occupied by the proteoglycans. In most tissues, ECM proteoglycans appear as convoluted filaments with abundant lateral projections. They are often found associated with collagen fibres, cell surfaces, and other ECM components.

Basement membranes

BMs are ubiquitous, specialized ECMs, located at the boundary between cells and adjacent stroma (Pierce and Nakane 1969; Kefalides et al. 1979; Kuehn et al. 1982; Martinez-Hernandez and Amenta 1983). BM separate epithelial cells of most organs (but not hepatocytes), such as the GI tract, glands, and epidermis from the adjacent connective tissues, as well as individually surrounding many mesenchymal cells, i.e., lipocytes, skeletal, smooth, and cardiac muscle cells. They also separate epithelial and endothelial cells, such as in the glomerulus and alveolus. The central nervous system has only vascular BM; whereas, in the peripheral nervous system Schwann cells are encased by BM. All endothelial cells are lined by a continuous BM, except those of the sinusoids in the liver, spleen, lymph nodes, and bone marrow. Synovial cells, fibroblasts, and histiocytes lack a BM.

By light microscopy, BM appear as pale, eosinophilic structures (1–1.5 mm). By electron microscopy, most BM are only 60–90 nm in width and have 2 distinct layers when stained with the conventional metallic stains (Martinez-Hernandez et al. 1981b). The layer adjacent to the cell membrane is electron lucent, the lamina rara (or lucida), and the layer adjacent to the interstitium, characterized by higher electron density, is the lamina densa. There are exceptions, such as the endothelial BM of the cornea and the lens capsule, which has a single, thick lamina densa. The glomerular BM and segments of the alveolar BM are trilaminar, with two 30 nm laminae rarae sandwiching a thick (60 nm) lamina densa. This trilaminar appearance is due to the fusion of the endothelial and epithelial BM (Martinez-Hernandez 1978). BM are formed by the interaction of several components. The best characterized of these components are collagen type IV, laminin, entactin, and BM-HSPG.

Collagen type IV. The presence of a collagenous component in BM was first demonstrated by immunological methods and by X-ray diffraction (Pierce and Nakane 1967; Lee et al. 1969). Collagen type IV was first identified in BM and has been found in all BM studied to date (Kefalides 1971; Timpl et al. 1977, 1978; Furthmayr et al. 1983; Martinez-Hernandez 1984, 1985a; Haralson et al. 1985; Timpl et al. 1985; Kleppel et al. 1986; Ogawa et al. 1986; Karkavelas et al. 1988). In addition, collagen type IV is found in hepatic sinusoids, unassociated with a morphologically recognizable BM (Martinez-Hernandez 1984). Rotary shadowing of collagen type IV demonstrates threads, with a globular domain (NC1) at the carboxyterminus and a tightly helical, 7S, domain at the aminoterminus. The globular NC2 domain is adjacent to the 7S domain. Dimers attached at the globular, NC1, domain and tetramers centered at the 7S domain have been identified. The latter associations provided the rationale for the proposed chicken wire arrangement of collagen type IV, as the structural backbone of BM. Recent studies (Yurchenco and Ruben 1987, 1988; Yurchenco and Schittny 1990) have proposed a more complex side to side association of collagen type IV molecules in BM. The exact nature of the supramolecular aggregates of collagen type IV in Disse's space is not presently known.

Laminins. Laminin (800 kDa) is a cruciform macromolecule, being the most abundant and best characterized non-collagenous BM glycoprotein (Kleinman et al. 1985; Yurchenco and Schittny 1990). SDS-PAGE reveals that laminin consists of three chains, the A chain 400 kDa, B1, and B2 chains each 200 kDa (Ohno et al. 1983, 1985; Barlow et al. 1984; Timpl and Dziadek 1986). Laminin has been present in all BM studied to date, with preferential distribution in the lamina rara (Hahn et al. 1980; Courtoy et al. 1982; Ekblom et al.

