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Comparative Studies on the Morphology of Chondrocytes, Adipocytes and Adipochondrocytes in New Zealand White Rabbits

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Abstract

Adult cartilage comes in three different types: hyaline cartilage, elastic cartilage, and fibrocartilage. In several forms of cartilage, chondrocytes are described as a one-cell population. Chondrocytes are the manufacturers of the surrounding ECM and collagen type II fibers in hyaline cartilage besides the elastic fibers in elastic cartilage. Whereas the white adipocytes mainly compose the white adipose tissue and they are specialized in production, storage and mobilization of triglycerides. Early studies explored a unique type of chondrocyte in mouse, rat, and rabbit auricular cartilage having morphology similar to white adipocytes and identified it as "adipochondrocyte". The objective of the current study was to explore the differences between chondrocyte, adipocyte and adipochondrocyte morphologies in white New Zealand rabbits and to ascertain if adipochondrocyte is more comparable to chondrocyte or adipocyte morphology. The auricles, articular cartilage of carpal joint, and pre-renal white fat of adult male white rabbits were harvested and processed for histological examination with light and transmission electron microscopy. The adipochondrocytes appeared as hypertrophic white adipocyte-like chondrocytes occupied the auricular cartilage plate of the white New Zealand rabbits, similar to the characteristic "signet ring" appearance of the white adipocytes in pre-renal white fat. The adipochondrocytes were housed in lacunae within an ECM similar to chondrocytes of articular cartilage. The TEM examination had illuminated that the adipochondrocyte cytoplasm contained large lipid globule that flattened the eccentric nucleus and sparse organelles. Further studies are suggested to exploring molecular and functional features of adipochondrocytes, chondrocytes and adipocytes.

Keywords: Adipochondrocytes, chondrocytes, adipocytes, Rabbit.

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Introduction

In animals, cartilage is a specialized type of connective tissue (Prydz, 2015). Adult cartilage comes in three different types: hyaline cartilage, elastic cartilage, and fibrocartilage. Each type supports particular musculoskeletal system functions (Amerman, 2021, Mescher, 2018). The C rings of the trachea, the laryngeal, costal, and nasal cartilages, as well as the articulating surfaces of the majority of bones include hyaline cartilage (Bissell and Steele, 2010, Carballo et al., 2017, Huber et al., 2000). The epiglottis, external ear, ear canal, and some of the smaller laryngeal cartilages include elastic cartilage (Gartner, 2020). Nonetheless, there are a few locations where fibrocartilage can be found, including some symphyses, the eustachian tube, intervertebral discs, and some articular discs (Gartner, 2020). Every form of cartilage is an avascular tissue made up of chondrocytes that are embedded in lacunae and are surrounded by extracellular matrix (ECM), which is produced and maintained by the chondrocytes themselves (Ahmed, 2007, Teng et al., 2017). The ECM consists of glycosaminoglycans (GAGs), proteoglycans, and fibers. Based on the majority and type of each structure of the ECM, the three cartilage subtypes could be differentiated (Martini et al., 2011, Naumann et al., 2002). The most prevalent and distinctive type of cartilage is the hyaline cartilage, which is enclosed by a well-defined perichondrium and is made up of a translucent matrix that seems glassy. Collagen fibers type II, water, and ground substances are the components of the matrix (Eyre, 2002, Gartner, 2020, Teng et al., 2017).

