A Scanning and Transmission Electron Microscopic Study of Fiber Arrangement in the Hepatic Capsule

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Summary: The arrangement of fibrous elements in the rat hepatic capsule was examined under a scanning electron microscope (SEM) after alkaline or acid maceration of the serous coat, in conjunction with examination of the thin sections using a transmission electron microscope (TEM).

The elastic fibers appeared as thin threads in a densely meshed network, lying just beneath the serous coat. Their surface was granular with short rods in the materials fixed with paraformaldehyde. In contrast, the collagen fibers were observed as relatively thick threads, with fascicles of collagen fibrils that were uniform in size. These fascicles extended in various directions to form rough meshes that were traversed by small fascicles and anastomosed with each other.

The fibrous branches of the elastic fibers extended on or into the underlying collagen fibers to be anchored, while the collagen fibers converged on many areas of the liver surface, and were transferred into the interlobular connective tissues.

The findings of the present study thus suggest that the fiber arrangement plays an effective role in the mechanical protection of the fragile liver cells and delicate serous cells from pressure and friction damage by the neighboring abdominal organs and walls of the abdominal cavity due to the elastic mobility of the subserosal elastic network in addition to the possible slippery cushion of a serous layer on the serous cells.

The hepatic capsule in mammals is generally held to be composed of two layers: the serous coat and the fibrous capsule. In contrast to the well-known structures of the serous coat, especially serous cells (Popper and Schaffner, 1957; Andrew and Porter, 1973; Tanikawa, 1976; Motta et al., 1976), relatively few studies have been conducted on the fibrous capsule. In addition to our knowledge of the histological structures of the connective fibrous layer (Pfuhl, 1932), the fine structure of the interstitial cells and fibrous elements has been previously observed in sections under TEM (Grisham et al., 1975; Tanikawa, 1979; Grisham et al., 1980). The fibrous capsule was examined under SEM through serous defects that were mechanically caused (Motta, Muto and Fujita, 1976; Inoue, Osatake and Tanaka, 1984). Motta and his co-workers (1976) observed that dense bundles of collagen fibers were oriented in different directions. Although they did not describe the existence of elastic fibers, Inoue and his co-workers (1984) first identified elastic fibers among the collagenous bundles. However, the three-dimensional arrangement of the fibrous elements and the mutual relationship between the two kinds of fibers have not been described in the liver capsule.

Recently, new techniques in specimen preparation have been introduced for the observation of fibrous elements under SEM. Ohtani (1987) used sodium hydroxide for the selective demonstration of collagen fibers in various organs. The elastic fibers were exposed to formic acid in the dermis (Tsujii et al., 1979) and blood vessels (Wasano and Yamamoto, 1983; Crissman and Guilford, 1984), which were fixed with glutaraldehyde. In order to preserve more detailed surface structures of the elastic fiber, Ushiki (1992) recommended paraformaldehyde for tissue fixation. Furthermore, Shimada, Nakamura, Kitahara and Sachi (1983) directly observed Purkinje fibers of the myonal cells under SEM after the removal of the endocardial coat using sodium hypochlorite.

The present study was undertaken to examine the three-dimensional arrangement of fibrous elements in the hepatic capsule after the selective exposure of elastic and collagen fibers by the chemical maceration methods mentioned above. The following findings are discussed from the viewpoint of the mechanical protection of the serous coat from the pressure and friction exerted on the liver by neighboring abdomi-
nal organs.

Materials and Methods

Adult Wistar rats were used in the present study. Following anesthesia by an intraperitoneal injection of Nembutal, the livers of the animals were perfused via the aorta with 50 ml of Ringer’s solution and then 500 ml of fixative. The fixative used here was 2.5% glutaraldehyde or 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The removed livers were immersed in the same fixative for a couple of days or longer at room temperature before preparing the specimens.