1982; Martinez-Hernandez et al. 1982b; Engvall et al. 1983; Barlow et al. 1984; Gil and Martinez-Hernandez 1984; Martinez-Hernandez and Chung 1984; Wan et al. 1984; Mark and Kuhl 1985; Martin and Timpl 1987; Karkavelas et al. 1988). Recently, it has become apparent that EHS laminin is only one member of a family of heterotrimeric proteins. Four isotypes have been identified B1-chain containing laminin (A-B1-B2), S chain containing laminin (A-S-B2), B1-containing merosin (M-B1-B2) and S-containing merosin (M-S-B2) (Engvall et al. 1990). The distribution of these isotypes has been determined in normal adult, developing, and regenerating liver (Wewer et al. 1992). The roles of these isotypic forms remains undefined.

Entactin. Entactin/nidogen (150 kDa) was originally isolated from cell cultures of a murine teratocarcinoma (Carlin et al. 1981). Also referred to as nidogen, entactin is a highly sulphated, dumbell-shaped glycoprotein restricted to BM (Chung 1977; Martinez-Hernandez and Chung 1984; Horiguchi et al. 1988, 1989). It is known to interact at one globular domain with the center of the laminin cross (Martin and Timpl 1987). The functions of entactin remain a unclear; however, some postulate that by its interaction with the cell attachment site of laminin, it may modify the latter cell binding properties (Martin and Timpl 1987). In a recent report, entactin could not be detected in Disse's space (Wewer et al. 1992).

The major characteristics of these four BM components are summarized in Table 2.

Although for practical reasons we discuss the ECM components individually, we should keep in mind that the ECM in any organ is a single functional unit with multiple interactions and associations.

Hepatic distribution of the extracellular matrix in adult liver

Even in humans, whose liver has a higher ECM content than most mammals (Popper and Udenfriend 1970), the ECM constitutes only a minor part of the normal liver. Nevertheless, the hepatic ECM has received considerable attention because of its prominent role in fibrosis and cirrhosis. To discuss the ECM distribution it is convenient to divide the liver into four major compartments: capsule, portal spaces, lobular interstitium (subsinusoidal space or space of Disse), and central space.

Capsule. The normal liver capsule is a thin, semi-transparent membrane, containing collagen types I, III, V, and VI, FN, and probably proteoglycans, although the latter have not been specifically demonstrated. The basic structural plan is that of a single layer of mesothelial cells resting on a delicate BM containing laminin, entactin, collagen type IV, and perlecan. Beneath this BM there is a matrix formed by cross-banded, collagen type I fibres as a primary scaffold, upon which are inserted thin fibres and filaments of collagen types III, V, VI, and FN. Finger-like projections of the capsular ECM are in continuity with the lobular ECM.

Portal spaces. The portal spaces contain bile ductules, hepatic artery and portal vein radicles embedded in an ECM. The epithelial cells of the bile ducts are separated from the surrounding parenchyma by a delicate (30 nm) BM. From studies on injury, regeneration, and ontogenesis (Martinez-Hernandez 1985a, b; Martinez-Hernandez et al. 1991), it appears that ductal epithelial cells are responsible for the synthesis and secretion of their own BM components.

The hepatic artery and portal vein radicles, like the vessels of similar calibre in other organs, contain BM and other ECM components. Occasionally, nerves with Schwann cells and BM are present in portal spaces (Martinez-Hernandez 1984). The ECM of portal spaces contains collagen types I, III, V, VI, FN, and elastic fibres. Cross-banded fibres of collagen type I from portal spaces are in continuity with similar fibres in the immediately adjacent lobular interstitium, that in turn are in continuity with those in central spaces. In this manner, collagen type I fibres and bundles (together with connecting elements), form the structural scaffold of the liver lobule (Martinez-Hernandez 1984). The distribution of ECM components in portal triads is summarized in Fig. 3.