Chondrocytes in hyaline cartilage scattered throughout the matrix attach via transmembrane proteins to the macromolecular framework they synthesize (Casale and Crane, 2019). Chondrocytes are flattened cells near the perichondrium (immature chondrocytes) and more rounded in deeper regions (mature chondrocytes). Chondrocytes in deeper regions are often arranged in pairs or groups of four to six, known as an isogenous nest because they are the offspring of a single chondrocyte during development (Sophia Fox et al., 2009, Nahian and Sapra, 2022). Even though the elastic cartilage has the same structure as hyaline one, the matrix also features collagen type II fibers embedded in a tiny amount of amorphous extracellular ground substance in addition to a thick, interwoven network of elastic fibers. The spherical chondrocytes in lacunae resemble those in hyaline cartilage in appearance, but they are more closely packed and are frequently seen singly, and isogenous nests are uncommon (Wachsmuth et al., 2006, Standring, 2021). A perichondrium surrounds elastic cartilage, just like it does hyaline cartilage (Gartner, 2020). Elastic cartilage in adult rats, mice, and rabbit's auricle is unique as its chondrocytes are inhabited by large fat droplets resembling the white adipocytes, and its ECM is sparse; however it is rich in elastic fibers and has never been mineralized (Sanzone and Reith, 1976, Mallinger and Böck, 1985, Kostović-Knežević et al., 1981, Bradamante et al., 1991, AHMED and Abdelsabour-Khalaf, 2018). According to Sanzone and Reith (1976), these chondrocytes are known as "lipochondrocytes," but more recently, these cells in the rabbit's auricular cartilage

are known as "adipochondrocytes." (AHMED and Abdelsabour-Khalaf, 2018). White adipocytes, on the other hand, are cells of the white adipose connective tissue (WAT) that are specialized in the production, storage, and mobilization of triglycerides (Walther and Farese Jr, 2012). The white adipocyte is a spherical cell that has a single cytoplasmic lipid globule that occupies the majority of its volume and is encircled by a cytoplasmic film (Mota de Sá et al., 2017). The current study was carried out with the aim of exploring the variance in the morphology of chondrocytes, adipocytes, and adipochondrocytes in white New Zealand rabbits and to determine if adipochondrocyte morphology is more similar to chondrocyte or adipocyte morphology.

Materials and Methods

This study was performed with the approval from the Research Bioethics Committee (RBC) at Faculty of Veterinary Medicine, South Valley University research ethics under approval number "VM/SVU/23(2)-26".

Study area

The current study was performed in the Histology Research Laboratory, Department of Histology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Sampling

Apparently healthy, mature (6-month-old) male New Zealand White rabbits (n = 20) were obtained from VACSERA (Serum and Vaccine Center, 51 Ministry of Agriculture St., Dokki, Cairo, Egypt). The animals were slaughtered, and small pieces (0.5 mm) of auricles (Adipochondrocyte), knee joint articular cartilage (Chondrocyte), and pre-renal white fat (Adipocyte) were dissected. Sample specimens were washed well with normal saline (0.9% sodium chloride) and were fixed in 10% neutral buffered formalin (NPF; pH = 7.4) for 24 hours for the light microscopy and in 2.5% glutaraldehyde (10% neutral buffered formalin: 2.5% glutaraldehyde = 1:1) for 48 hours at 4 °C for examination with the transmission electron microscopy (TEM).

Light microscopy

Neutral-buffering formalin-fixed samples were dehydrated in ascending degrees of ethanol, cleared in methyl benzoate, then impregnated and embedded in paraffin wax. Paraffin sections (3-5 m thickness) were undertaken by the LEICA HistoCore AUTOCUT- Automated Rotary Microtome and were stained with hematoxylin and eosin (H&E) as a general stain, periodic acid–schiff (PAS) for neutral GAGs, alcian blue for acidic GAGs, combined PAS-alcian blue for both types of GAGs, Masson's trichrome for collagen fibers, Verhoeff-Van Gieson (VVG) for elastic fibers, and osmium tetraoxin (Osmium) (Abd-Elhafeez et al., 2020a, Abd-Elhafeez et al., 2020b, Bancroft and Stevens, 1990, Gates et al., 2016). Sections were examined with the light microscope (Leica DMLS). Photographs were captured with Leica digital camera (Leica ICC50) using ×4, ×10, ×40 and ×100 objectives in JPEG format.

Transmission Electron microscopy

The samples were post-fixed in 1% osmium tetraoxide and washed by buffer phosphate, then were dehydrated in ascending grades of ethanol, then in propylene oxide. After that, the samples were infiltrated and embedded in Epon's resin. Semi-thin (0.5 m) sections were taken, stained with toluidine blue, and were examined by light microscopy.