To remove the serous coat or selectively demonstrate the fibrous elements, three kinds of chemical solutions were applied to the tissue blocks (10 x 10 x 5 mm³) of either the glutaraldehyde- or paraformaldehyde-fixed livers. The tissue blocks were immersed in (1) 5% sodium hypochlorite solution for 10 min at room temperature, (2) 88% formic acid for 2 or 3 days at 45°C, or (3) 10% sodium hydroxide for 70 days at 10°C. The immersion time in each solution was decided by preliminary application in a series of time sequences. After performing the chemical maceration described above, the tissue blocks were rinsed in either water or 0.02 M HCl for the blocks immersed in formic acid, transferred into 2% tannic acid overnight, then postfixed in 1.33% osmium tetroxide in the buffer mentioned above. The postfixed blocks were dehydrated with a graded series of acetone, transferred into isoamyl acetate, dried by the critical point method of CO₂, coated with metal in an ion coater and observed in either a Hitachi S-450 or S-900 SEM.

Small pieces of aldehyde-fixed livers were immersed in 2% tannic acid for 10 min, and postfixed with 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). After dehydration with a graded series of ethanol and propylene oxide, the tissue specimens were embedded in Epoxy resin. The resin-embedded specimens were cut into thin sections, doubly stained with uranium and lead, then observed in a Hitachi H-500 TEM. Furthermore, small blocks of aldehyde-fixed livers were embedded in paraffin, cut into sections and stained by Masson-Goldner’s method combined with aldehyde-fuchsin stain.

Results

Light Microscopy

The elastic fibers stained deep violet by aldehyde-fuchsin were delicate threads that were seen in a densely meshed network in the graded sections of hepatic capsules (Fig. 1). The collagen fibers stained with light green appeared as coarse threads, extending on or between the groups of liver parenchymal cells (Fig. 1).

Transmission Electron Microscopy

The outer surface of the liver was covered with a thin cytoplasmic layer of serous cells, which contained a small number of minute organelles and vesicles around the flattened nuclei (Fig. 2). Their peripheral cytoplasm was adjoined with that of neighboring cells, and overlapped with the lamellated cytoplasmic processes equipped with junctional structures (Figs. 3a, b).

The elastic fibers were selectively impregnated with tannic acid, revealing both an interior homogeneous substance and granular structures on the outer surface (Figs. 2, 3a, b). Those fibers consisted of either thin threads (0.5 µm thick) of fragmental masses arranged in beaded lines. The collagen fibers demonstrated relatively large fascicles (about 5 µm thick) of fibrils that were uniform in size (about 50 nm thick) (Figs. 2, 3a, b, 11).

The subserosal space was filled with the connective tissues of the fibrous capsule, which was formed with the fibrous elements of both elastic and collagen fibers (Figs. 2, 3a, b, 13) and interstitial fibrocytes (Fig. 13).

The elastic fibers were mostly situated just beneath the serous coat (Fig. 2). However, their extensions were frequently observed in the fascicles of collagen fibrils or were entangled with small fascicles of collagen fibrils (Figs. 3a, b).

Scanning Electron Microscopy

1. Formic acid-treated capsules, which were previously fixed with one of two different kinds of glutaraldehyde, provided a mossy structure, which was seen as a spongy network of delicate threads (about 0.5 µm thick) on a roughly meshed network of thick threads (about 5 µm thick). The delicate threads were highly anastomosed to form a three-dimensional network in dense meshes (Figs. 4, 6). The network was almost the same in profile as that of the aldehyde-fuchsin-stained elastic fibers described in the section on light microscopy. The underlying thick threads extended in various directions and were interwoven with each other. However, no obvious anastomosis between thick threads was observed. Thin threads were frequently connected with thick threads (Fig. 8). In the specimens fixed with paraformaldehyde before the formic acid treatment, thin threads showed granular surfaces (Fig. 5), as reported by Ushiki (1992) for elastic fibers of the rat aorta, whereas those threads in the glutaraldehyde-fixed specimen were smooth (Figs. 7, 8). The granular
surface caused by the parallel arrangement of short rods (about 500 nm long and 75 nm thick) was almost the same as that of the elastic fibers in sections, previously mentioned in the transmission electron microscopic findings.

