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Review

Cartilage: A new parameter for the determination of the postmortem interval?

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ABSTRACT

The determination of the time of death or the postmortem interval (PMI) is one of the most important and frequently asked questions in forensic medicine. The methods used for PMI determination are based largely on early and late postmortem changes. The determination of the PMI during the late postmortem changes is based primarily on a subjective assessment and is less precise due to the lack of objective methods. Different studies have presented a gradual decrease in chondrocytes' viability but these researches did not answer the question whether we can use the decrease of chondrocytes' viability for an objective PMI determination. The structure and anatomical location of the cartilage together with its mechanical, physical and chemical properties enable chondrocytes to survive for several weeks after the individual's death, and give cartilage the attributes of a compartment. Therefore, cartilage could be a new parameter for PMI determination. This idea had been partially confirmed by a few *in vitro* studies. The next step in testing this idea should be an extensive *in corpore* study.

1. Introduction

The determination of the time of death or postmortem interval (PMI) is one of the most important and most frequently asked questions in forensic practice. Despite the fact that the determination of PMI is among the most researched areas of forensic medicine, the results of these studies are relatively modest.

Eighteen years ago a group of leading experts in the field of forensic medicine collected the results of these studies and published the first book on the topic of PMI determining,[1] especially during the development of early postmortem changes, such as body cooling, *rigor mortis*, and *livores mortis*, whose dynamics help to determine the PMI, but only during the first 24–36 h after death or until the postmortem changes due to putrefaction. In 2002, the same group of experts prepared the second edition,[2] which was a reprint of the first edition with an added chapter on determining the PMI based on the stage of digestion after a meal and gastric emptying. This was particularly emphasized by other experts in the field of forensic medicine,[3,4] but in the foreword to the second edition Knight explained that in seven years since the first edition relatively few original researches of PMI determination had been published. Even in the last decade important progress on this issue has not been reported.[5–8]

Among the other methods which could be used for PMI determination in the first hours and days after death are the assessment of the supravital reactivity of the skeletal muscles after mechanical or electrical stimulation, and the chemical or histological changes in the compartments (parts of the body that are anatomically separate from their surroundings and thus less affected by putrefaction) mainly changing the concentration of potassium in the vitreous humor.[9–12] According to the available databases, the last survey of changes in the compartments for PMI determination considered the postmortal decline in the number of odontoblasts in the dental pulp.[13] Histological and biochemical changes in other organs, such as in the heart or liver, were also studied for PMI determination but their practical use is questionable.[14,15]

However, Henssge and Madea pointed out that every forthcoming parameter for the practical relevance in evaluating the PMI should fulfill the following criteria: the quantitative measurement, mathematical description, taking into account the influencing factors quantitatively, a declaration of the precision and the proof of precision on independent material.[16]

The intention of this paper was to answer whether cartilage could be determined as a compartment, and if cartilage could be used for the determination of PMI. Therefore, in the paper there were described the particular attributes of the cartilage and the facts that supported this idea.

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2. Could cartilage be determined as a compartment?

Cartilage is a highly specialized connective tissue that acts as a support tissue which consists entirely of cartilage cells (chondrocytes), and a large amount of intercellular mass called the extracellular matrix (ECM). There are three types of cartilage:[17–21]

- hyaline cartilage (the surface of articular ends, parts of the nose and respiratory branches, and the ends of the ribs)
- elastic cartilage (the outer ear, Eustachian tube, and epiglottis)
- fibrocartilage (the mandibular and sternoclavicular joint, intervertebral discs, symphysis pubis, menisci, and tendon insertions)

Hyaline cartilage in the synovial joints is a highly specialized tissue that is adapted to operate in a highly stressed environment, and usually without malfunctioning during an individual's entire life. The main tasks of the joint cartilage are the distribution of loads over a wider surface, and minimizing articular friction and damage during articular movements.[22–25] Articular cartilage is an isolated tissue without nerves or blood and lymphatic vessels. Cartilage such as in the ribs is exceptionally minimally vascularized. The chondrocytes in the articular cartilage are supplied only by diffusion through the ECM. These elements limit the final thickness of the cartilage, which is typically 1–6 mm.[19,25]

2.1. Cartilage cells (chondrocytes)

The cartilage solely contains a homogeneous cell population of chondrocytes. The density of the cells in the cartilage tissue is lower than in any other tissue and occupies up to 10% of the volume.[26]