Lobules. In the hepatic lobule there are sinusoids, an almost imperceptible (by light microscopy) interstitial space (of Disse or subsinusoidal space), and hepatocyte cords. In order to describe the lobular ECM and its changes in pathological conditions, it is convenient to discuss the relationship among these three lobular components.

a) Sinusoids. The small vessels interposed between the arterial and venous circulation are called capillaries. The capillary wall consists of endothelial cells, occasional pericytes, and BM. According to the characteristics of the endothelial cells and their BM, capillaries are classified (Simionescu and Simionescu 1988) into: continuous; fenestrated; and discontinuous or sinusoids (Fig. 4). Continuous capillaries have uninterrupted endothelium with overlapping cytoplasmic tongues, specialized plasmalemmal junctions, a continuous BM, and occasional pericytes embedded in the BM. In fenestrated capillaries the endothelium has several transcellular openings called fenestrae, each fenestra being closed by a single-layered unit membrane. Nevertheless, the endothelium of fenestrated capillaries, still has overlapping cytoplasm, specialized junctions, and a continuous BM. A sub-type of fenestrated capillary is found in the renal glomerulus, where the fenestrae lack diaphragms (open fenestrations), but a continuous BM (double the usual thickness) is present (Martinez-Hernandez et al. 1981a; Martinez-Hernandez and Amenta 1983; Mynderse et al. 1983; Martinez-Hernandez and Chung 1984). Sinusoids (discontinuous capillaries) are characterized by the presence of large (up to 300 nm) cytoplasmic fenestrae, without intervening diaphragms, gaps between endothelial cells (therefore no specialized plasmalemmal junctions), and the absence of a BM. In general, continuous capillaries (the most abundant type) are the least permeable,

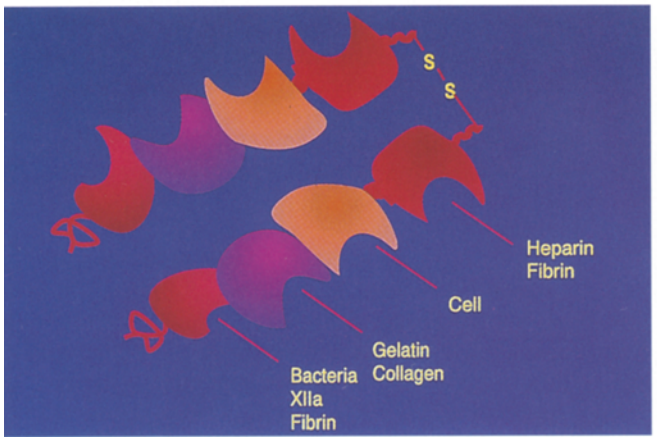


Fig. 1. Diagram of fibronectin molecule. Two identical chains (in the case of plasma fibronectin) are held together by disulphide bonds. Several domains in the chain contain binding sites for extracellular matrix (ECM) molecules, cell surfaces, DNA, and bacteria

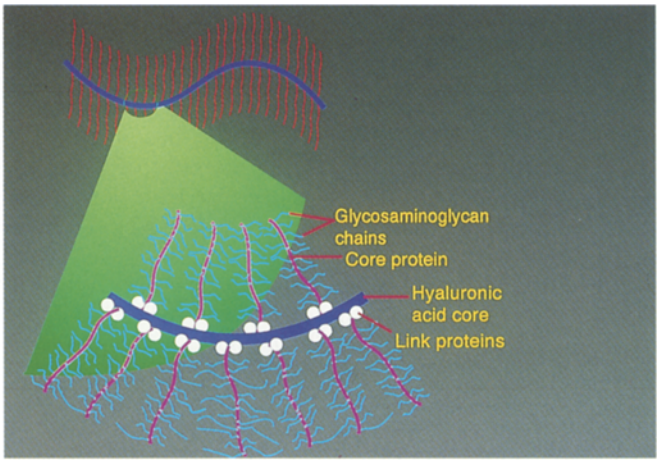


Fig. 2. Diagram of a typical proteoglycan. Attached to a central core of hyaluronic acid there are side chains. These chains consist of a core protein with a variable number of glycosaminoglycan side chains. The proteoglycan chains are bound to hyaluronic acid by link proteins

