Three-dimensional Ultrastructure of Synoviocytes in the Horse Joint as Revealed by the Scanning Electron Microscope*

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Summary. The synovial membrane displays a superficial cellular lining composed of two types of synoviocytes: “absorptive” macrophages (type A cells) and “secretory” fibroblast-like cells (type B cells). The types are intermingled and extend a variety of processes, rendering the cellular architecture of the synovial membrane difficult to visualize. Previous electron microscopic and histochemical studies failed to demonstrate the entire shape of synoviocytes, except our immunohistochemical study for protein gene product 9.5 in the horse joint. The present SEM study is the first to demonstrate the three-dimensional ultrastructure of synoviocytes as well as their distribution in the synovial membrane, using macerated samples from the horse carpal joints. The equine synovial membrane was largely covered by conspicuously developed synovial villi. Type A synoviocytes were closely similar to macrophages in regard to surface structure, and showed uneven distribution with the densest occurrence around the tips of the synovial villi. In the basal half of villi, type B synoviocytes, which were situated in close proximity to the synovial cavity, projected thick processes horizontally and intertwined to form a regular network of processes on the synovial surface. Those in the upper half of the villi were located in the abluminal layers and protruded an antenna-like process into the joint cavity with tips covered with long microvilli, in addition to forming the superficial plexus of processes. Type B cells were also provided with fine, membranous extensions that tended to cover the surface of synovial intima. The meshwork of horizontal processes, the antenna-like processes, and the membranous processes imply advantages in not only secretion but also sensation and regulation of the barrier function in the synovial membrane.

The synovial intima contains two morphologically

different cell types: cells of macrophage lineage and fibroblast-like cells (KREY and COHEN, 1973; GRAABAEK, 1982, 1984; EDWARDS, 1994). The latter cells, called type B synoviocytes, are proper cells which secrete collagen (FELL et al., 1976), fibronectin (MATSUBARA et al., 1983; MAPP and REVELL, 1985), hyaluronan (CASTOR and CABRAL, 1989) and other proteoglycans into the interstitium and joint cavity. Transmission electron microscopic (TEM) studies have characterized type B cells as dendritic cells possessing abundant rough endoplasmic reticulum, distinguishable from subintimal fibroblasts (BARLAND et al., 1962; WYLLIE et al., 1964). They often display an epithelium-like arrangement, developing junctional devices between the adjacent synoviocytes and possessing fragmented basal lamina (GROTH, 1975), but are different from authentic epithelia. Several markers have been identified which have a degree of specificity for type B cells. These include the enzyme prolyl hydroxylase (WILKINSON et al., 1992) and uridine diphosphoglucose dehydrogenase, which are involved in collagen synthesis and hyaluronan synthesis, respectively (WILKINSON et al., 1992), and vascular cell adhesion molecule 1 (VCAM-1) (WILKINSON et al., 1993). However, these markers for type B synoviocytes when used in enzyme histochemistry and immunohistochemistry present problems in specificity and stainability, and are not satisfactory for revealing the detailed morphology including ultrastructure of the cells. In contrast, many marker substances and antibodies are available for histochemical detection of the macrophagic type A cells (FORRE et al., 1982; BURMESTER et al., 1983; HOGG et

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al., 1985; IZUMI et al., 1990; EDWARDS, 1994).

Our recent study demonstrated the selective localization of protein gene product (PGP) 9.5, a neuron-specific protein, in type B synoviocytes of the horse joint and showed that this protein is a useful cytochemical marker of the cells (KITAMURA et al., 1999). In subsequent observations, we noticed a more complicated shape of type B cells than that described in previous TEM studies. Scanning electron microscopy (SEM) is the most useful to analyze structures three-dimensionally, but previous SEM studies failed to visualize the whole cell shapes of synoviocytes (WOODWARD et al., 1969; SHIVELY and VAN SICKLE 1977; STEINBERG and HODDE, 1990; BREIT and KONIG, 1995; MURASHIGE et al., 1999). We report here the three-dimensional structure of type B synoviocytes as well as type A cells in the carpal joints of the horse as revealed by SEM observation of macerated samples and conventional TEM. The characteristic shapes of the cells suggest their active function such as secretion towards the joint cavity and sensation for signals from the joint cavity.

