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The Connective Tissue Framework of the Hepatic Ligaments in the Human Liver

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Objectives: To demonstrate the three-dimensional structure of the collagen fiber framework in the human liver ligaments and capsule. **Methods:** We studied the collagen fiber framework of relatively normal human liver specimens using a cell maceration method and scanning electron microscopy. **Results:** The collagen fibers of the hepatic falciform ligament subdivided into three types depending on the direction and location. The outer collagen fibers of the hepatic teres ligament formed the longitudinal plate, and the inner fibers had a loop-like structure. The coronary ligament contained two parallel collagen bundles toward the hepatic capsule. The hepatic capsule possesses the outer thicker and inner interlaced layers of the collagen fibers. The hepatic ligaments' outer layer connected with the hepatic capsule collagen fibers, while the inner layer tended to merge with the hepatic lobular parenchyma's connective tissue. **Conclusions:** The hepatic ligaments and liver capsule are layered structures of collagen fibers differing in direction. The hepatic ligaments' outer layer connected with the liver capsule's collagen fibers and the inner layer merged with the hepatic lobular parenchyma's connective tissue.

Keywords: Cell-Maceration Method, Human Liver Ligaments, Collagen Fibers, Scanning Electron Microscopy

Introduction

It has long been considered that the connective tissue fibers in the liver provide the endothelial cells of the hepatic sinusoids with a scaffold [1], while their excessive accumulation in the hepatic parenchyma is associated with chronic of liver disease and the development of cirrhosis [2, 3].

The cell-maceration method has demonstrated "Gitterfasern" or the liver's so-called reticulum fibers in various species, including humans [4]. Recent immunohistochemical techniques have visualized type I, III and IV collagens in both normal and fibrotic livers [5-8].

Scanning electron microscopic techniques have contributed significantly to visualizing the liver's three-dimensional microstructure [9-11]. The three-dimensional demonstration of human liver collagen fibrils has been studied by the cell-maceration method in their natural form and location [4, 12].

It has been reported that type XI collagen is restricted to thin fibrils, suggesting that a correlation of various collagen fibrils exists between their diameter and the collagen's biochemical components [13].

Glisson's sheath's collagen fibrils contain two different collagen populations derived from two distinct cell origins, fibroblast-associated and bile epithelium-associated [14]. These collagen fibrils are thought to help maintain the structural and functional integrity against frictional stresses caused by the movement of the neighboring abdominal wall and organs [15]. Parry et al. measured the fibrils' diameter in various tissues and species and found that their diameter correlated with the tissue's tensile strength [16].

An over-deposition of collagen in the liver constitutes hepatic fibrosis and is associated with diminished liver function, as observed in hepatic cirrhosis [3].

The pathological changes in liver architecture were essentially similar in the intralobar and subcapsular areas, and that capsule thickness reflected intralobar non-parenchymal changes [17]. For this reason, medical imaging studies tend to focus on the hepatic subcapsular area as a surrogate expression of hepatic microstructure changes.

Recent studies of connective tissues of hepatic fibrosis have identified that not only the liver parenchymal tissues but also the Glisson's capsule, and the hepatic ligaments, were changed. Kawasaki et al. reported from the structural status of liver capsules and ligaments, it is possible to diagnose hepatic cirrhosis early [3]. The thickening of hepatic falciform and teres ligaments in these ligaments extending to the capsule was identified during ultrasound examination [17].

Recently, the hepatic capsule and ligaments' cooperative function forming the connective tissue framework supporting the biliary and blood flow was described. However, it is unclear if this connective tissue framework rhythmically moves with respiration and diaphragm movement [14].

Mongolia has the highest prevalence of hepatitis B, C, D virus infections, and more than 30% of chronic viral hepatitis progresses to liver cirrhosis, a degenerative structural change of liver parenchyma [18]. We are unable to find a study of the three-dimensional structure of the Mongolian human liver. Noninvasive methods to evaluate a liver connective tissue mass, such as an ultrasound, transient elastography, computed tomography, have been used at a macroscopic level. But these do not prove the information necessary to understand the three-dimensional structure and integration of the human livers' connective tissue framework. This study aimed to examine the liver capsule and hepatic ligaments' detailed three-dimensional collagen fiber network microstructure using scanning electron microscopy.

Materials and Methods

Sample collection

We used a cross-sectional study design. All cadaver tissue specimens (n=12) were obtained from the subjects who died of the non-hepatic causes. Their ages at the time of death ranged from 36 – 55 years, and the male to female ratio was 1.4:1. Preliminarily light microscopy determined that the liver tissue had no significant pathological changes.