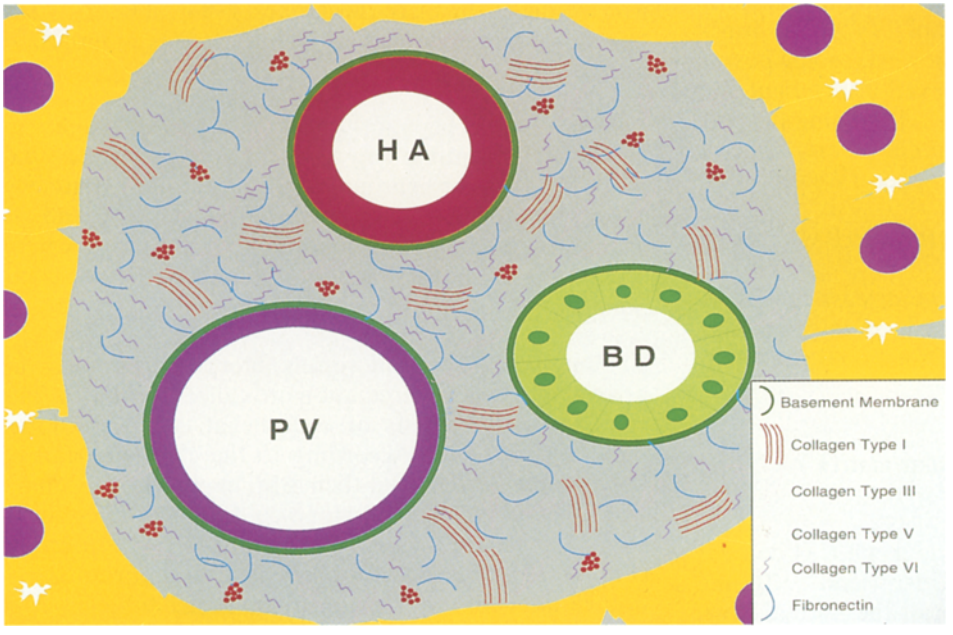


Fig. 3. The portal area usually contains a hepatic arteriole, portal venule, and bile ductule. Each of these structures is encased by a typical basement membrane containing: laminin, collagen type IV, entactin, and heparan sulphate proteoglycan (HSPG). The surrounding portal interstitium contains collagen types I, III, V, and VI, fibronectin and tenascin

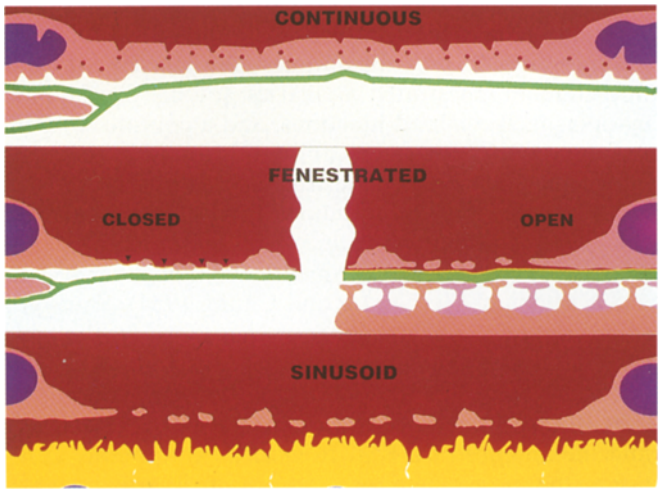


Fig. 4. Diagram of the major capillary types. Continuous capillaries are characterized by a continuous endothelium with specialized junctions, a continuous basement membrane and occasional pericytes. They are the most abundant capillaries in the body. Fenestrated (closed) capillaries have endothelial cells with cytoplasmic attenuations (fenestrae) closed by a single unit membrane (diaphragm). They have a continuous basement membrane (BM) and occasional pericytes. Fenestrated capillaries are found in endocrine organs, gastrointestinal tract mucosa, and renal interstitium. A variant of fenestrated capillaries (*open*) is found in the renal glomerulus where the endothelial cells lack diaphragms. They also have a continuous BM. Sinusoids have fenestrated endothelium, without diaphragms, and without BM. They are found in the liver, bond marrow, and lymphoid organs.