MATERIALS AND METHODS

Scanning electron microscopy (SEM)

One male and six female Thoroughbred horses without any abnormalities in the joint were used in this study. All experiments were performed under protocols following the Guidelines for Animal Experimentation, Graduate School of Veterinary Medicine, Hokkaido University. The animals were deeply anesthetized with both pentobarbital and thiopental sodium, and were sacrificed by bloodletting from the cervical artery.

The synovial membrane including the synovial villi was obtained from joint capsules in the carpal joints on both sides. The tissues were dissected into small pieces ($1 \times 1 \times 1$ cm) and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) overnight at 4°C.

The specimens were conductive-stained by the tannin-osmium method according to MURAKAMI (1974), and dehydrated through a graded series of ethanol. The tissue pieces were dipped in isoamyl acetate and critical-point dried using liquid carbon dioxide. The dried specimens were evaporation-coated with platinum-palladium and examined under a Hitachi S-4100 SEM (Tokyo, Japan).

Some fixed specimens were processed by a modification of the NaOH maceration method (TAKAHASHI-IWANAGA and FUJITA, 1986) before the conductive staining. The fixed specimens were rinsed in 0.1 M PB and subsequently placed in 6N NaOH at

60°C for 8–30 min. After the alkaline maceration, the tissue blocks were degraded into small fragments with a rigorous stream of 0.01 M PB (pH 7.2) ejaculated through a fine pipette. Subsequent processes were identical to the procedure mentioned above.

Transmission electron microscopy (TEM)

Fresh tissues of the synovial membrane from the carpal joints were dissected into small pieces ($2 \times 2 \times 2$ mm), fixed in 2.5% glutaraldehyde in 0.1 M PB for 6 h and post-fixed in 1% OsO₄ dissolved in distilled water for 1.5 h. They were dehydrated through a graded series of ethanol and embedded in Epon 812 according to the conventional method. Ultrathin sections were prepared by use of a diamond knife (Diatome, Switzerland) and stained with uranyl acetate and lead citrate for observation with a transmission electron microscope (H-7100; Hitachi, Tokyo, Japan).

RESULTS

The synovial membrane of horses, except at a junctional area between synovial membrane and articular cartilage, was largely covered by densely arranged villi (synovial villi), which were far longer than those in other mammals (Fig. 1, inset). The villi varied in length and thickness; longer ones were often branched in their tips. The two types of synoviocytes were lined—one to three cells deep—on the surface of the synovial membrane to form the synovial intima. The cellular lining was discontinuous, being especially fragmental in the basal region of the synovial villi. Because the synoviocytes were very low in density in the non-folded region of the synovial membrane, we focused on the villous regions in the present observation.

The nomenclature of synoviocytes varies according to authors. For macrophagic synoviocytes such names as type A cells, synovial macrophages, M cells and A (absorptive) cells have been given, whereas for fibroblast-like synoviocytes, type B cells, synovio-blasts, F cells and S (secretory) cells. Due to the lack of a general consensus, classical terms “type A and type B synoviocytes” were adopted in the present study.

Type A synoviocytes

Since type A synoviocytes faced the joint cavity, they were easily identified in the conventional specimens for SEM (Figs. 1, 2). They were spherical in shape and densely covered with filo- and lamellipodia, struc-

Fig. 1. SEM observation of a synovial villus in the horse carpal joint. Round cells, identifiable as type A synoviocytes, gather densely at the villous tip with deep indentations. The **inset** shows a bush of synovial villi that cover the synovial membrane. $\times 430$, inset: $\times 20$

Fig. 2. Higher magnification of the luminal surface at a villus tip. Type A synoviocytes (A) are spherical in shape and possess many lamellipodia and microvilli. The **arrow** shows a tuft of long microvilli extending from a type B cell. $\times 3,800$

Figs. 3 and 4. SEM views of the middle region of a synovial villus in macerated samples. The primary processes of type B synoviocytes extend radially and interlace to form a characteristic meshwork along the luminal surface. At a higher magnification (**Fig. 4**), their processes are frilled with membranous appendages and filopodia. Fig. 3: $\times 1,200$, Fig. 4: $\times 3,900$