The density and distribution of chondrocytes in the uncalcified part of the articular cartilage is different (Fig. 1). In the superficial tangential zone (layer) there is a high density of oval-shaped chondrocytes, whose longer axis is parallel to the surface of the cartilage. In the middle zone (intermediate layer) there are round chondrocytes, which are randomly distributed and diluted. The chondrocytes in the deep zone are rounded and arranged in the form of pillars, which are vertical to the boundary (tidemark) between the uncalcified and calcified part of the cartilage. Chondrocytes are supposed to act differently in the different layers.[25,27,28]

Chondrocytes, though small in number, produce, secrete, set up and maintain the organic parts of the ECM, whose ingredients are in a constant state of dynamic equilibrium, homeostasis (continuous “turn over”), which means that the state of the ECM depends entirely on the chondrocytes. In comparison to other cells, chondrocytes live in modest conditions: having a low supply of nutrients, reduced oxygen concentration and acidosis. Increased oxygen concentration even inhibits chondrocyte functioning, on the contrary hypoxia induces collagen crosslinking and enhances the mechanical properties of the articular cartilage.[17–19,25,28–33]

Medical conditions such as osteoarthritis increase the permeability of the cartilage which lowers its resistance to pressure. The changed load carrying capacity has an influence on the chondrocytes and forms an imbalance between the chondrocytes' anabolic and catabolic activities, which leads to a vicious circle of progressive cartilage degeneration.[25,34,35]

2.2. Extracellular matrix (ECM)

The ECM of the articular cartilage forms a dense network of collagen fibers, especially type II with small amounts of collagen types V, VI, IX, X and XI, which are encased in a ground substance, a concentrated solution of proteoglycan (PG).[30,32,36–38]

Parts of the articular cartilage are usually: collagen (15–22%), PG (4 and 7%), and the rest is water with inorganic salts and a small amount of matrix proteins, glycoproteins and lipids. Collagen fibers and the PGs are able to create a highly robust network structure by themselves but the real biomechanical properties of these structures are only shown with water.[24,28,39–46]

2.2.1. Collagen

Collagen fibers in hyaline cartilage are usually arranged in a three-dimensional structure that resembles felt. Collagen is secreted in the ECM as tropocollagen, which is the basic biological unit made of three procollagen polypeptide chains, alpha helices, twisted to the left, which are further wrapped one around the other to the right triple helix. Tropocollagen rod-shaped molecules polymerize into large collagen fibrils.[17,19,36,37]

The most important mechanical feature of the collagen fibers is their resistance to elongation, which is enabled by an arrangement of tropocollagen molecules, wherein each tropocollagen molecule overlaps the other molecules by one quarter of its length. This provides a striation to the collagen fibers. Covalent cross-links between the tropocollagen molecules further contribute to the stretching resistance of the collagen fibers.[25,37]

The collagen fibers of the articular cartilage are arranged in a non-homogenous way and give it the appearance of tissue with three layers, zones (Fig. 1). The superficial, tangential zone is 10–20% of the total thickness of the articular cartilage. It contains densely packed fibers, which run parallel to the smooth surface of the articular cartilage, and is called *lamina splendens*. In the middle zone, which is 40–60% of the total articular cartilage thickness, the collagen fibers are orientated in all directions, homogeneously dispersed, and less densely packed with a greater spacing between

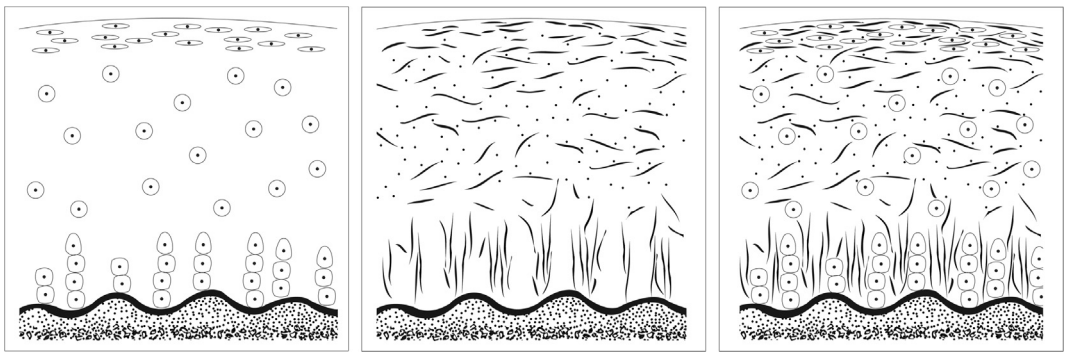

Fig. 1. Histological structure of articular cartilage. *Left*: position and shape of chondrocytes in layers (zones). *Middle*: distribution of fibers in the extracellular matrix and zones. *Right*: chondrocytes and the extracellular matrix together.