Cell maceration method

The collagen fibrils of the liver were extracted and examined according to the cell-maceration method [4, 12]. The materials were fixed in 2.5% glutaraldehyde in a 0.01 M phosphate buffer solution (pH 7.3) for more than a day and cut into small pieces (1 x 1 x 1 cm3). The pieces were immersed in 2N NaOH for 3-7 days at room temperature (about 25°C). And then rinsed in distilled water for 2-3 days or until the pieces became transparent.

Scanning electron microscopy (SEM)

The specimens were post-fixed with 2% OsO4 for 1 or 2 h at -4°C and then dehydrated in a series of graded concentrations of ethanol and in isoamyl acetate every 30 minutes. The specimens were dried at critical point using CO2 (Critical Point Dryer Hitachi HCP-2, Tokyo, Japan) and coated with the gold of 200 Å thickness (Ion Coater IB-3, Ibaragi, Japan), and examined with scanning electron microscope/SEM (S-800, Hitachi, Tokyo, Japan) at an accelerating voltage of 10-15 kV. The structural data and the scanning electron micrographs were produced on the RS Image software (Media Cybernetics, Maryland 20910 USA).

Ethical statement

The current study was approved by the Ethics and Research Committees of the Mongolian National University of Medical Sciences (№74/3). The tissues were harvested after obtaining ethical permission following the guidelines of the Helsinki Declaration.

Results

Hepatic falciform ligament

The primary component of hepatic falciform ligament was the dense regular connective tissues that contain collagen fibers. The collagen fibers were subdivided into three types, depending on direction and location. The falciform ligament's outer thick longitudinal collagen bundles gradually blended into the collagen fibers wrapping the umbilical vein. In the marginal side of the left and right hepatic lobes, two different collagen fibers were found differing in direction. The hepatic falciform ligament's outer layer of the collagen fibers was longitudinal, and the inner layer was oblique in direction. The third intermediate structure of this ligament was located only in the portion bordering the teres ligament and formed the collagen network surrounding the umbilical vein. This loose collagen network connects the falciform and teres ligaments and may protect the collagen fibrous plate from detachment (Figures 1 and 2).

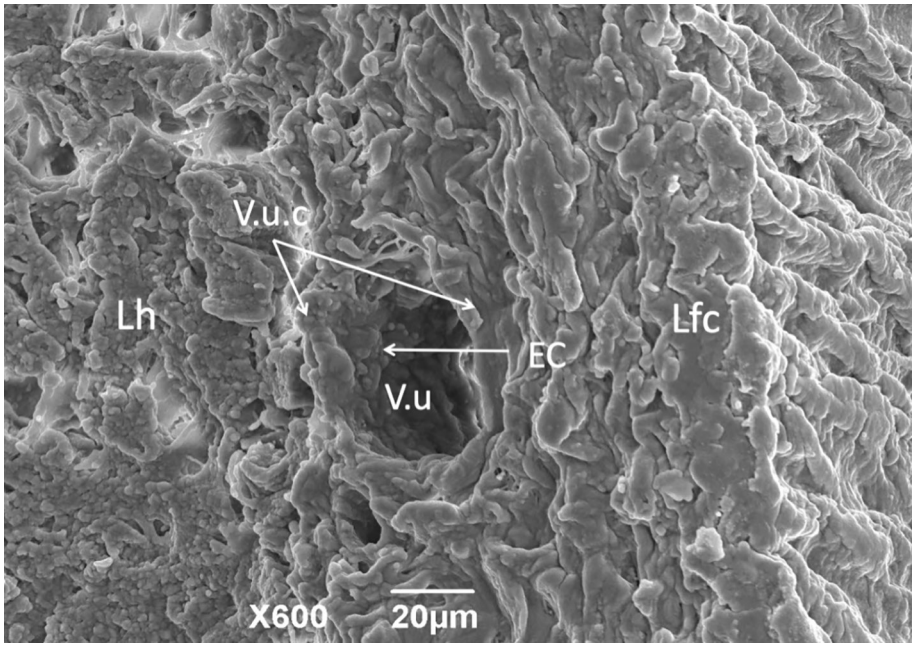

Figure 1. Scanning electron micrograph of the hepatic falciform ligament, magnification x600. The thick and longitudinal collagen bundle of the falciform ligament (Lfc) gradually blended into the collagen fibers (V.u.c), wrapping the umbilical vein (V.u). The umbilical vein was lined by endothelium (EC) and separated by the thin connective tissue wall from the hepatic lobule parenchyma (Lh).