Figs. 5-7. SEM and TEM images of processes of type B synoviocytes which protrude into the joint cavity. The rod-shaped processes extend vertically or obliquely from cell bodies in the deeper layer, whose tips are endowed with long microvilli. **Fig. 6.** An isolated type B synoviocyte that projects a vertical process with a microvillous crown. The TEM image (**Fig. 7**) demonstrates that the microvillous crowns (*arrows*) in the lumen correspond to the apical end of B cell processes. *A* type A cell, *B* type B cell, *C* joint cavity. Fig. 5: $\times 2,600$, Fig. 6: $\times 1,800$, Fig. 7: $\times 6,300$

Figs. 8 and 9. SEM and TEM images of membranous processes extending from type B synoviocytes. The membranous processes, flat and pleomorphic, develop particularly in the area where the microvillous crowns gather (arrows in **Fig. 8**) and cover the surface of the synovial intima (arrowheads in **Fig. 9**). An arrow in **Fig. 9** indicates a single cilium. C joint cavity. Fig. 9: $\times 6,500$, Fig. 10: $\times 11,000$

Fig. 10. Illustrations of type B synoviocytes in different portions of synovial villi. **a.** Type B cells in the basal region of synovial villi extend several rod-shaped processes radially along the surface of synovial intima. **b.** Type B cells, which exist close to the synovial surface in the middle region of the synovial villi, issue horizontal processes endowed with membranous processes. **c.** Type B cells in the upper half of the villi are located in a deeper layer of the synovial intima and extend a primary process with the microvillous crown towards the joint cavity. Some type B cells develop membranous processes covering the synovial surface.

tures unique to macrophages (Fig. 2). The density of type A synoviocytes on the surface of the synovial intima varied along the length of villi: the cells tended to gather in the upper region of the synovial villi (Fig. 1), while they were distributed sparsely in the middle region of the villi, with hardly any in the basal region of the villi. TEM observation of type A cells confirmed the rich existence of vacuoles and lysosomes throughout the cytoplasm as well as folds or projections on the luminal cell surface (KREY and COHEN, 1973; GRAABAEK, 1984).

Type B synoviocytes

Type B synoviocytes were constantly distributed along the entire extent of the synovial villi. The cells were mostly embedded in an amorphous or somewhat fibrous matrix, which obstructed the observation of their whole cell shapes under conventional SEM. In macerated samples, type B cells in the basal region of the villi revealed a round cell body and issued several long, rod-shaped cytoplasmic processes radially along the surface of the synovial intima (Fig. 10 a). The horizontal processes crossed and overlapped with each other to form a characteristic meshwork

on the luminal surface (Figs. 3, 4). The cell bodies and cytoplasmic processes were essentially smooth, except for sparse microvilli. Underlying subintimal tissue contained slender cells, which extended thin and tapered processes without any regularity. Considering their location and shape, these cells were identifiable as fibroblasts, apparently different from type B synoviocytes. In the upper region of the synovial villi, type B synoviocytes existed in the deeper layer of the synovial intima and extended an apical process vertically or obliquely towards the joint cavity (Fig. 10 c); the process ramified near the joint cavity, forming a network similar to that seen in the basal region of the villi. The vertical or oblique processes frequently protruded into the joint cavity like antennae, and their tips exposed to the cavity were covered with a tuft of long microvilli, displaying a unique microvillous crown (Figs. 5-8). Such microvillous crowns appeared as a group in the upper region of the villi (Fig. 5). In the middle region of the villi, two areas showing the different morphologies of type B cells mentioned above could be identified; the location of the boundary between the two areas markedly differed among various villi.

Membranous processes of type B synoviocytes

Type B synoviocytes possessed membranous processes extending from both their cell bodies and rod-shaped primary processes (Figs. 8, 10 b, c). The membranous processes were flat and pleomorphic, having a few filopodia. They were generally 2–4 μm in size but occasionally enlarged to be bigger than the cell body (over 10 μm). Membranous processes of various sizes were scarce in the basal region of synovial villi, and gathered in the middle and upper regions of the villi (Fig. 8). They formed in total a flat sheet covering the surface of the synovial intima, though the covering was still fragmental. TEM observation showed that the membranous processes, frequently interdigitating with each other, covered the synovial surface to form an apparent limiting margin (Fig. 9).

Distribution pattern of type A and type B synoviocytes in synovial villi

The synovial villi were distinguished into three areas due to the distribution pattern of two types of synoviocytes: 1) the area covered predominantly by type A synoviocytes (Fig. 2); 2) the area rich in the microvillous crowns of type B cells (Fig. 5); and 3) the area occupied by a meshwork of rod-shaped processes extending from type B cells (Fig. 3).