Ultrathin sections (60–80 nm) were taken by ultratome (LEICA UCT), stained with uranyl acetate and Reynolds stain, and were examined by a transmission electron microscope (TEM) (JIOL 1010) at the Central Laboratory of the SVU.

Results

Morphology of Chondrocytes in the Articular Cartilage Covering Femur Bone in Knee Joint of the White New Zealand Rabbits

Articular cartilage covering femur bone in knee joint of the white New Zealand rabbits was hyaline cartilage that wasn't surrounded by a perichondrium (Fig. 1A). This articular hyaline cartilage was composed of the chondrocytes, which were located inside lacunae surrounded by a dense extracellular matrix (ECM). The chondrocyte appeared oval to spherical cells with large oval to spherical nucleus in transitional zone of the articular cartilage, and some chondrocytes were observed in isogenous groups (Fig. 1A, Fig. 2A). The cytoplasm of the chondrocyte contained few small lipid droplets that were appeared as empty spaces in paraffin section stained with H&E (Fig. 2A) and were clearly observed as small black droplets in osmium tetroxide stained specimens (Fig. 3A). The ECM was subdivided into pericellular matrix, which surrounded the chondrocyte in the vicinity of the lacunae, a territorial matrix which surrounded the pericellular matrix, and the interterritorial matrix which was the biggest zone of ECM between the chondrocytes (Fig. 2A). Proteoglycans and collagen fiber type II were the main components of the ECM secreted by chondrocytes and were stained positively with Crossman's Trichrome stain (Fig. 1D, Fig. 2D). As well, collagen fibers were stained red by Verhoeff-Van Gieson (VVG) stain (Fig. 1G, Fig. 2G). The pericellular matrix positively reacted with alcian blue and PAS because it contained both of acidic and neutral glycosaminoglycan-rich cartilage extracellular matrix (GAGs), while, the interterritorial matrix gave positive with PAS and weak positive with alcian blue (Fig. 4A, D and G).

The Transmission Electron microscope (TEM) examination shown that the chondrocyte contained large nucleus had euchromatin and heterochromatin (Fig. 5A) and was bounded by a well-defined nuclear envelope with dilated nuclear intramembrane space and outer nuclear membrane studded with ribosomes. The cytoplasm was full with a well-developed rough endoplasmic reticulum (RER). The RER was consisted of parallel arrays of narrow cisternae studded with ribosomes and had a dilated ends of cisternae. The chondrocyte was characterized by having cell processes and cell extrusions (Fig. 5D). The pericellular matrix had a finely fibrillar appearance and the surrounding territorial matrix and interterritorial matrix contained type II collagen fiber (Fig. 5G).

Adipocytes Morphology in the Pre-renal White Fat

The white adipose connective tissue in the pre-renal white fat was formed mainly by white adipocyte surrounded by loose connective tissue (Fig. 1B). The white adipocyte was composed of a single large lipid globule occupies almost the entire cell cytoplasm and the nucleus rejected at the periphery of the cell (Fig. 2B). The lipid globule was dissolved through preparation of paraffin section leaving large empty space surrounded by a rim of cytoplasm, the characteristic "signet ring" appearance of the adipocytes (Fig. 2B). For

demonstration of fat on paraffin sections, the specimens were stained with osmium tetroxide and the lipid was appeared as large black globule almost filling the cell (Fig. 3B). Also the lipid globule was clearly observed in semithin section as large black or gray single droplet full all the cell leaving a thin rim of cytoplasm stained with toluidine blue (Fig. 3E). The extracellular loose c.t reacted positively with Crossman's Trichrome and Verhoeff-Van Gieson (VVG) stains (Fig. 1E, H). Additionally, the white adipose c.t presented positive reaction with PAS and gave weak reaction with alcian blue stain (Fig. 4B, E and H).

The ultrastructure of the white adipocyte by TEM revealed that the thin rim of cytoplasm surrounded the single large lipid globule was contained most cytoplasmic organelles including small flattened periphery located nucleus where heterochromatin and euchromatin were existed (Fig. 5B). The thin rim of cytoplasm contained minute lipid droplets beside the large globule, mitochondria, Golgi apparatus and a few cisternae of RER (Fig. 5E, H).