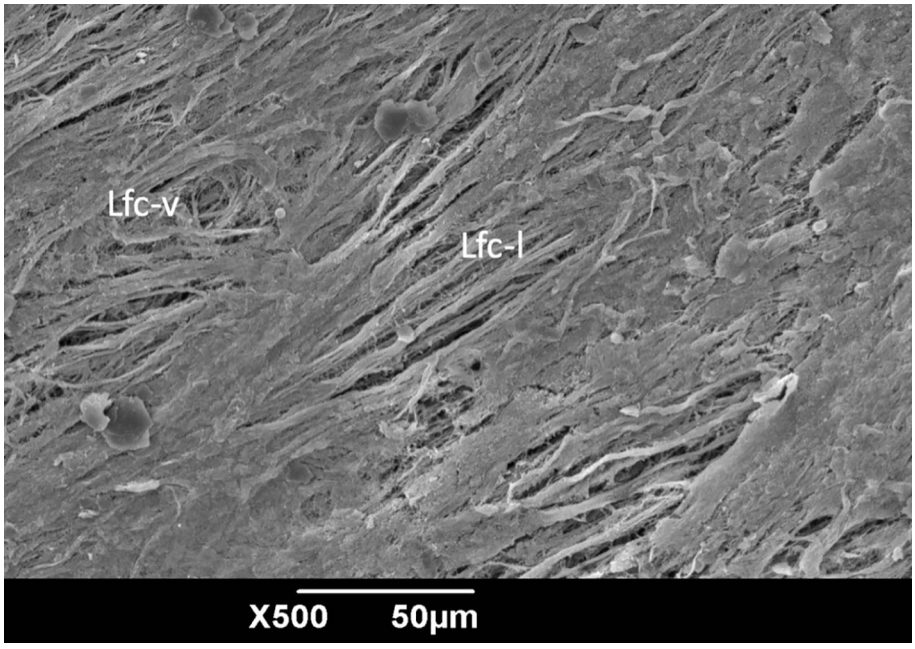

Figure 2. Scanning electron micrograph of collagen fiber network of the hepatic falciform ligament, magnification x500. The outer layer of the collagen fibers of the hepatic falciform ligament (Lfc-l) was longitudinal, and the inner layer was (Lfc-v) oblique in direction.

Hepatic teres ligament

A dense connective tissue plate lined the teres ligament. The umbilical vein was situated deep in the teres ligament and branched into the hepatic lobule parenchyma. The collagen fiber bundles sheltered the vein (Figure 3).

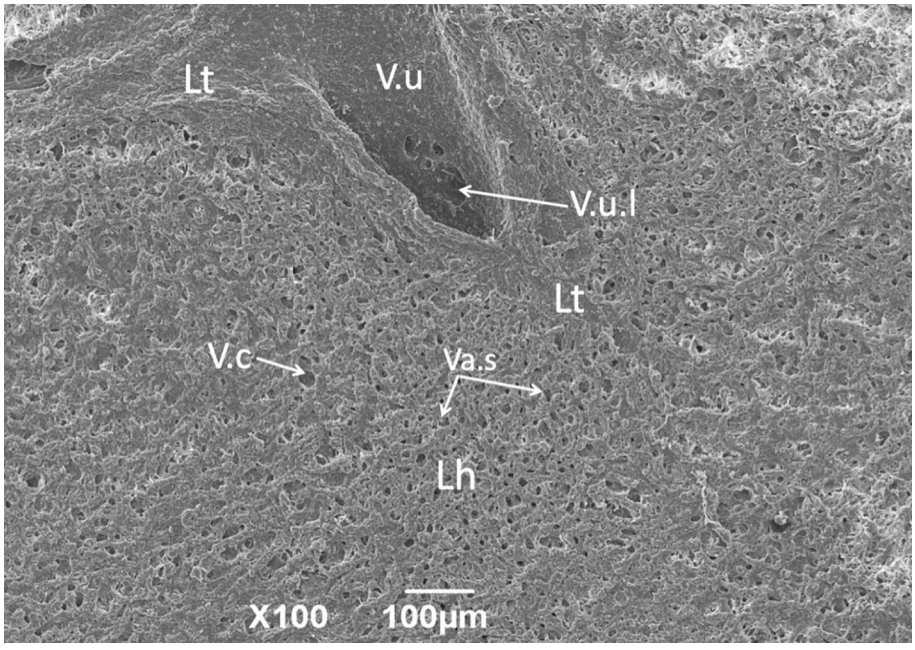

Figure 3. Scanning electron micrograph of the hepatic teres ligament, magnification x100. A dense connective tissue plate lined the hepatic teres ligament (Lt). The umbilical vein (V.u) was situated in the deep of the teres ligament and branched (V.u.l) deep into the hepatic lobule. The central vein (V.c) and sinusoid vessels (Va.s) are shown in the inside of the hepatic lobule parenchyma (Lh).

