Three-dimensional Ultrastructure of Synoviocytes in the Knee Joint of Rabbits and Morphological Changes in Osteoarthritis Model*

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Summary. The synovial intima is composed of two types of synoviocytes: absorptive macrophages and secretory, fibroblast-like F cells. Many studies have tried to observe synoviocytes by scanning electron microscopy (SEM) but failed to reveal the entire shape of synoviocytes because they are deeply embedded in the interstitial matrix. The present study, primarily employing SEM observation of NaOH macerated samples, reveals the distribution and three-dimensional ultrastructure of the synoviocytes in the normal knee joint of rabbits, and the morphological changes of synoviocytes in an osteoarthritis model of this animal. F cells were broadly distributed throughout the synovial intima, while macrophages showed a restricted distribution on fatty tissues around the patella. F cells were classified into a flat type, which covered the surface of synovial membrane like an epithelium, and a dendritic type, which extended long processes to form a characteristic meshwork on the surface. The flat type predominated in regions adhering to the femur, while the dendritic type predominated in ambilateral parts of both the patella and tendon of the musculus quadriceps femoris, and on the periarticular fatty tissue. Intermediate forms of flat and dendritic types appeared in middle regions between the patella and periphery of the joint capsule. In the synovial membrane of the osteoarthritis model, both types of synoviocytes increased in number and changed their morphology, indicating their elevated activities in absorption and secretion. It is suggested that the ultrastructural changes in synoviocytes reflect pathological conditions of the synovial membrane, and synoviocytes play important roles in the pathogenesis of osteoarthritis.

The synovial intima is a cell-rich layer consisting of two morphologically different cell types: cells of macrophagic origin (type A synoviocytes), and fibroblast-like F cells (type B synoviocytes) (Barland, 1962; Linck and Porte, 1978; Graaabaek, 1982, 1984). The synovial macrophages develop microvilli and microvilli on their cell surface, and can actively phagocytose cell debris and wastes wandering in the joint cavity, to refresh the components of synovial fluid (Graaabaek, 1982, 1985). F cells are proper cells that secrete collagen, fibronectin, hyaluronic acid, and other proteoglycans into the interstitium and joint cavity (for review, Iwanaga et al., 2000). While F cells vary in shape, they are commonly characterized by branched cytoplasmic processes, which extend toward the joint cavity. Transmission electron microscopic (TEM) observations have showed that F cells are dendritic and possess abundant rough endoplasmic reticulum (rER) in their cytoplasm, distinguishable from subintimal fibroblasts (Edwards, 1994). Although F cells often display an epithelioid nature and arrangement, developing junctional devices between the adjacent synoviocytes and possessing fragmented basal lamina, they differ from authentic epithelial cells (Groth, 1975; Nozawa-Inoue et al., 1999; Iwanaga et al., 2000).

The scanning electron microscope (SEM) is useful for analyzing the three-dimensional ultrastructure of cells, especially cells with complicated shapes. Although many researchers have tried to reveal the definite morphology of synoviocytes by SEM, they failed to clarify the entire aspect of synoviocytes since these are embedded in an abundant intercellular

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matrix of the synovial intima (WOODWARD et al., 1969; WYsocki et al., 1972; DATE, 1979; McDonald and Levick, 1988; Delrio et al., 1999; Murashige et al., 1999). Recently, our SEM observation succeeded in revealing the three-dimensional ultrastructure of synoviocytes in the horse carpal joint by use of NaOH macerated samples, in which the intercellular matrix and fibers were perfectly removed (Shikichi et al., 1999). It is expected that F cells change their shapes depending on the region of synovial intima; however, no previous studies are available regarding such regional differences. These differences under normal conditions will need to be considered for an exact understanding of morphological changes of synoviocytes in pathological cases such as arthritis.

Osteoarthritis is a destructive disease of articular cartilage characterized by a sequence of changes starting with fibrosis and progressing to the erosion and ulceration of articular cartilage, followed by the formation of periarticular osteophytes, sclerosis of the subchondral bone, and osteochondral remodeling (Weisbrode and Doige, 2001). Many studies have dealt with morphological changes of the joint in osteoarthritis models and clinical cases, but their description has focused on changes in articular cartilages or other periarticular structures, and not been available on the ultrastructural changes in synoviocytes (Hefti et al., 1991; Yoshioka et al., 1996; Bray et al., 1997; Sah et al., 1997; Shimizu et al., 1997; Shymkiv et al., 2001).

This study examines the distribution and morphology of two types of synoviocytes in whole synovial intima of the normal knee joint in rabbits as well as the definite morphological changes of synoviocytes in a rabbit osteoarthritis model.