Adipochondrocytes Morphology in the Auricular Cartilage Plate of the White New Zealand Rabbits

The adipochondrocytes were appeared as a hypertrophic white adipocyte-like chondrocytes occupied the auricular cartilage plate of the white New Zealand rabbits. The adipochondrocytes were housed in rounded to oval lacunae and occupied most of the central zone of the auricular cartilage. They were separated from each other by a sparse ECM and were enclosed by collagenous tissue of perichondrium. Moreover, there were smaller ovoid cells between the central hypertrophied adipochondrocytes and the perichondrium containing variable amounts of lipid droplets (Fig. 1C). Adipochondrocytes cytoplasm was mostly occupied by single large rounded to oval lipid globule, but some cells were contained smaller lipid droplets. Through paraffin section preparation, the lipid droplets of the adipochondrocytes dissolved leaving empty spaces surrounded by a rim of cytoplasm, similar to the "signet ring" appearance of the adipocytes (Fig. 2C). In osmium tetroxide-stained specimens, the lipid was look like as large droplets almost filling the cell in paraffin section (Fig. 3C) and in semithin section it was leaving a thin rim of toluidine blue-stained cytoplasm (Fig. 3F). The adipochondrocytes had flattened peripherally located nucleus, while some cells were binucleated (Fig. 1C, Fig. 2C). The interterritorial and extracellular matrix between the adipochondrocytes contained abundant amount of elastic fibers stained positively with Verhoeff-Van Gieson (VVG) stain (Fig. 2I) besides the collagen fibers, which were demonstrated by Crossman's Trichrome stain (Fig. 2F). Moreover, the interterritorial matrix gave positive with PAS (Fig. 4C, I), but weak positive with alcian blue (Fig. 4F, I). Also, pericellular matrix stained strongly positive with alcian blue and positive with PAS because it contained both of acidic and neutral glycosaminoglycan-rich cartilage extracellular matrix (GAGs) (Fig. 4I).

The TEM examination illuminated that the adipochondrocyte contained large lipid globule that was surrounded by amorphous dark areas in the electron dense cytoplasm and dark nucleus (Fig. 5C). The cytoplasm contained sparse organelles and secretory granules (Fig. 5F). The plasma

membrane showed many cellular processes extended into a proteoglycan-rich ECM (Fig. 5C, I). The interterritorial matrix was consisted of a network of dense-granule proteoglycans integrated with amorphous areas of fibers were seen in some areas (Fig. 5I).