2. The sodium hydroxide-treated capsules showed a knitting-ball profile. Threads measuring about 5 μm thick extended in various directions to form a rough network. These threads also crossed over each other. However, no direct anastomosis was observed between them. At high magnification, the threads revealed fine filamentous elements that were uniform in size (about 50 nm thick), and extended in parallel fashion in the fascicles (Figs. 8, 9). The small fascicles of the filaments diverged from the large fascicles of the thick threads, while extending through the meshes and becoming confluent with other fascicles at various distances (Figs. 7, 8). The filamentous elements in the fascicles were almost the same in structure as those in the collagen fibrils described above in the transmission electron microscopic findings.

3. In the sodium hypochlorite-treated specimens, the serous coats were removed, but the hepatic cells remained intact (Fig. 12). Variously sized threads extended over the hepatic cells. The threads also consisted of fascicles of filamentous subcomponents, as seen in the sodium hydroxide-treated specimens, and were recognized as collagen fibers. These fibers converged at many points on the liver surface, and extended into the interhepatocytic spaces (Fig. 12). Extension of the collagen fibers from the fibrous capsule to the interlobular connective tissue was also seen in the TEM observation of thin sections (Fig. 13).

Discussion

The chemical treatment applied to tissue blocks in the present study selectively exposed the intercellular fibers after the removal of cellular elements. However, sodium hypochlorite was effective in the gradual removal of all tissue elements from the surfaces of the tissue blocks, although some differences in maceration grade were observed in the tissue elements. The combination of time, tissue block size and concentration of the chemicals applied in the present study removed the serous coat, elastic fibers and thin fascicles of collagen fibrils, whereas the thick threads of collagen fibers and the underlying hepatic cells remained almost intact. The gradual removal of all tissue elements with sodium hypochlorite was also supported by the experiments of Shimada and his co-workers (1983), who applied commercial kitchen detergents containing sodium hypochlorite to cardiac tissue. They succeeded in observing the conduction of myocardial cells on the usual myocardial cell layer after removal of the endocardium and its underlying connective tissue.

The thin threads in the densely meshed network in the formic acid-treated hepatic capsule eventually correspond to elastic fibers. Their size and extension in the capsule closely resemble those of the aldehyde-fuchsin stained elastic fibers. Furthermore, the surface fine structures of the threads in the present study are almost the same as those of the branching or anastomosing elastic fibers in both the formic acid-treated arterial wall (Wasano and Yamamoto, 1983) and loose connective tissues (Imayama and Braverman, 1988). The granular structures on elastic fibers, which Inoue and his co-workers (1984) observed under SEM after mechanically tearing off the serous coat of the liver, were observed even after formic acid treatment in the paraformaldehyde-fixed materials reported by Ushiki (1992), but not in glutaraldehyde-fixed ones. However, the three-dimensional arrangement of elastic fibers in the network was well preserved in the glutaraldehyde-fixed materials. The significance of the difference in fixation of the granular structures on the elastic fibers between the two kinds of aldehyde was not clarified in the present study.

The thick threads under the densely meshed network of elastic fibers are considered definitely to be thick fascicles of collagen fibrils, which remained almost completely intact by a short-term immersion in formic acid, because the prolonged immersion of tissue blocks completely removed these thick threads in the arterial walls in Wasano and Yamamoto's study (1983) and also in the hepatic capsule in the preliminary experiments of this study.

Andrews and Porter (1973) and Motta, Muto and Fujita (1978) have previously suggested that the serous cells of the liver capsule may be protected by the slippery cushion of a layer of serous exudate enveloping the microvilli on the free surface of the serous cells from friction damage. In addition to this slippery cushion, the present study may also suggest that the subserosal development of the elastic fiber network endows the capability of elastic mobility to the serous coat of the liver capsule in its close contact with other abdominal organs or cavity walls including the diaphragm.