MATERIALS AND METHODS

Animals and surgical treatment

Thirteen male and three female Japanese White rabbits, weighing 3.0 to 4.0 kg and without any abnormalities in their joints and general conditions, were used in this study. Nine rabbits were sacrificed to investigate the normal structures of the synovial membrane, and the others sacrificed for observation of pathological changes in an experimental osteoarthritis model.

Seven male rabbits were put under sedation by an intramuscular injection of midazolam (2 mg/kg) and generally anesthetized by the inhalation of isoflurane. The anterior cruciate ligament of the right knee was completely transected in five rabbits to induce osteoarthritis. For the sham control, two rabbits received the same incision and exposure of the joint as the transection surgery, but retained the ligament intact. Post-operatively, the animals were monitored for infections and other complications, and permitted cage activity with the limb without immobilisation. The animals were assessed 2 and 4 weeks after the operation. All experiments were performed under protocols following the Guidelines for Animal Experimental, Graduate School of Veterinary Medicine, Hokkaido University.

Tissue sampling

The animals were deeply anesthetized with pentobarbital and perfused via the external iliac artery with physiological saline followed by 2% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The anterior region of the knee joints including both the patella and the infrapatellar fat pad was removed as a whole, and dipped in 2% glutaraldehyde in 0.1 M PB overnight at 4°C for electron microscopic observation. The synovial membrane obtained from one rabbit perfused with 4% paraformaldehyde (0.1 M PB) was used for hematoxylin and eosin (H-E) staining. Paraformaldehyde-fixed specimens were cut into a series of small pieces and dipped in 4% paraformaldehyde in 0.1 M PB overnight. The tissues were dehydrated through a graded series of ethanol and embedded in paraffin. Five-micron thick sections were cut and stained with H-E according to the conventional method for observation under a light microscope.

Transmission electron microscopy (TEM)

Some parts of samples perfusion-fixed with 2% glutaraldehyde (0.1 M PB) were dissected into small pieces (2×2×2 mm), fixed in 3% glutaraldehyde (0.1 M PB) for an additional 4 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 Quetol 812 according to the conventional method. Semi-thin sections were prepared from these blocks by use of a diamond knife and stained with toluidine blue for observation under a light microscope. Ultra-thin sections were stained with uranyl acetate and lead citrate, and observed with a TEM (JEM-100SX; JEOL, Tokyo, Japan).

Scanning electron microscopy (SEM)

The glutaraldehyde-fixed tissues were dissected into small pieces (3×3×3 cm) and processed according to the NaOH maceration method before the conductive staining (Takahashi-Iwanaga and Fujita, 1986). The fixed specimens were rinsed in 0.1 M PB and subsequently placed in 6 N NaOH at 60°C for 15 to 40
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. The specimens were then 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 SEM (S-4100; Hitachi, Tokyo, Japan).

RESULTS

Normal synovial membrane

The inner surface of synovial membrane was mostly smooth, but was rough and folded over the fatty tissue of the infrapatellar fat pad (Fig. 1a). Light microscopically, synoviocytes lined almost all parts of the synovial membrane to form the synovial intima. Only on the tendon of musculus quadriceps femoris were cellular elements scarce in the intimal layer. The synovial intima usually consisted of 1 to 2 cell-layers, but the underlying layer (subintima) differed depending on the region, being occupied by densely arranged collagen fibers, adipose tissue or loose connective tissue, largely in accordance with Key’s classification (Key, 1928; Wysoki and Brinkhaus, 1972).

Fibroblast-like F cells (Type B synoviocytes)

The major population of the cells lining the synovial intima was F cells characterized by unique cytoplasmic processes. Under the SEM, they were classified into flat and dendritic types, which differed in their shapes, location of cell bodies in the intimal layer, and distribution (Fig. 1b).

The flat type F cells were densely arranged over the surface of the synovial membrane to show an epithelial arrangement (Fig. 2a). The cell bodies were superficially located in the synovial intima and possessed flat and short processes, being interdigitated with each other. The surface of cell bodies and processes did not develop microprojections, and were smooth in appearance. TEM observation (Fig. 2b) confirmed that flat type F cells possessed the
Fig. 2. SEM (a) and TEM images (b) of flat type-F cells. a. F cells are densely arranged to cover the surface of the synovial membrane like an epithelium. b. The cell bodies of F cells (F) are superficially located in the synovial intima and possess flat processes, which overlap with those of adjacent cells. JC joint cavity. Bars: 5 μm (a), 2 μm (b)