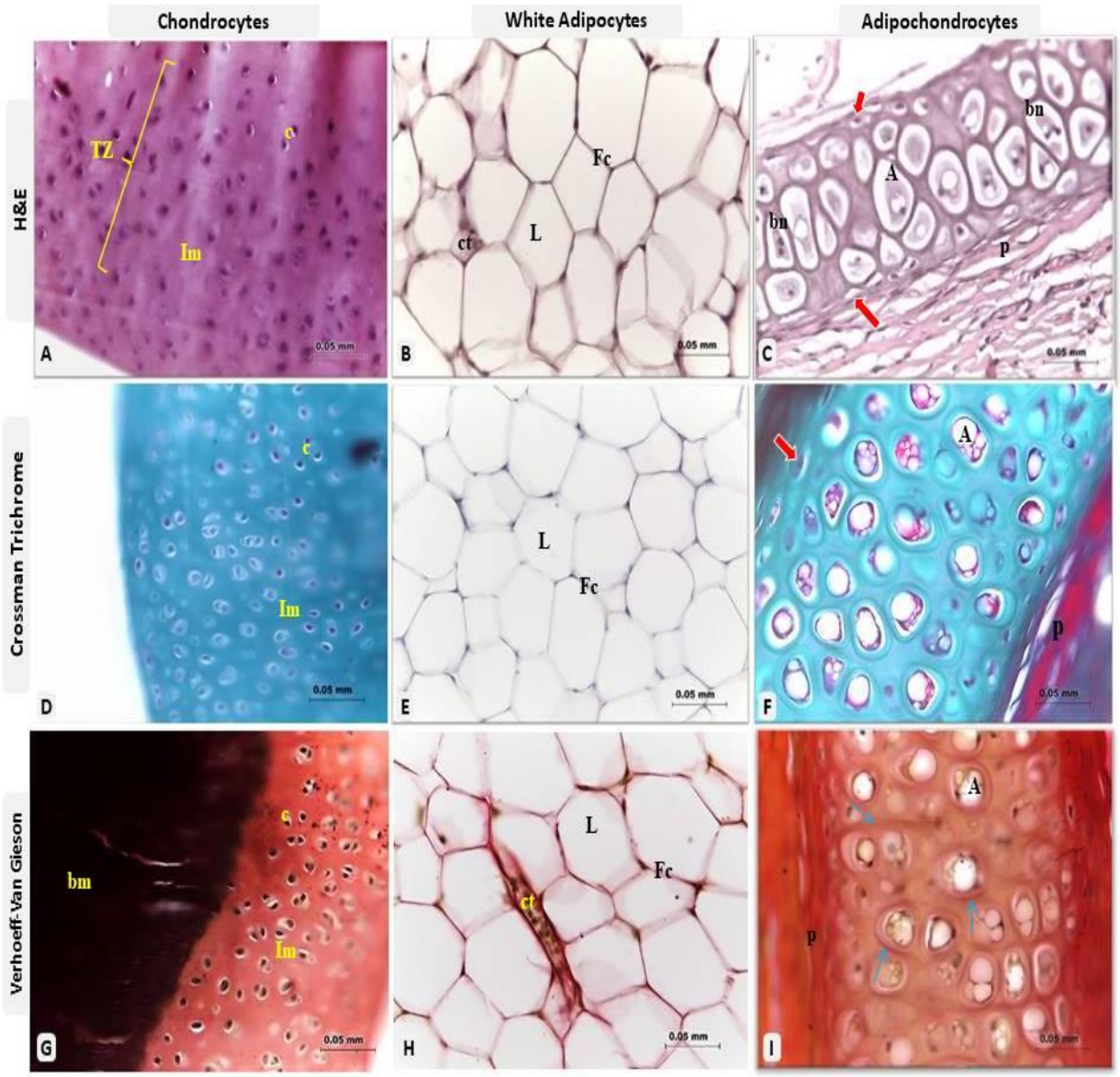

Figure 1: Histological Structure of Articular Cartilage, Pre-Renal White Fat and Auricular Cartilage in Adult White New Zealand Rabbits

Light micrographs of paraffin sections of articular cartilage covering femur bone in the knee joint (A, D, G), white adipocytes in pre-renal white adipose connective tissue (B, E, H) and adipochondrocytes in auricular cartilage (C, F, I) stained with H&E (A-C), Crossman trichrome (D-F), and Verhoeff–Van Gieson Elastic (G-I) stains. Transitional zone of articular cartilage (TZ), inter-territorial ECM (Im), chondrocyte inside lacune (c), bone matrix (Bm), interstitial connective tissue (ct), lipid globule (L), fat cell (FC), adipochondrocyte inside lacune (A), binucleated cell (bn), perichondrium (p), ovoid chondrocyte (red arrow) and elastic fibers (blue arrows); bars= 0.05 mm.