fenestrated are intermediate, and sinusoids have maximal permeability. In the adult, sinusoids are present only in haematopoietic, lymphoid organs, and the liver (Simionescu and Simionescu 1988). The hepatic sinusoid has abundant (up to 40% of the sinusoidal surface) endothelial fenestrations, these fenestrations, together with the gaps between endothelial cells, and the lack of BM make the hepatic sinusoid ideally suited to facilitate the bidirectional exchange of macromolecules normally taking place between plasma and hepatocytes. Unlike most epithelial cells, hepatocytes lack BM; therefore, the liver is unique among epithelial organs in lacking any continuous barrier (neither endothelium nor BM) between the plasma space and the epithelial cell surface.

b) Space of Disse. This minute (<1 μm) space between endothelial cells and hepatocytes has received considerable attention. Strictly speaking, it represents the hepatic interstitium and therefore, all molecules exchanged between plasma and hepatocytes must traverse this space. When studied by conventional electron microscopy, it not only lacks BM, but appears almost devoid of ECM except for some cross-banded collagen fibres and occasional, poorly defined filaments. A different image is obtained by immunohistochemistry: Thick, cross-banded, collagen type I fibres can be found at any point between the portal space and central veins. Small bundles (8–12 fibres) tend to be present at points of inflection, where the liver cell cords branch or change directions. In these locations, the collagen type I bundles are often found in a symmetrical distribution. The bundles are more prominent in subsinusoidal regions proximal to portal and central spaces. The continuity between the collagen type I bundles in portal, subsinusoidal, and central spaces can be compared to a system of cables providing physical support to the hepatic lobule.

In the space of Disse, the most abundant ECM component is FN. By light microsopy immunohistochemistry (5–7 μm sections), FN appears as an almost continuous lining along the full length of the hepatic sinusoids, from portal triads to central veins. By electron microscopy (60–80 nm sections) individual, discontinuous deposits of FN can be resolved. FN forms either granular or delicate filamentous structures on the surface of hepatocyte microvilli, coating collagen type I fibres, intermixed with other fibres and filaments (collagen types III, V, and VI), and is in contact with the interstitial surface of endothelial cells. It seems that, in addition to its multiple functions, FN connects the cell surface of endothelial cells and hepatocytes to the collagen type I bundles and in this manner transforms the different elements of the hepatic lobule into a single physical unit.

Collagen type III is not prevalent in the normal space of Disse. When present, it appears as thin, beaded, delicate fibres lacking any prominent striations. Collagen type III fibres are often found associated with cross-banded fibres of collagen type I and with FN. It tends to be more prominent in the proximity of portal and central regions.

The localization of collagen type V in the liver has been reported by light microscopy (Biempica et al. 1980; Schuppan et al. 1986), but not by electron microscopy. It has been described as irregular, discontinuous deposits along the space of Disse. Based on chemical analysis it does not seem to a major constituent of the hepatic ECM.

Collagen type VI is relatively abundant in the space of Disse forming thin filaments, often associated with cross-banded fibres of collagen type I, with beaded fibres of collagen type III and FN. Its distribution throughout the lobule is relatively homogeneous.

The distribution in the liver of the basement membrane components laminin, perlecan, and collagen type IV has been reported (Martinez-Hernandez 1984, 1985a, b; Stow et al. 1985a; Schuppan et al. 1986). Collagen type IV is found in the space of Disse as discrete, discontinuous, aggregates. These aggregates are associated neither with laminin nor with perlecan (Martinez-Hernandez 1985a; Stow et al. 1985a). The presence of collagen type IV deposits, unassociated with other BM components, is unique to the space of Disse. In every other organ studied, collagen type IV is found in morphologically recognizable BM associated with laminin, entactin, and perlecan. The reason for the presence of free collagen type IV, and its functional significance, remain unclear.