Fig. 3. SEM (a) and TEM observation (b) of dendritic type-F cells. a. Thick processes of F cells extend radially on the surface of synovial intima to form a characteristic meshwork. b. F cells (F) possess cell bodies deeply located in the synovial intima and develop rough endoplasmic reticulum in their cytoplasm. JC joint cavity. Bars: 5 μm (a), 2 μm (b)
Fig. 4. SEM (a) and TEM (b) observation of intermediate forms between flat and dendritic types of F cells. a. The cells possess longer processes and are more loosely arranged than F cells of the flat type. b. The cell bodies of F cells (F) are deeply located, and extend irregularly shaped thin processes toward the joint cavity (JC). Bars: 5 μm (a), 2 μm (b)

Fig. 5. SEM (a, b) and TEM images (c) of synovial macrophages. Macrophages (M) are rich on the fatty tissue around the patella. They are spherical (a) or slender (b) in shape, and intervene among the projections of dendritic type F cells. Macrophages develop microvilli on their surface but only a small number of vacuoles in their cytoplasm (c). Bars: 5 μm (a, b), 0.5 μm (c)
Fig. 6. Legend on the opposite page.
superficially located cell bodies and extended short processes, which overlapped with those of adjacent cells. The cytoplasm contained moderately developed rER, Golgi apparatus, and mitochondria. This type of synovial lining cell occupied the region adhering to the femur (Fig. 1b).

In dendritic type F cells, the cell bodies were located so deeply in the synovial intima that they were hardly visible by SEM. The cells extended a major projection toward the surface of the synovial intima, where this projection branched out radially. In this way, a characteristic meshwork composed of long and thick processes was formed on the surface of synovial membrane (Fig. 3a). TEM observation revealed that the cell bodies were located deeply in the synovial intima and were surrounded by wide interstitial spaces between them (Fig. 3b). They developed abundant rER in their perinuclear cytoplasm and processes. The dendritic type F cells were distributed in ambiateral parts of both patella and the tendon of musculus quadriceps femoris (Fig. 1b).

These two types of F cells gradually changed their

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**Fig. 6.** Morphological changes of F cells in osteoarthritis model shown by semi-thin plastic sections (a–d) and SEM specimens (e–g). The synovial intima of the sham control is thin and contains dispersed synoviocytes (a). F cells increase in number at 2 (b, c, e, f) and 4 weeks (d, g) after surgery to form a thick cellular lining. At 2 weeks after surgery, the mitosis of a F cell is found in the cell lining composed of F cells (e). The increased F cells possess thick and short processes, which perpendicularly extend to the joint cavity (e, f). At 4 weeks after surgery, F cells extend processes parallel to the surface of the synovial membrane; many macrophages (arrows) occupy the front line of the intima, and many capillaries are observed in the subintima (d). A characteristic meshwork composed of long and thin processes is observed in the surface of the synovial membrane (g). Bars: 10 μm (a–d), 5 μm (e–g)
morphologies, and intermediate forms of flat and dendritic types (Fig. 4a, b) appeared in middle region between the patella and periphery of the joint capsule (Fig. 1b). Therefore, the projections of F cells tended to become shorter and flatter, and the cell bodies tended to locate more superficially when leaving the patella and the tendon of the musculus quadriiceps femoris.

_Macrophages (Type A synoviocytes)_

In SEM observation, macrophages were identifiable as round cells covered with developed microvilli and microvilliæ (Fig. 5) and were densely distributed on the fatty tissue around the patella (Fig. 1b). In this region, macrophages were embedded in the meshwork formed by projections of dendritic type-F cells (Fig. 5a, b). Macrophages were rarely observed in regions other than the fatty tissues. The shapes of macrophages were spherical (Fig. 5a) or slender (Fig. 5b), the latter extending projections from both sides of the cells.

Under the TEM, macrophages showed irregularly shaped microprojections on their surface but only a small number of vacuoles in their cytoplasm. The nuclei of macrophages were darker in appearance than those of F cells, due to rich heterochromatin (Fig. 5c).

_Synovial membrane of experimental osteoarthritis model_

All of the osteoarthritis model and Sham-operated rabbits did not show any abnormal signs of general body condition throughout the experimental period. Macroscopically and histologically, the synovial membrane of the Sham control showed no difference as compared with the normal one (Fig. 6a).

In the osteoarthritic model, the knee joint swelled up, and the synovial fluid slightly or moderately increased in volume 2 and 4 weeks after surgery. The surface of lateral epicondyle of the femur became rough 4 weeks after surgery. Light microscopic
observation of semi-thin sections stained by toluidine blue demonstrated that both types of synoviocytes increased in number, forming 3 to 5-cell layers in the synovial intima; blood capillaries of the subintima also increased in number, suggesting neo-
vascularization (Fig. 6d).