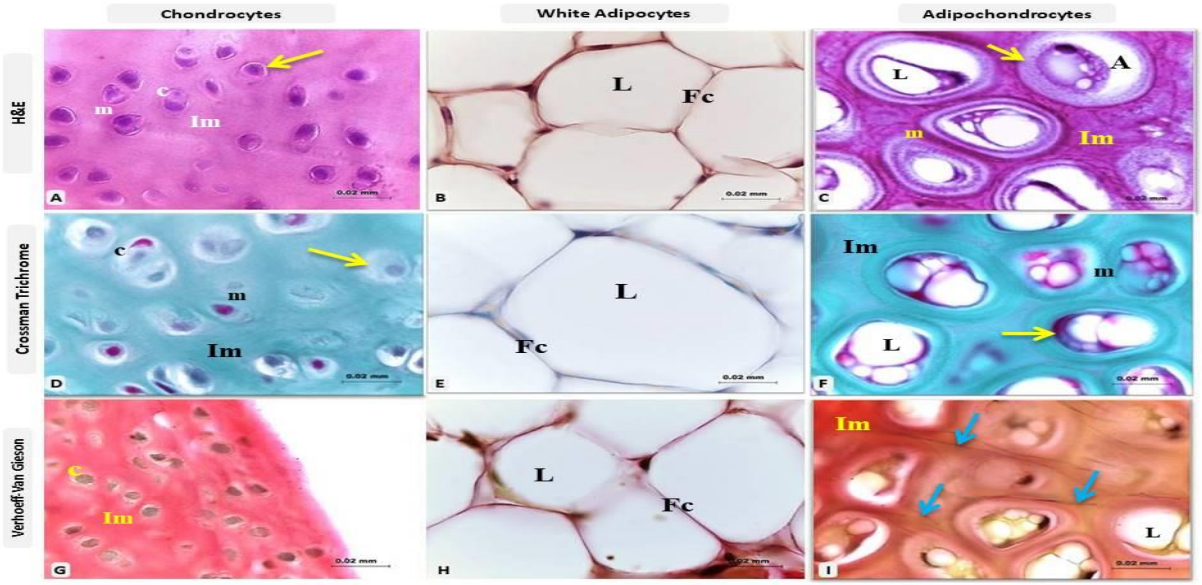

Figure 2: Chondrocytes, Adipocytes and Adipochondrocytes Morphology in Adult Rabbits Articular Cartilage, Pre-Renal White Fat and Auricular Cartilage

Light micrographs of paraffin sections illustrate chondrocytes in articular hyaline cartilage covering fumer bone (A, D, G), white adipocytes in pre-renal white adipose connective tissue (B, E, H) and adipochondrocytes in auricular cartilage (C, F, I) stained with H&E (A-C), Crossman trichrome (D-F) and Verhoeff–Van Gieson Elastic stain (G-I). Chondrocyte inside lacune (c), pericellular matrix (yellow arrow), inter-territorial matrix (Im), territorial matrix ECM (m), lipid globule (L), fat cell (FC), adipochondrocyte inside lacune (A) and elastic fibers (blue arrows); bars= 0.02 mm.

