Equine Synovial Villi: Distinctive Structural Organization of Vasculature and Novel Nerve Endings

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ABSTRACT. The structural arrangement and cellular distribution of endothelial and lining cells of the synovial villi were studied in the equine palmar/plantar recess of the metacarpo- and metatarsophalangeal joints by light microscopy and electron microscopy. The extent and distribution of blood vessels varied with villous shape and length. The majority of vessels formed concentric circles in cross and longitudinal sections and probably are arranged in a convoluted, spiral or helical pattern. The villi do not contain smooth muscle cells or typical capillaries as observed in other organs. Under the electron microscope, the endothelium is surrounded by connective tissue and discontinuous circular cells, presumably fibroblasts. The outermost layer was sometimes surrounded by type A and/or B synovial cells. The lumen of the blood vessels at the top of villus appeared to be constricted in most cases, with a diameter of about 12 ± 3 μm. Blood vessels formed by more than six endothelial cells in the middle portion of villus generally were not constricted. Well-developed cytoplasmic processes extended into the lumen of blood vessels. The constriction of blood vessels with no apparent smooth muscle presence and the observation of numerous intermediate filaments in the cytoplasm of the endothelial cells suggests that these villous blood vessels consist through contraction of their own endothelial cells. Lining cells were distributed unevenly even within a single villus; the villous lining cells seemed to have directional preferences with domination of synovial type A cells. Surprisingly, structures resembling myelinated nerve ends (~0.2 μm) were observed between juxtaposed endothelial cells as well as directly on an endothelial cell, suggesting that these nerve endings may be a sensor detector of either pressure or temperature or have a proprioceptive-like function. Synovial villi have a distinctive structural arrangement of vessels, lining cells, and nerve endings. — KEY WORDS: blood vessel, equine synovium, nerve ending, synovial villi, TEM.


The synovium is the tissue layer that lines the inner surface of the articular capsule. It is usually smooth and glistening, and is formed into folds and villi in some regions [26, 42]. The interior structure of synovium consists of several types of synovial fibroblasts, loose connective tissue, fat deposits, blood vessels, lymphatic vessels and a nerve supply [3, 14, 25, 29, 42].

The synovium is highly vascularized. The vascular arrangement of the synovial tissue has been described as consisting of an innermost capillary layer, sub-synovial layer and fibrous layer by using light microscopy [15]. Arterioles that originate from the basal arteries extend to supply the villi [15]. A rich blood supply combined with the large surface area of the synovial villi supports the potential of the synovium for absorption and secretion [30]. Lindstrom and Brandemark [22] have described the presence of superficial anastomoses of synovial capillaries into a net-like or arcade arrangement; however, it is unclear whether these are located near the villi or in flat areas of the synovium. Vessels, such as arteriovenous anastomoses [37], arterioles or venules provide the source of synovial fluid that is as the major source of nutrition for the articular cartilage [1]. It has been hypothesized that the vessel proximity to cartilage may explain, in part, the central, focal nature of early degenerative joint disease in the articular surface [42].

Barland et al. [3] defined two synovial cell types: A cell (macrophage-like cell) and B cell (fibroblast-like cell) in the human synovium through electron microscopy. Since then, there have been many ultrastructural investigations of synovium, particularly of these two distinctive cells, in a variety of species [3, 14, 19, 29, 40, 42]. A systematic ultrastructural examination of the villous arrangement of the synovium, however, has not been performed, and villi may differ from flat areas of synovium. The villous arrangement may be very important to understanding the functional structure of synovium.

A large number of somatic nerves are reported to enter the synovium and terminate in nerve loops, globular extensions of simple, specialized nerve endings [9, 10, 35, 38], and nerves staining positive for substance P have been demonstrated in equine synovium [28]. In this study, we report the histology and ultrastructure of blood vessels within the synovial villi in the palmar and plantar recesses of the equine metacarpo- and metatarsophalangeal joint.

MATERIALS AND METHODS

Synovial membranes from 4 palmar and 3 plantar recesses of the metacarpo- and metatarsophalangeal joints from 2 clinically healthy Standardbred horses were obtained immediately after euthanasia. They were prefixed in 2.5% glutaraldehyde (GA) solution consisting of 2.5% GA, 0.1% tannic acid, 100 mM K-phosphate (pH 7.2), 0.1 M sucrose,
1 mM NaN₃, for 2 hr at room temperature. Prefixed specimens were observed to determine the shape and distribution of villi under a dissecting microscope. Some of the villi were dissected from the base, rinsed in the Ringer's solution and post-fixed with 1% osmium tetroxide for 1-3 hr at room temperature. They were dehydrated in a graded series of ethanol (50%, 70%, 95%, and 100%) and 100% acetone. Specimens were embedded in Epon 812 consisting of 46.9% Medcast, 39.7% NMA, 11.5% DSSA, and 1.9% DMP-30. The specimens were oriented to obtain exact cross and longitudinal sections. The resin was polymerized at 5°C. The embedded blocks were trimmed under a dissecting microscope.