Morphological changes in F cells
F cells in the osteoarthritis model dramatically changed their densities and shapes. At 2 weeks after surgery, F cells increased in number and densely lined the intima (Fig. 6b, e). Mitoses of F cells were occasionally found in the cell lining composed of F cells (Fig. 6c). These F cells possessed deeply located cell bodies and thick and short processes, which extended perpendicularly toward the surface of the synovial intima (Fig. 6f). The tips of the processes were covered with ruffle- and bubble-like microprojections, appearing cauliflower-like in shape. Such F cells were never seen in the normal synovial membrane. At 4 weeks after surgery, F cells tended to extend long processes parallel to the surface of the synovial membrane to form a fine, dense meshwork composed of processes longer than those of normal F cells on the surface of the synovial membrane (Fig. 6g). Both the cell bodies and processes of F cells developed ruffle- and bubble-like projections entirely on their cell surface. In TEM observation at both 2 and 4 weeks after surgery, F cells were found to develop more abundant rER in their cytoplasm than normal cells, and the lumens of rER were conspicuously extended (Fig. 7a, b).

Morphological changes in macrophages
Macrophages also increased in number at 2 weeks after surgery, but the increments of macrophage numbers were more remarkable at 4 weeks after the operation (Fig. 8a, b). SEM revealed many spherical macrophages aggregated on almost the entire surface of the synovial membrane in the osteoarthritis model (Fig. 8b). These increased macrophages developed many vacuoles in their cytoplasm (Fig. 8a). In TEM observation, the cytoplasmatic vacuoles showed various sizes and densities of their contents (Fig. 8c).

DISCUSSION

Morphology and distribution of synoviocytes in the normal synovial membrane
The present study classified fibroblastic F cells into a flat type and a dendritic type. These two types of F cells have been separately reported in the synovial membrane of the dog (Wysocki, 1972), rat (Murashige et al., 1999), and rabbit (Date, 1979; McDonald and Levick, 1988; Delrio et al., 1999). However, their topographical relationship remains unclear, although some previous SEM studies have suggested that, under normal conditions, F cells change shape depending on the region (Wysocki et al., 1972; Date, 1979; McDonald and Levick, 1988; Delrio et al., 1999). In the rabbit knee joint that we observed, F cells of the flat type predominated in the regions adjacent to the femur, while dendritic type cells predominated in ambilateral parts of both the patella and tendon of the musculus quadriceps femoris, and on the fatty tissue around the patella. The region-dependent difference seems to reflect their different circumstances and functions. Since joint movement yields intense pressure near the regions adhering to the femur, the epithelium-like arrangement of flat type-F cells might be constructed to resist this pressure. The existence of similar synovial lining cells has been documented by SEM observation in rats (Murashige et al., 1999) and rabbits (McDonald and Levick, 1988; Delrio et al., 1999), but their distribution in the joint has remained unclear. The characteristic meshwork formed by the processes of dendritic type-F cells on the synovial surface has been observed in the synovial membrane of the human (Barland et al., 1982), rabbit (Date, 1979; Delrio et al., 1999), and horse (Shikichi et al., 1999). An important function of F cells is to secrete glycosaminoglycans such as hyaluronic acid and fibronectin into the synovial fluid and intercellular matrix (for review, Iwanaga et al., 2000), so the dendritic shape of F cells may assist in the secretory function. This idea is supported by the finding that dendritic type-F cells developed more abundant rER in their cytoplasm than the flat type.

Macrophages in the rabbit knee joint developed microvilli and microprojections on their cell surface and were identical to typical macrophages. Joint macrophages absorb the extracellular constituents, cell debris, microorganisms and antigens in the synovial fluid and intimal matrix and degrade them by a well-developed lysosomal system (Graabeek, 1982, 1985; Asari et al., 1995). Our previous study of horse carpal joints (Shikichi et al., 1999) demonstrated the aggregation of macrophages at the tips of the synovial villi. In the rabbit synovial membrane, macrophages aggregated on the peripatellar fatty tissues, in which the synovial membrane was folded. The peripatellar fat pad is close to the anterior and posterior cruciate ligament, supplying nutrition to ligamental elements and receiving metabolites from
them (McDougall and Bray, 1998). The aggregation of macrophages on the fat pad is advantageous for disposing metabolites arising in association with joint movement.