The first area was not always recognizable; if present, it occupied the tips of synovial villi, in which a small number of microvillous crowns of type B cells protruded among type A cells. The second area was seen in the upper and middle regions of the synovial villi: type B cells were lined in the deeper layer of synovial intima showing an epithelium-like arrangement. The antenna-like processes of type B cells were densely distributed, intermingling with a small number of type A cells. The third area appeared chiefly in the basal region of synovial villi: type B cells with horizontal processes ranged at lower degree of density, and type A cells were absent. It is worth to note that extents of these three areas varied from villus to villus; in some villi the second area made up essentially the entire synovial villus.

DISCUSSION

Unique structures of type B synoviocytes revealed by SEM observation

Fibroblast-like type B synoviocytes are proper and fixed cells in the synovial intima (KREY and COHEN, 1973), in contrast to type A cells which are originated

from macrophages (DREHER, 1982; EDWARDS and WILLOUGHBY, 1982; BROKER et al., 1990). Detailed morphology of type B cells as well as their origin and pathological changes remain unclear, due to a lack of specific staining methods. Nevertheless, previous histochemical and electron microscopic studies have noted their irregular, frequently dendritic shape in various mammals (BARLAND et al., 1962; WYLLIE et al., 1964; FELL et al., 1976; LINCK and PORTE, 1978; NISHIJIMA, 1981; GRAABAEK, 1982, 1984; WILKINSON et al., 1992, 1993). BARLAND et al. (1962) who observed synoviocytes in the human joint by TEM presented a three-dimensional drawing of synoviocytes, in which the cells projected long cytoplasmic processes overlapping with those from other cells, thus forming a plexus of processes in the synovial intima. This image was partially realized by our recent immunohistochemical study for PGP 9.5 in the horse joint (KITAMURA et al., 1999).

On the other hand, SEM is one of the most powerful tools for the three-dimensional viewing of whole cell, being especially effective for complicated structures. However, previous SEM studies on the synovial membrane were restricted to viewing the synovial surface (WOODWARD et al., 1969; SHIVELY and VAN SICKLE 1977; STEINBERG and HODDGE, 1990; BREIT and KONIG, 1995; MURASHIGE et al., 1999). In order to obtain the SEM images of cells embedded in the tissue, researchers have developed maceration techniques that remove the intercellular matrix by use of enzymes or alkaline solutions. By using such macerated samples, we first tried in the present paper to demonstrate the entire shape of type B synoviocytes and successfully revealed: 1) a prominent surface meshwork of cytoplasmic processes in the synovial intima, 2) antenna-like microvillous crowns projecting into the joint cavity, and 3) membranous processes covering the synovial surface.

Functional significance of processes issued from type B synoviocytes

Functional significances of the network composed of synoviocyte processes on the synovial surface are unclear. Since type B synoviocytes are engaged in the secretion of hyaluronate, glycoproteins and other components into the synovial fluid (LINCK and PORTE, 1978; OKADA et al., 1981; EDWARDS, 1994), their contact with the synovial cavity via the broad cell surface may be for the effective exertion of this function. However, the processes, as revealed by the present study, are too complicatedly and elaborately organized to account for the simple secretion of those substances into the joint cavity. We therefore would

like to propose a sensor function of synoviocytes to monitor certain mechanical and chemical conditions of the joint cavity, such as pressure, viscosity, and change of chemical composition.

PGP 9.5, a neuronal marker, is shared by several paraneuronal sensory cells including gustatory cells, olfactory receptor cells, and Merkel cells (IWANAGA et al., 1992). The selective existence of PGP 9.5 in type B synoviocytes of the horse joint (KITAMURA et al., 1999) may support the idea that they might also be involved in a sensory function, although the cells in question could not be categorized as neuron-related forms. Rodent's type B synoviocytes have been known to contain secretory granules in the cytoplasm and it was observed that they were released in response to stimuli noxious to the synovial membrane (LINCK and PORTE, 1981). Gut endocrine cells, which also contain PGP 9.5, essentially reach the apical cytoplasm to the lumen and have been designated as open-type cells (FUJITA and KOBAYASHI, 1977). Tips of the processes are endowed with a characteristic tuft of microvilli, functioning as a receptor site for chemical information in the gut lumen (FUJITA and KOBAYASHI, 1977; HASHIMOTO et al., 1999). The present study was able to demonstrate the antenna-like processes of the type B synoviocytes that protruded into the joint cavity and were covered by long microvilli, closely similar to the open-type gut endocrine cells. Possible sensation by the synoviocytes is not transmitted to the brain, due to a lack of synaptic contact with sensory neurons (our unpublished data). It is suggested that, in response to conditions in the joint cavity, the synoviocytes may autoregulate, via a paracrine fashion, the secretion of components into the intimal matrix and joint cavity.