For light microscopy, longitudinal sections (1 µm) at the center of the villus and cross sections (1 µm) were taken exactly at the tip and 0.5 mm from the tip of the villus by using a superscript glass knife. Sections were stained with toluidine blue and observed under an inverted microscope. Photomicrographs of longitudinal sections of villi (n=15) were used for counting the number of synovial lining cells, other cells and cross sections of blood vessels in a 500 µm² area, number of blood vessels in longitudinal section, and the villous width at 0.25 and 0.5 mm from the tip of the villus (Table 1). Each parameter was measured at 4 sites per horse (n=8).

For electron microscopy, ultrathin sections (50 nm) were made at exactly the same positions as selected for light microscopic observation with an LKB ultratome V with a diamond knife. The sections were placed upon 400 mesh grids and double-stained with uranyl acetate (2% solution in H₂O) and with lead citrate. Magnifications ranging from × 3.4K to × 60.4 K were performed with a transmission electron microscope (Hitachi HU11-DS) with accelerating voltage at 75 kV. Electron micrographs were taken on Kodak electron image film. Subjective observations of cell type, matrix organization and blood vessel formation were made. The diameter (µm) of the blood vessels at the villous tip was measured at 10 locations within the section.

RESULTS

Gross distribution and shape of synovial villi: The distribution of synovial villi on the caudal wall of the palmar/plantar recess demonstrated four communities of villi, one above and below the concavity between the medial and lateral sesamoid bone, and one at each abaxial margin (Fig. 1-Top-rectangular area).

Four types of villi were distinguished on the basis of shape and length and were designated as bell type, tongue type, leaf type, and sword type (Fig. 1-bottom). An intermediate type, intermittently observed between the leaf type and the sword type, had a leaf shape villus with multiple secondary villi. The percentage of individual villus types depended on the area of synovial membrane. The sword type villus comprised the greatest percentage.

Light microscopy findings: The sword type villous structure under the light microscope varied depending on section orientation (Fig. 2). Numerous cross sections of blood vessels were observed in the subintimal layers from the base to the tip of longitudinal sections of most villi (Fig. 2a). Most vessels were circular in cross sections (Fig. 2b and c). The longitudinal section of blood vessels was unusual except at the narrow region at the base of the villus (Fig. 2a) (≤ 10 µm). No typical capillaries composed of a single endothelial cell were observed in the villi. The blood vessels of the tongue type villus (Fig. 3a) were not straight (i.e., sectioned longitudinally), but could be convoluted or helical.

At the surface of villi, cells which were heavily stained with toluidine blue formed several layers (Fig. 2). The
number of the cells in the layers increased from the bell type villi to sword type villi (Table 1). If two villi originated close to each other, the apposing sides had fewer cell layers than the outer sides. Bell type and tongue type villi, the cells of which had unstained nuclei, formed the layer near the base of villi.

Electron microscopy findings: The blood vessels at the tip of the sword type villus were comprised 3–5 endothelial cells and red blood cells in the lumen (Figs. 4 and 5). In most cases, the vessel was constricted. The diameter of these vessels was 12 ± 3 μm (n=10). Numerous number of intermediate filaments were observed in the cytoplasm of the endothelial cells (Fig. 6). Structures immediately adjacent to the endothelium contained loosely packed elastic fibers (EF) encircled by a discontinuous layer of thick (with a nucleus) and thin (cell process) cells, presumably fibroblasts. Various elongated cells were distributed unevenly among EF layers. Circularly (and sometimes randomly) oriented collagen fibers were observed in this outer layer of elongated cells. The blood vessels were surrounded peripherally by several types of synovial cells which had well-developed cell processes. Another larger type of blood vessel was located in the middle portion of the villus and was formed by more than 6 endothelial cells (Fig. 7). These vessels were in a non-constricted stage, and filled with serum and red blood cells. Structures surrounding these vessels were of the same composition as stated above, but with more cells. Endothelial cells in both types of blood vessels were characterized by the presence of numerous rough endoplasmic reticula, mitochondria, and, uniquely, well developed cytoplasmic processes. The processes (projections) were obvious in the lumen of the blood vessel (Fig. 8).