Some authors have reported the presence of some laminin along the space of Disse (Abrahamson and Caulfield 1985; Bissell et al. 1987), but others fail to detect this antigen in this space (Grimaud et al. 1980; Hahn et al. 1980; Carlsson et al. 1981; Sell and Ruoslahti 1982; Martinez-Hernandez 1984; Clement et al. 1986; Martinez-Hernandez et al. 1991). Our personal experience is that two polyclonal antibodies, one directed against intact laminin (extracted without protease digestion) of rat origin (Martinez-Hernandez et al. 1982b), the other directed against human laminin obtained after pepsin digestion (Heyl), and a monoclonal antibody (Wan et al. 1984) fail to demonstrate laminin in the normal space of Disse of livers processed with a variety of protocols (frozen, acetone-fixed; frozen, formaldehyde-fixed; and paraffin-embedded, formaldehyde-fixed with or without pepsin or glycosidase digestion). The same antibodies with all of the above protocols clearly demonstrate laminin in all BM in portal areas and central veins. Recently (Wewer et al. 1992), the expression of the laminin A, B1, B2, S, and M chains has been examined in the liver. In the adult rat, laminin was present as short streaks radiating from the portal tracts and central regions. Rarely laminin was also present in the perisinusoidal space as focal deposits. Vascular and biliary BM at all ages contained B1, B2, and S-laminin domains. In contrast, the A chain was found only in larger blood vessels, but not in small vessels and biliary ductules.

Perlecan is not present in the space of Disse (Stow et al. 1985a), although a HSPG of lower molecular weight (syndecan) is abundant in the sinusoidal aspect of the hepatocyte cell membrane (Stow et al. 1985a). The absence of perlecan is in agreement with the lack of laminin and of a recognizable BM in the space of Disse. Entactin is not demonstrable in the hepatic sinu-

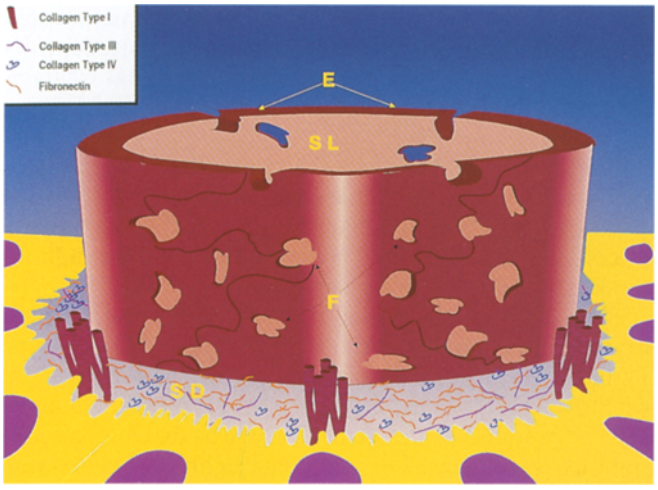


Fig. 5. Schematic diagram of a typical hepatic sinusoid and the ECM in Disse's space. Collagen type I forms a network of "cables" within the hepatic perisinusoidal space. The remaining components are present as discontinuous deposits around the collagen fibres. The endothelial cells forming the sinusoid have abundant fenestrations and lack BM

soidal space. The sinusoidal structure and ECM distribution in Disse's space is summarized in Fig. 5.

c) Hepatocytes. All epithelial cells in the organism are separated from the interstitium by a BM. Hepatocytes are the exception to this rule, they lack a BM separating them from the interstitial space. FN, in particular, and other ECM components can be seen in close apposition to the hepatocyte microvilli (Martinez-Hernandez 1984).

Central space. Central veins (terminal hepatic veins) consist of an endothelial monolayer resting on a delicate BM containing laminin, collagen type IV, and perlecan (Martinez-Hernandez 1984; Stow et al. 1985a). Outside of this BM, there are fibres of collagen types I, III, VI, sporadic filaments of collagen type V and abundant FN, this central matrix imperceptibly blends with the matrix of the space of Disse resulting in a continuity of the lobular ECM.

It seems that the hepatic ECM in the capsule, portal triads, and central veins is comparable to the ECM in other epithelial organs such as kidney, lung, etc. The uniqueness of the hepatic ECM resides in the lobule, where the space of Disse lacks a BM and the major component of the tenuous ECM present in this space is FN. Clearly the lack of any continuous filtration barriers (fenestrations and gaps in the endothelial cells, and absence of BM) seems ideally suited to facilitate the bidirectional exchange of macromolecules constantly taking place between plasma and hepatocytes.

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