Leaf-like processes are structures first demonstrated by the present SEM and TEM observation. The membranous processes tend to cover the surface of the synovial intima and demarcate it against the joint cavity, being reminiscent of the glial limiting membrane composed of astrocytes' processes. The synovial fluid is largely derived from blood vessels in the synovial membrane, but proteins in the synovia are quite different from those in the blood plasma (CURTISS, 1964), suggesting the existence of a blood-synovial barrier. BARLAND et al. (1962) considered that the passage of proteins and smaller molecules from capillaries to the joint cavity is influenced by a physical barrier of the overlapping cytoplasmic processes on the synovial intima. It appears more likely that the synovial limiting margin composed of membranous processes may function as cellular elements of the blood-synovial barrier, rather than the coarse

network of rod-shaped processes of BARLAND et al. (1962).

Morphology and function of type A synoviocytes

Surface structure of type A synoviocytes, as revealed by the present SEM observation, is identical to typical macrophages: round cells characterized by surface ruffles or lamellipodia. Type A cells are known to be immunoreactive to several monoclonal antibodies against macrophages and macrophage-derived substances (THEOFILOPOULOS et al., 1980; BROKER et al., 1990; IZUMI et al., 1990). The macrophagic origin of type A cells is supported by the fact that in macrophage-deficient (osteopetrotic op/op) mice, this type of synoviocyte conspicuously decreased in number (NAITO et al., 1991). Type A cells are active in the uptake of foreign substances injected into the joint cavity (BALL et al., 1964; GRAABAEK, 1982, 1985). Physiologically, they may absorb and degrade, by a well-developed lysosomal system, extracellular constituents, cell debris, microorganisms, and antigens in the synovial fluid and intimal matrix. The present observations seem to support the general view that type A synoviocytes play a role in maintaining normal joint homeostasis including the turnover of components in the synovial fluid, and also regulating inflammatory reactions.

No previous studies have referred to any regional distribution of this cell type in the synovial intima. In the equine synovial villi, type A cells showed region-dependent density with the densest distribution in the upper region of the villi, but were few in the basal region of the villi in contrast to a rather constant distribution of type B cells. Some previous TEM studies documented the predominance of type A cells in the human (BARLAND et al., 1962), guinea pig (WYLLIE et al., 1964) and cat (GROTH, 1975), in disagreement with histochemical and other TEM studies (KREY and COHEN, 1973; GRAABAEK, 1982, 1984; EDWARDS et al., 1994; KITAMURA et al., 1999). This discrepancy may be explained by the uneven distribution of type A cells as well as the misinterpretation of many type B cells as intermediate cells (cf. GRAABAEK, 1982). The rich existence of type A cells in the villous tips is reasonable in consideration of their contacting and uptaking substances and cellular elements wandering in the joint cavity. Type A cells, originating from the bone marrow and migrating into the synovial intima, can carry ingested material to the lymphatic channels of the subintima, and eventually remove it from the joint (KEY et al., 1929; NAGAI, 1987).