Myelin-like structures were observed frequently between juxtaposed endothelial cells and/or directly on an endothelial cell (Fig. 5). They were approximately 0.2 μm in diameter. The myelinated sheath possessed a repeating light and electron-dense pattern with a periodicity of 10–18 nm (Fig. 9).

Several types of synovial cells were observed in the intimal layers. One was the type A synoviocyte rich in lysosomal particles, indicative of a phagocytic nature, with many cell processes (Fig. 10). The cell also had a well-developed Golgi apparatus. Another distinctive cell type identified was the type B synoviocyte, which possessed well-developed cell processes, with abundant mitochondria and rough endoplasmic reticulum (Fig. 11). We also observed a third cell type, an intermediate filament-rich cell with a large nucleus (Fig. 12). The cell does not possess many cell processes.

DISCUSSION

We have provided the first detailed description of the structure of the synovial villus using gross, light, and electron microscopy. The villi do not contain smooth muscle cells or typical capillaries as defined by a luminal diameter up to 8 μm, three or less endothelial cells and prominent pericytes [7, 27, 33]. The structure of these blood vessels is unique. Studies reported in the literature on the ultrastructure of the synovial membrane have not specified in the exact location of the source of the specimens. This could be critical since the basal cell bed (intravillous area) may be different from the villus. A needle biopsy was used for specimen collection and may be clinically useful [31], but restricted information is obtained. We demonstrated variation in villus structure both grossly and histologically that is location dependent and probably varies from joint to joint.

We demonstrated that synovial villi in the cul de sac region of the joint surface had several shapes. The location
and shape may be related to joint function and biomechanics. Villi range from short and thick villi (i.e., bell type) at the articular edge to long and thin villi (i.e., sword type) at a greater distance from the cartilage. Sword type villi comprised the most surface area of the synovial layer and were the most numerous. This may suggest that sword type villi function as the mature stage and are the most functional or provide the greatest surface area for synovial fluid production. If long villi were close to the cartilage, they might get pinched and traumatized. Others have described that synovial villous formation is a normal reaction of the synovial membrane to repeated trauma by the cartilage surface during joint movement [37].

In our study, blood vessels appeared as concentric circles in cross and longitudinal sections of villi. This suggests that the blood vessels in the villi have a nonlinear arrangement, possibly convoluted, spiral or helical. In another study, light microscopic evaluation showed that most individual villi contained an arterial trunk and that the afferent arteriole invading the villi formed glomeruli [15]. Spiral structure of the blood vessels in the villus results in a many-fold increase of surface area that may be needed for synovial fluid absorption and secretion as has also been described in the small intestine [5]. We speculate that this classic advanced structure assists in absorption of blood components, secretion of synovial fluid and thermoregulation.

The myelinated structure identified between endothelial cells may represent possible innervation. This location of a nerve structure has not been previously observed in synovial villi. This may be a reflection of species differences or sectioning. The villi in this study were chosen from the synovial membrane under a dissection microscope and therefore known to comprise solely the villus. Subsequently villous blood vessels were shown to be ultrastructurally distinct from others described, which contain smooth muscle cells and typical capillaries [39, 43]. Previous studies have described the ultrastructure of the basal bed layer, which
Table 1. Morphometrical analysis (mean ± SEM) of histological sections of equine synovial villi stained with toluidine blue. Each value indicates mean ± SE