The collagen fiber bundles of the teres ligament were subdivided into two layers, outer and inner. The outer collagen fibers beneath the mesothelium formed the longitudinal plate, and the inner fibers located under the plate and wrapping the umbilical vein were angled 15-20°, forming a loop-like structure. The outer plate of the ligament gradually connected with the hepatic capsule and the inner plate deeply blended into the collagen fiber network of the hepatic capsule's central diaphragmatic surface (Figure 4).

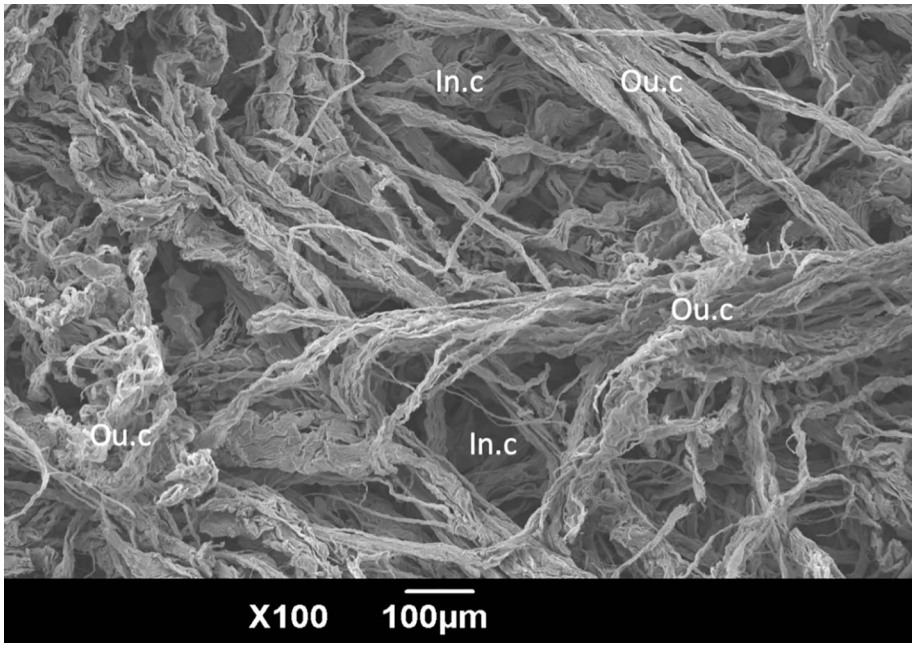

Figure 4. Scanning electron micrograph of hepatic teres ligament's collagen fiber network, magnification x100. The hepatic teres ligament contained the outer (Ou.c) and the inner collagen fibers (In.c), differing in direction and thickness..

Hepatic coronary ligament

The coronary ligament was composed of loose connective tissue fibers rich in blood vessels and had two parallel collagen bundles toward the hepatic capsule. These collagen bundle sheets enclosed the hepatic vein at their junction and gradually merged with the hepatic capsule's connective tissue. The loose connective tissue fibers of the bundle structure were observed in the innermost part of the coronary ligament and hepatic capsule (Figure 5).