The collagen fibers observed in the liver capsule treated with sodium hydroxide were fascicles of the fibrils, a subcomponent which was also revealed in the TEM observations. The large fascicles were interwoven with each other without any direct connection even at their mutual cross-overs.
study clearly demonstrated that collagen fibers were interconnected by either the divergence or the convergence of thin fascicles of fibrils between the large fascicles. Furthermore, the entanglements of elastic fibers with collagen fibrils, as demonstrated in the present study, may be the structures that anchor the elastic fibers to the underlying collagen fibers.

The existence of reticular fibers in the capsule was first suggested in a histological examination (Pfuhl, 1932). The present study did not demonstrate exact images of the reticular fibers under SEM. However, the isolated collagen fibrils or their fine fascicles in the meshes of the collagen network may well represent reticular fibers. A similar conclusion has also been presented by Ohtani (1987) in his observations of reticular fibers in the pancreatic islet.

References

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Explanation of Figures

Plate I

Fig. 1. Alddehyde-fuchsin, Masson-Goldner’s stain. The elastic fibers stained deep violet appear to be delicate and are visible in a net of dense meshes. While collagen fibers stained light green are coarse and demonstrate a rough meshwork in which hepatic cells are grouped. ×600
Plate II

Fig. 2. A TEM graph of the hepatic capsule. A thin cytoplasmic layer of serous cells (M) rests on the basal lamina (BL) and its underlying fibrous layer of elastic fibers (E), which are impregnated with tannic acid, and also on a layer of collagen fibers (C). H: hepatic cells. \( \times 22,000 \)

Fig. 3. TEM graphs of the capsular fibers. The elastic fibers (E) are observed either a) in the fascicles of collagen fibrils (C) or b) under them. The arrow indicates the junctional structure on the lamellated peripheral cytoplasm of the serous cells (M). a: \( \times 37,000 \), b: \( \times 37,000 \)
Plate III

Fig. 4. An SEM graph of the fibrous capsule fixed with glutaraldehyde prior to immersion in formic acid. Delicate elastic fibers (E) form a densely meshed network above a rough network of coarse fibers of collagen (C). ×610

Fig. 5. Close-up of the network of elastic fibers. Slender threads of elastic fibers are branched and anastomosed with each other and line the small meshes in various sizes. C: collagen fibers. ×2,300
Plate IV

Fig. 6. High magnification of the elastic fibers (E) in Fig. 5. On the smooth surface, thin threads of elastic fibers extend either on (arrow) or into (arrowhead) coarse fibers of collagen (C). ×9,500
Fig. 7. SEM graphs of elastic fibers fixed with paraformaldehyde prior to formic acid treatment.

a): The fine meshes that are formed with delicate threads of elastic fibers are almost the same as those in Fig. 5.

b): High magnification of the elastic fibers in Fig. 7 a). Rods which are similar in size appear in a parallel arrangement on the elastic fibers. a: x2,300, b: x27,500
Plate VI

Fig. 8. SEM graphs of a fibrous capsule after immersion in sodium hydroxide.

a) Thick fibers of collagen extend in various directions while forming a network.

b) Most of the fibers are interwoven, crossing over each other. Small fibers (arrows) diverge from the thick fibers to extend across the meshes and anastomose with fibers either nearby or far away. a: ×150, b: ×470
Plate VII

Fig. 9. Close-up of meshes of collagen nets. Each fiber is a fascicle of fine fibrils, some of which extend in different directions and are confluent with the neighboring fascicles. ×520

Fig. 10. High magnification of the collagen fibers. The fibers contain uniformly sized fibrils extending parallel to each other. ×5,600
Fiber Arrangement in the Hepatic Capsule

Plate VII

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Plate VIII

Fig. 11. An SEM graph of a fibrous capsule after immersion in a sodium hypochlorite solution. Thick fibers of collagen (C) appear to converge at some points (*), extending between the groups of hepatic cells (H). ×2,300

Fig. 12. A TEM graph of the collagen fibers (C) with interstitial cells (I) between the neighboring groups of hepatic cells (H). ×11,000