<table>
<thead>
<tr>
<th>Number</th>
<th>Villus Type</th>
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<tbody>
<tr>
<td></td>
<td>Sword</td>
<td>Leaf</td>
<td>Tongue</td>
<td>Bell</td>
</tr>
<tr>
<td>Lining cells (per 500 μm²)</td>
<td>350.6 ± 67.6</td>
<td>126.5 ± 15.9</td>
<td>120.9 ± 9.3</td>
<td>107.9 ± 16.9</td>
</tr>
<tr>
<td>Other cells (per 500 μm²)</td>
<td>383.1 ± 117.8</td>
<td>241.8 ± 41.4</td>
<td>135.0 ± 15.9</td>
<td>155.0 ± 18.0</td>
</tr>
<tr>
<td>Blood vessels in cross section</td>
<td>7.4 ± 1.1</td>
<td>10.5 ± 3.4</td>
<td>8.6   ± 0.9</td>
<td>10.6 ± 0.6</td>
</tr>
<tr>
<td>Blood vessels in longitudinal section</td>
<td>3.8 ± 1.1</td>
<td>6.3 ± 1.2</td>
<td>3.5   ± 0.9</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>Width of villus 0.25 mm from tip</td>
<td>4.0 ± 0.9</td>
<td>5.3 ± 0.4</td>
<td>5.1   ± 0.3</td>
<td>5.7 ± 0.9</td>
</tr>
<tr>
<td>Width of villus 0.5 mm from tip</td>
<td>4.8 ± 1.2</td>
<td>8.6 ± 0.7</td>
<td>7.4   ± 0.4</td>
<td>8.5 ± 2.0</td>
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Fig. 4. Electron micrograph of cross section of vessel in tip of villus. Endothelial cells (3–5) are packed together with part of a red blood cell in the lumen. Loosely packed elastic fibers (EF) are attached to the outer layer of the endothelium. The EF layer is encircled by a discontinuous layer of scattered thick (with nucleus) and thin (cell process) cells, presumably fibroblasts. Various types of elongated cells are distributed nonuniformly among EF layers. Circularly (and sometimes randomly) oriented collagen fibers are observed also in the outer layer of the elongated cells. The blood vessel is surrounded finally by several types of cells with well-developed processes, presumably synovial cells. The diameter of the cross section through endothelium is approximately 12 μm (Mag. x 5,700).

may not be representative of villi. These differences may be species related or location related, but were regularly identified.

Our study showed that the villi seem to lack the smooth muscle cells associated with contraction of the blood vessel and yet many small vessels were in a constricted state. This suggests that the constriction of blood vessels might have been controlled by the endothelial cells.
Fig. 5. The cross section of the blood vessel which is constituted of 5–6 endothelial cells and with a highly contracted lumen. The structural arrangement of the cells and connective tissue fibers surrounding the endothelial cells are similar to Fig. 4. Myelin-like structure closely associates with an endothelial cell in the villi (arrow). The outermost cells are a mixture of type A and B cells (Mag. × 4,000).

Fig. 6. In a higher magnification of blood vessel with a highly contracted lumen, the presence of numerous rough endoplasmic reticula and mitochondria reflect the high metabolic activity of the cells. The well-developed cytoprocesses (projections) are obvious in the lumen of the blood vessel, often observed as white empty holes. Numerous number of intermediate filaments occur in the cytoplasm (Inset: High magnification of the rectangular area, Mag. × 40,000). The endothelial cells possess several cell junctions including tight and gap junctions (Mag. × 12,000).
Postcapillary venules that are distinguished by tall endothelial cells have been reported in human rheumatoid synovium [20] but were not seen in joint villi [17, 18, 21]. The electron microscopic appearance of post capillary venules has been described in the lymphoid tissue, and lymphocytes emigrate from the blood into the paracortical region through postcapillary venules [8, 23].

The arrangement of endothelial cells in synovial villi was quite distinctive as compared to that in blood vessels of other organs. The lumen almost always appeared constricted at the tip of the villus but less constricted in the middle portion. Layers of unpacked elastic filaments, discontinuous circularly oriented cells or cell processes, and an outer layer containing circularly oriented collagen fibers and type A and/or B synovial cells characterized villi. This type of cell and connective tissue filament arrangement was not previously documented. The constricted blood vessels were formed by 4–6 endothelial cells at the tip of the villi, loosely surrounded by elastic fibers and a few discontinuous circular cells. Generally, the wall of blood vessels is composed of smooth muscle cells. The constriction of blood vessels is directly performed by the smooth muscle contraction under the regulation of autonomic nerves. However, the association of these components was not observed in the villous small vessels.

The discontinuous circular cells around the vessels were characterized by large nucleus, rich rough endoplasmic reticulum with numerous ribosomes, and many mitochondria. Dense bodies, which are a characteristic structure of smooth muscle cells and are an attaching place of actin myofilaments, were not identified at the electron microscopic level. These anatomical findings suggest that the discontinuous circular cells may be fibroblasts and are probably not smooth muscle cells. It is possible that these villous blood vessels are constricted by contraction of their own endothelial cells.