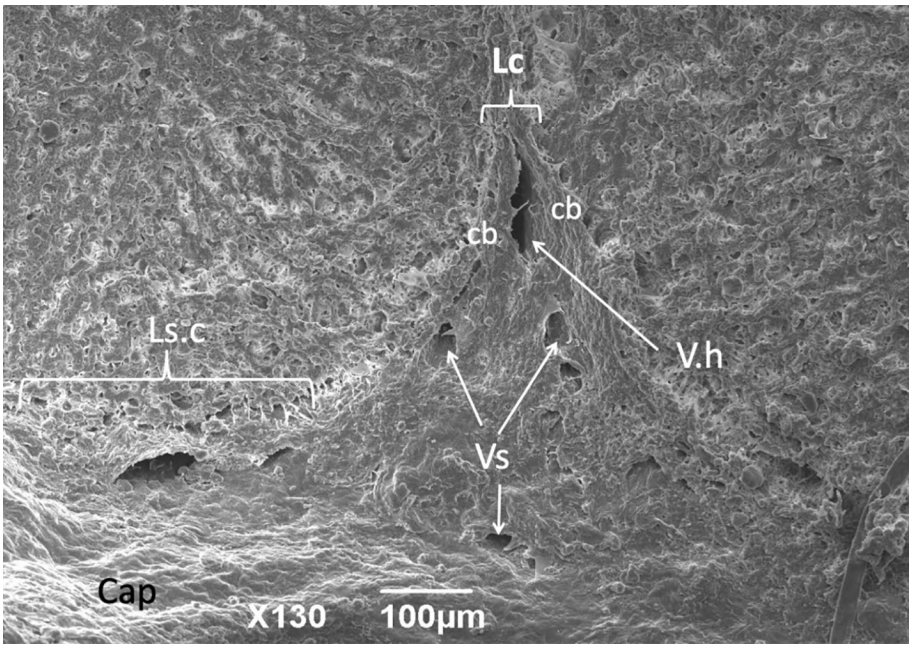

Figure 5. Scanning electron micrograph of the hepatic coronary ligament, magnification x130. The hepatic coronary ligament (Lc) contained the collagen bundles (Cb) rich in blood vessels (Vs). The collagen bundle sheets of the coronary ligament enclosed the hepatic vein (V.h) and gradually merged with the connective tissue of the hepatic capsule (Cap). The loose connective tissue fibers (Ls.c) are shown in the coronary ligament and hepatic capsule's innermost parts.

The hepatic capsule structure

The hepatic capsule possesses two main layers of the outer and inner. The outer layer had the thicker collagen fiber bundles, and the inner layer of this collagen fiber network had an interlaced structure. The outer layer of the hepatic capsule was composed of the thickest collagen fiber bundles. However, we could not measure their diameter in the current study (Figure 6).

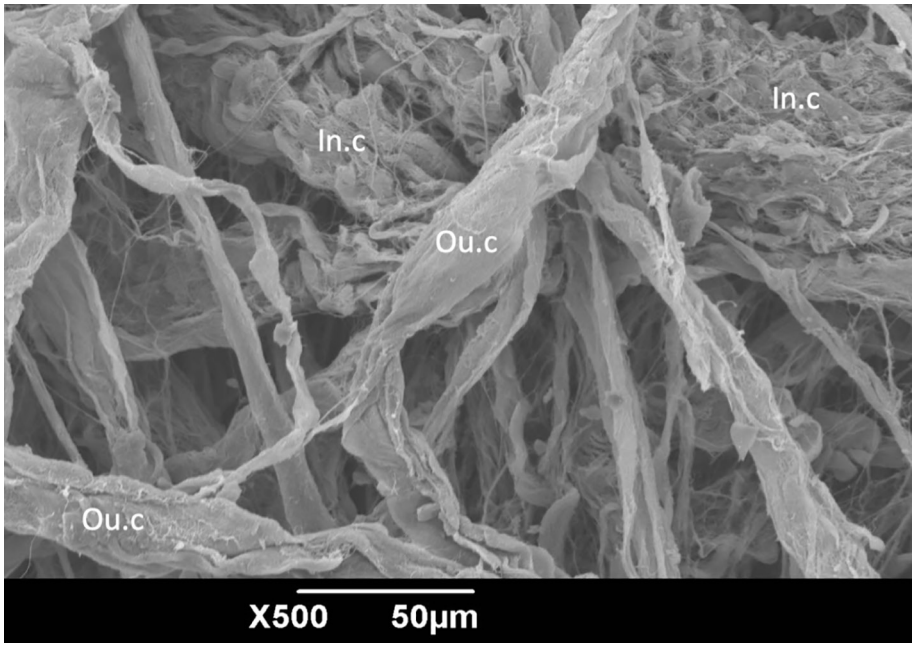

Figure 6. Scanning electron micrograph of collagen fiber network of the hepatic capsule, magnification x500. The hepatic capsule contained the outer collagen fiber bundles (Ou.c) and the inner interlaced plates (In.c).