REFERENCES

- BALL, J., J. A. CHAPMAN and K. D. MUIRDEN: The uptake of iron in rabbit synovial tissue following intra-articular injection of iron dextran. A light and electron microscope study. *J. Cell Biol.* 22: 351-364 (1964).
- BARLAND, P., A. B. NOVIKOFF and D. HAMERMAN: Electron microscopy of the human synovial membrane. *J. Cell Biol.* 14: 207-222 (1962).
- BREIT, S. and H. E. KONIG: Scanning electron microscopy of the synovial membrane of the distal phalangeal joint and the navicular bursa in horses. *Tierarztl. Prax.* 23: 273-279 (1995).
- BROKER, B., J. C. W. EDWARDS, M. FANGER and P. LYDYARD: The prevalence and distribution of macrophages bearing FcRI, FcRII and FcRIII in synovium. *Scand. J. Rheumatol.* 19: 123-135 (1990).
- BURMESTER, G. R., A. DIMITRIU-BONA, S. J. WATERS and R. J. WINCHESTER: Identification of three major synovial lining cell populations by monoclonal antibodies directed to Ia antigens and antigens associated with monocytes/macrophages and fibroblasts. *Scand. J. Immunol.* 17: 69-82 (1983).
- CASTOR, C. W. and A. R. CABRAL: Connective tissue activating peptides. *Methods Enzymol.* 163: 731-748 (1988).
- CURTISS, P. H.: Charges produced in the synovial membrane and synovial fluid by disease. *J. Bone Joint Surg.* 46-A: 873-888 (1964).
- DREHER, R.: Origin of synovial type A cells during inflammation. *Immunobiology* 161: 232-245 (1982).
- EDWARDS, J. C. W.: The nature and origins of synovium: experimental approaches to the study of synoviocyte differentiation. *J. Anat.* 184: 493-501 (1994).
- EDWARDS, J. C. W. and D. A. WILLOUGHBY: Demonstration of bone marrow derived cells in synovial lining by means of giant intracellular granules as genetic markers. *Ann. Rheum. Dis.* 41: 177-182 (1982).
- FELL, H. B., A. M. GLAUERT, M. E. J. BARRATT and R. GREEN: The pig synovium. I. The intact synovium *in vivo* and in organ culture. *J. Anat.* 122: 663-680 (1976).
- FORRE, O., J. THOEN, T. LEA, G. H. DOBLOUG, O. J. MELLBYE, J. B. NATVIG, J. PAHLE and B. G. SOLHEIM: In situ characterization of mononuclear cells in rheumatoid tissues, using monoclonal antibodies. *Scand. J. Immunol.* 16: 315-319 (1982).
- FUJITA, T. and S. KOBAYASHI: Structure and function of gut endocrine cells. *Int. Rev. Cytol. Suppl.* 6: 187-233 (1977).
- GRAABAEEK, P. M.: Ultrastructural evidence for two distinct types of synoviocytes in rat synovial membrane. *J. Ultrastr. Res.* 78: 321-339 (1982).
- : Characteristics of the two types of synoviocytes in rat synovial membrane. *Lab. Invest.* 50: 690-702 (1984).
- : Absorption of intraarticularly injected horseradish peroxidase in synoviocytes of rat synovial membrane: an ultrastructural-cytochemical study. *J. Ultrastr. Res.* 92: 86-100 (1985).
- GROTH, H.-P.: Cellular contacts in the synovial membrane of the cat and the rabbit: an ultrastructural study. *Cell Tiss. Res.* 164: 525-541 (1975).
- HASHIMOTO, Y., T. USHIKI, T. UCHIDA, J. YAMADA and T. IWANAGA: Scanning electron microscopic observation of apical sites of open-type paraneurons in the stomach, intestine and urethra. *Arch. Histol. Cytol.* 62: 181-189 (1999).
- HOGG, N., D. J. PALMER and P. A. REVELL: Mononuclear phagocytes of normal and rheumatoid synovial membrane identified by monoclonal antibodies. *Immunology* 56: 673-681 (1985).
- IWANAGA, T., H. HAN, H. KANAZAWA and T. FUJITA: Immunohistochemical localization of protein gene product 9.5 (PGP 9.5) in sensory paraneurons of the rat. *Biomed. Res.* 13: 225-230 (1992).
- IZUMI, S., M. TAKEYA, K. TAKAGI and K. TAKAHASHI: Ontogenetic development of synovial A cells in fetal and neonatal rat knee joints. *Cell Tiss. Res.* 262: 1-8 (1990).
- KEY, J. A.: Experimental arthritis: reactions of joints to mild irritants. *J. Bone Joint Surg.* 11: 705-738 (1929).
- KITAMURA, P. H., H. YANASE, H. KITAMURA and T. IWANAGA: Unique localization of protein gene product 9.5 in type B synoviocytes in the joints of the horse. *J. Histochem. Cytochem.* 47: 343-351 (1999).
- KREY, P. R. and A. S. COHEN: Fine structural analysis of rabbit synovial cells. I. The normal synovium and changes in organ culture. *Arthritis Rheum.* 16: 324-340 (1973).
- LINCK, G. and A. PORTE: B-cells of the synovial membrane. I. A comparative ultrastructural study in some mammals. *Cell Tiss. Res.* 187: 251-261 (1978).
- : B-cells of the synovial membrane. IV. Ultrastructural evidence of secretory variations in hypophysectomized or propylthiouracil-treated mice. *Cell Tiss. Res.* 218: 123-128 (1981).
- MAPP, P. I. and P. A. REVELL: Fibronectin production by synovial intimal cells. *Rheumatol. Int.* 5: 229-237 (1985).
- MATSUBARA, T., M. A. SPYCHER, J. R. RUTTNER and K. FEHR: The ultrastructural localization of fibronectin in the lining layer of the rheumatoid synovium: The synthesis of fibronectin by type B lining cells. *Rheumatol. Int.* 3: 75-79 (1983).
- MURAKAMI, T.: A revised tannin-osmium method for non-coated scanning electron microscope specimens. *Arch. Histol. Jap.* 36: 189-193 (1974).
- MURASHIGE, N., T. GOTOH, Y. ICHIKAWA and K. YAMASHITA: Normal structure and morphological effects of a corticosteroid on the peripatellar synovial membrane of the rat. *Acta Anat. Nippon.* 74: 183-189 (1999).
- NAGAI, S.: The basic study on the synovial membrane of the normal rabbit knee joint by using the induction system of carbon particles. *J. Tokyo Med. Univ.* 45: 488-498 (1987).
- NAITO, M., S. HAYASHI, H. YOSHIDA, S. NISHIKAWA, L. D. SHULZ and K. TAKAHASHI: Abnormal differentiation of tissue macrophages in osteopetrosis (op) mice defec-