It is well documented that numerous blood vessels, particularly capillaries, are present in subintima of flat
Another cross section of the blood vessel in the area about 0.5 mm from tip of the villi. Some red blood cells and well-developed endothelial cells with distinctive projections are clearly distinguished. Ordinary endothelial cells and capillary endothelial cells do not possess such projections. Thus, the blood vessels in the synovial villi are very unique; we can classify the tissue as synovial villi-specific endothelium. The exact role and the functions of these projections are not clear (Mag. × 13,500).

synovial membrane. A more recent study, using indirect immunoperoxidase technique, showed a high capillary density was present within 25 μm of the normal human synovial surface and small venules were major components beneath the capillaries [43]. In our study of villi, no typical capillaries were observed. This suggests that the vascular tree of the villus may be formed by an arteriole, venule alone or by arteriovenous anastomoses. We also observed that the endothelial cells had numerous projections toward the lumen side which were similar to short and irregular microvilli of the epithelial cells of distal convoluted tubule and renal medulla in kidney [34]. Abnormal microvilli formation on the endothelial cell surface has been observed in nicotine-treated rabbit aorta [4], cryoinjured cerebral capillaries [41], Ultraviolet B-irradiated veins [24], pulmonary artery with congenital heart defects and pulmonary hypertension [32], cholinergic toxin-effected retinal capillaries [13], and the microvessels during ischemia/reperfusion injury [2]. We do not know the significance of this cell processing; however, the projections may be related to the fundamental role of the endothelial cells in synovial villi, such as absorption and metabolism of blood substances.

We observed three morphologically distinct cell types in the villi in addition to the endothelial cell and fibroblast-like cell which surrounded the endothelial cells. One cell type contained prominent Golgi complexes, indicative metabolically active, many vesicles and many vacuoles but little rough endoplasmic reticulum, probably a type A cell. Another cell type had well developed rough endoplasmic reticulum and poorly represented Golgi complexes, probably a type B cell. In equine synovial villi, the synovial cells were dominated by type A cells (macrophage-like cell) with fewer type B cells (fibroblast-like cell). Similar cells were distributed as surrounding the outermost layer of blood vessels and randomly located between the blood vessels. We do not know how the shape and distribution of lining cells in the villi affect synovial membrane function; however, the fact that the distribution of lining cells has polarity and that the cells are unevenly distributed suggests that the villi play a distinct role dependent on the position and the shape of villi. The third cell type has numerous
Fig. 9. This micrograph shows a myelin-like structure associated with synovial cell processes in the synovial villus. Myelin-like structures often appear between juxtaposed endothelial cells or directly on the endothelial cell. The myelinated sheath possesses a repeating light- and electron-dense pattern with periodicity of 5–18 nm. Mag. × 53,000.

intermediate filaments and a large nucleus and does not possess cell processes. The function of this type of cell is unknown, however, one possibility is that this type of cell may act as a reserve cell for endothelial or other cell types. It is considered to be distinctive from the lining cell or the endothelial cell of villi.

One of the most interesting observations of this study was the structures associated with myelin-like material which possessed a repeating light- and electron-dense pattern with a periodicity of 5–18 nm. These structures were often found on endothelial cells or between juxtaposed endothelial cells. Myelin sheath is situated within Schwann’s cells in peripheral nervous system or formed by cytoplasmic sheets of oligodendrocytes in central nervous system. It is composed of a variable number of concentrically wrapped series of myelin lamellae, appearing under transmission electron microscope as light and dark lines [16]. The periodicity of dark to dark is about 24–30 nm. Giles et al. [12] has described that the small myelinated nerves are demonstrated in association with some capillaries in the synovial folds of human lumbo-sacral zygapophyseal joint. Their reported nerve diameters (~0.2 μm) and myelin periodicity (~15 nm) are very similar to ours. The small myelinated nerves have been expected that might have clinical importance in joint pain [6, 11]. The localization of nerve endings in synovium in the present study has not been described before. These nerve endings may be a sensor detector of either pressure or temperature or have a proprioceptive-like function [36]. At high magnification, the myelin-like structure has a microvaricosity-like appearance which corresponds to the nerve ending structure identified in the histochemical sections. Although our findings may not directly prove that these structures are nerve endings, this is the first time that such structures were observed closely associated with endothelial cells of the synovial villi.

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Fig. 10. A type A cell rich in lysosomal particles, indicative of a phagocytic nature, is shown in a synovial villus. Such cells often have many cell processes. Numerous empty spaces are observed in the extracellular area. The cell seems to be metabolically active, with a well-developed Golgi apparatus. Mag. x 18,000.
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Fig. 11. The synovial villi also contain distinctive type B cells, rich in mitochondria and rough endoplasmic reticulum. The type B cell is referred to as a fibroblast-like cell. We observed a Golgi apparatus. Mag. × 18,000.


Abbreviations

Fig. 12. This intermediate filament-rich cell with a large nucleus is considered to be distinctive from the lining cell or the endothelial cell of villi. The cell does not possess cell processes, and the function of this type cell is not clear. One possibility is that this type of cell may play act as a reserve cell for endothelial and/or other cell types. Mag. × 23,000.