**Synovial membrane in the osteoarthritis model**

Osteoarthritis is classified into two main types: primary osteoarthritis without an obvious initiating cause, and secondary osteoarthritis following some other abnormality of the joint, for example, rupture of the anterior cruciate ligament of the knee joint (Bennett, 1984). Osteoarthritis models in dogs and rabbits have been frequently produced by transection of the anterior cruciate ligament. However, pathological studies using osteoarthritis models have focused on changes in the articular cartilage, bone, and ligaments, with nothing available regarding the ultrastructural changes of synoviocytes (Hefti et al., 1991; Yoshioka et al., 1996; Bray et al., 1997; Shih et al., 1997; Shimizu et al., 1997; Shymkiv et al., 2001).

The initial change in osteoarthritis occurs with the destruction of the cartilage matrix composed of collagen and glycosaminoglycans. Subsequently, enzymes to resolve glycosaminoglycans are released and degrade them. Joint macrophages uptake these cleavage products and cell debris, and are activated to release inflammatory mediators, which stimulate chondrocytes, synoviocytes and subintimal fibroblasts, and induce the production of some proteinases such as cysteine proteinases, metalloproteinases and serine proteinases, resulting in destruction of the cartilage matrix (Brandt and Mankin, 1993). In addition to these alternations, mechanical stress made by abnormal movement of the joint causes typical pathological changes, for example, the fibrosis and ulceration of the articular cartilage, the formation of periarticular osteophytes, and sclerosis of the subchondral bone (Palmer, 1991). Yoshioka et al. (1996) reported that in a rabbit osteoarthritis model, the first ulceration of the whole strata of articular cartilage appeared 8 weeks after transection; in contrast, the tylosis of the synovial intima was detectable by 4 weeks after surgery. These authors considered that the onset of changes of secondary osteoarthritis in the synovial membrane continued up to 4 weeks after surgery, but they did not report which kinds of cells increased in the synovial intima. Therefore, in this study we focused on morphological changes of synovial lining cells in the early stages of osteoarthritis.

No morphological disorders were found in the Sham control of our osteoarthritis model except for the sutured region, indicating that the changes observed here were caused by transection of the anterior cruciate ligament.

**Morphological changes of synoviocytes in osteoarthritis model**

One remarkable change among F cells in the osteoarthritis model was an increase in cell number at 2 weeks after surgery. Since the mitosis of F cells was detectable in the synovial intima of osteoarthritis rabbits, it seems that F cells proliferated locally in the synovial intima, in agreement with previous studies that detected the mitosis of F cells in the synovial membrane in culture (Krey and Cohen, 1973) and in vivo (Linck and Porte, 1981). In early osteoarthritis, F cells may increase in number by mitosis and then gradually change their morphology in relation to their functional activity.

Another morphological change of F cells in the present osteoarthritis model was characterized by the extension of cytoplasmic processes, especially 4 weeks after surgery. McDonald and Levick (1988) reported that F cells became more dendritic when pressure of the synovial cavity was elevated. In the present osteoarthritis model, the synovial fluid volume actually increased in the knee joint whose anterior cruciate ligament had been transected, and was intimately related to neo-vascularization in the subintima. The F cells in the osteoarthritis model developed extensive rER with dilated cisternae, indicating their active protein synthesis. It is known that proteinous components of the synovial fluid such as metalloproteinases increase in various joint diseases, including osteoarthritis (Walakovits et al., 1992; Ishiguro et al., 1999a, b). F cells in the present osteoarthritis model also developed ruffle- and bubble-like projections on their cell surfaces. The development of microprojections of F cells has been documented in the joint with experimental hemarthrosis (Date, 1979) as well as when the joint pressure was artificially raised (McDonald and Levick, 1988). Since the development of microprojections increases the surface area of F cells contacting the joint cavity, this may be advantageous for the secretory and absorptive functions of the F cells.

The increase in cell number in the synovial intima was observed in both types of synoviocytes, but more remarkably so in macrophages. These macrophages in early osteoarthritis must be supplied by bone marrow-derived monocytes (Naito et al., 1991). In our osteoarthritis model, macrophages developed many vacuoles in their cytoplasm, indicating their stimulated phagocytotic activity. They may actively phagocytose cell debris and cleavage products in the
joint cavity and produce some inflammatory mediators. In some joint diseases, synovial fluid shows elevated concentrations of interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α (PARTSCH et al., 1997; KUBOTA et al., 1998), which may be released from activated macrophages.

In conclusion, the alkaline maceration method was very useful for revealing the ultrastructure of cells embedded in the interstitial matrix. Moreover, a comprehension of the ultrastructural changes of synoviocytes should prove helpful toward a deeper understanding of the pathogenesis of osteoarthritis.

REFERENCES


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