Discussion

The relationship between the diameter of collagen fibrils and mechanical stress acting on the tissue has been suggested by Parry et al., who pointed out that the diameter of fibrils and a tissue's tensile strength are correlated in several types of tissues [16]. Flint et al. studied the diameter of collagen fibrils from various parts of the skin of various species. They suggested a correlation between the distribution of the fibrils, biochemical conditions and the functional loading of the tissue. Different diameters of collagen fibrils have been reported in various tissues including cartilage [19-21], subepithelial connective tissue of oral mucosa [22, 23], eye [24, 25], peripheral nerve [26, 27], skeletal muscle [28, 29] and tendon [30-32]. From studying these works, it seems that collagen fibers generally have a large diameter in tendons and sclera, where they are exposed to substantial mechanical stress. Matthew and Moore compared the fibrils' diameter distribution in healing tissue after complete and partial transection and suggested that the different diameters can be explained by different levels of mechanical stress [33]. Although we could not measure the collagen fibers in the current study, usually, the outer layer of the hepatic capsule and ligaments appeared thicker than the inner layer. This may reflect the mechanical stress on the collagen framework of the human liver.

It is currently believed that the hepatic ligaments' function is to secure the liver in the epigastrium during the body's various movements [15]. Newer high-level medical imaging devices use the structural status of and the hepatic ligaments to obtain useful information to diagnose hepatic diseases, especially cirrhosis in the early stages [2, 3, 34]. Ryoo and Buschman reported that the part of hepatic ligaments that connect with the hepatic capsule was extensively thickened as in cirrhosis, and the amplitude of the livers' motion was quite diminished, during respiration [17]. Our study defined two or more different layers of the individual ligaments and capsule and with the collagen fibers of the hepatic ligaments blending into the collagen network of the hepatic capsule. We consider Glisson's capsule and the hepatic ligaments to form the entire collagen fiber network, structurally extending to each other and providing structural support for the liver to function.

The liver hilum is connected to the duodenal-hepatic ligament containing the bile duct, hepatic artery, and portal vein. The Glisson's sheath, which surrounds the larger bile duct and is located closer to the hepatic hilum, is thought to receive greater mechanical stress [14]. In the current study, the hepatic ligaments' collagen bundles surrounded the blood vessels, especially in the coronary ligament. These bundles

may play a role in the blood vessel's biological valves during respiration. Consequently, we speculate that the load on the hepatic ligaments increases during inspiration and the liver moves its rounded posterior margin towards the hilum, slightly compressing the bile ducts to excrete bile. During expiration, the diaphragm moves superiorly and posteriorly towards the thoracic cavity. As this occurs, the liver is pulled up by the hepatic coronary ligaments, while being pulled inferiorly by the lesser omentum and gastro-hepatic ligament. During this flapping-like movement of the liver, the mechanical tension in the deep duodenal-hepatic ligament enlarges the portal vein, and venous blood from the peritoneal cavity can easily flow into the portal vein crossing the hepatic hilum. Also, this movement assists with transferring the portal vein blood to the inferior cava vein. It was recently postulated that cholestasis could occur after liver surgery because of scaring, limiting the liver's rhythmic excursion with respiration. Therefore, the gastro-hepatic and duodenal-hepatic ligaments, and the hepatic ligaments, might have an important function in this process.

Research regarding the structural role of connective tissues in the body and their functional characteristics in the internal organs started in the 1970s. The previously referenced studies broadly described the collagen fiber types forming the human liver's connective tissue framework and its various biological functions. In the current study, we distinctly defined the hepatic ligaments' connective tissue structure as layered. However, previous works revealed the hepatic ligaments and capsule were connected. We examined the layered connective tissue structure of the individual ligaments and identified that they are divided mainly into two groups of fibers - outer and inner. The outer and inner groups of collagen fibers are clearly different in location and direction, playing different roles in the liver capsule and parenchyma's connective tissue. We did not define the intralobular connective tissue in this study. The collagen fiber network of the hepatic ligaments and capsule, the diaphragm's connective tissue, and the peritoneum are still not clearly defined.

In the current study, there were some limitations. We analyzed relatively few samples due to the difficulties of collecting normal hepatic tissue from the cadaver specimens. We used only morphological methods. Further studies are required to understand this connective tissue network entirely. We need to investigate the ligament's collagen types and the inter- and intralobular connective tissue's ultra-structure and their connection to the hepatic capsule and ligaments. Thereafter, we need to enrich our research methods with the novel invasive and noninvasive methods and to compare the connective tissue network of the normal and fibrotic liver in the living people instead of cadaver specimens.

Conclusion

The hepatic ligaments and liver capsule are a layered structure of collagen fibers differing in direction. The hepatic ligaments' outer layer connected with the liver capsule's collagen fibers and the inner layer merged with the hepatic lobular parenchyma's connective tissue.

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