- tive in the production of macrophage colony stimulating factor. *Amer. J. Pathol.* 139: 657-667 (1991).
- NISHIJIMA, T.:** The fine structure of the synovial membrane of the knee joint in rats with special reference to regional differences. *J. Jap. Orthoped. Ass.* 55: 601-613 (1981).
- OKADA, Y., I. NAKANISHI and K. KAJIKAWA:** Ultrastructure of the mouse synovial membrane. Development and organization of the extracellular matrix. *Arthritis Rheum.* 24: 838-843 (1981).
- SHIVELY, J. A. and D. C. VAN SICKLE:** Scanning electron microscopy of equine synovial membrane. *Amer. J. Vet. Scand.* 38: 681-684 (1977).
- STEINBERG, P. J. and K. C. HODDE:** The morphology of synovial lining of various structures in several species as observed with scanning electron microscopy. *Scanning Microsc.* 4: 987-1020 (1990).
- TAKAHASHI-IWANAGA, H. and T. FUJITA:** Application of an NaOH maceration method to a scanning electron microscopic observation of Ito cells in the rat liver. *Arch. Histol. Jap.* 49: 349-357 (1986).
- THEOFILOPOULOS, A. N., D. A. CARSON, M. TAVASSOLI, S. F. SLOVIN, W. C. SPEERS, F. B. JENSEN and J. H. VAUGHAN:** Evidence for the presence of receptors for C3 and IgG Fe on human synovial cells. *Arthritis Rheum.* 23: 1-9 (1980).
- WILKINSON, L. S., A. A. PITSILLIDES, J. G. WORRALL and J. C. W. EDWARDS:** Light microscopic characterization of the fibroblast-like synovial intimal cell (synoviocyte). *Arthritis Rheum.* 35: 1179-1184 (1992).
- WILKINSON, L. S., J. C. W. EDWARDS, R. N. POSTON and R. O. HASKARD:** Expression of vascular cell adhesion molecule-1 in normal and inflamed synovium. *Lab. Invest.* 68: 82-88 (1993).
- WOODWARD, D. H., A. GRYFE and D. L. GARDNER:** Comparative study by scanning electron microscopy of synovial surfaces of four mammalian species. *Experientia* 25: 1301-1303 (1969).
- WYLLIE, J. C., R. H. MORE and M. D. HAUST:** The fine structure of normal guinea pig synovium. *Lab. Invest.* 13: 1254-1263 (1964).

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