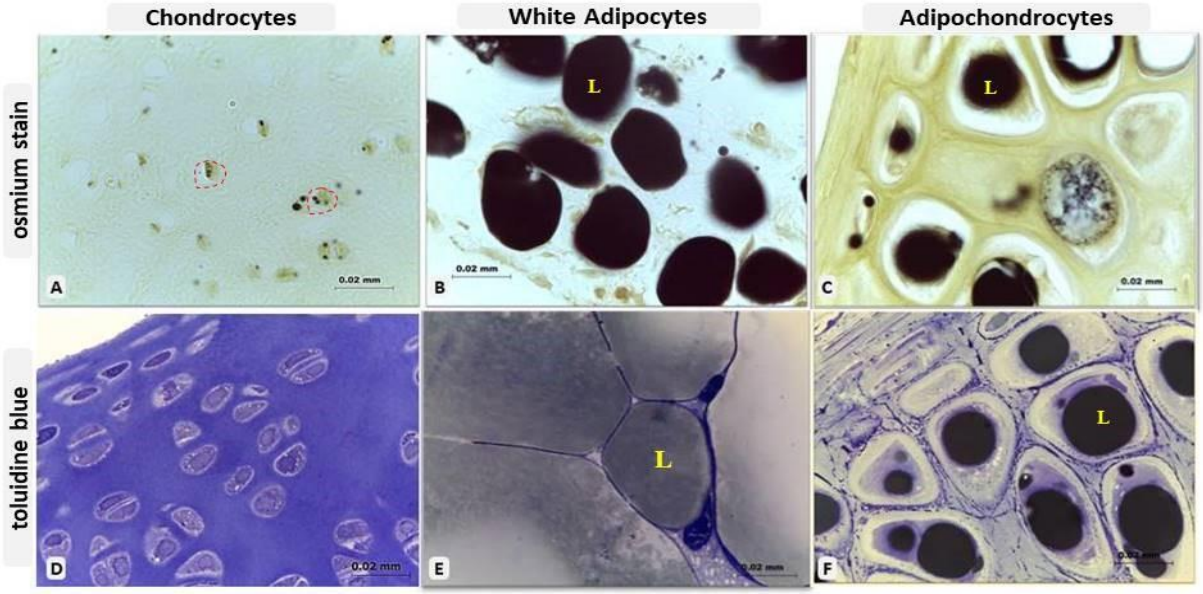

Figure 3: Variation of Lipid Content in Chondrocytes, Adipocytes and Adipochondrocytes in Adult White Rabbits

Light micrographs of paraffin sections stained by osmium (A, B, C) and Semithin sections stained by toluidine blue (D, E, F) in adult rabbit's articular hyaline cartilage (A, D), pre-renal white fat (B, E) and auricular cartilage (C, F). The figures clarify the lipid content (L); bars= 0.02 mm.

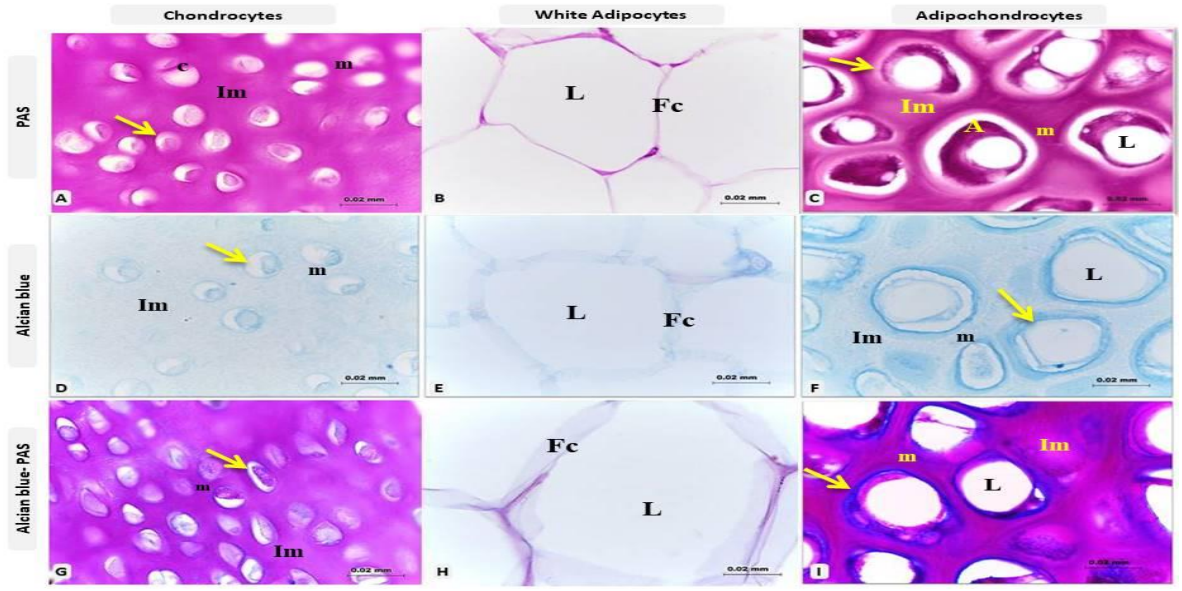

Figure 4: The ECM Morphology in Adult Rabbits Articular Hyaline Cartilage, Auricular Cartilage and Pre-Renal White Fat

Light micrographs of paraffin sections show ECM in articular hyaline cartilage (A, D, G), pre-renal white adipose connective tissue (B, E, H), auricular cartilage (C, F, I) stained with PAS (A-C), Alcian blue (D-F), and Alcian blue- PAS (G-I). Chondrocyte inside lacune (c), pericellular matrix (yellow arrow), territorial matrix ECM (m), inter-territorial matrix (Im), lipid globule (L), fat cell (FC) and adipochondrocyte inside lacune (A); bars= 0.02 mm.

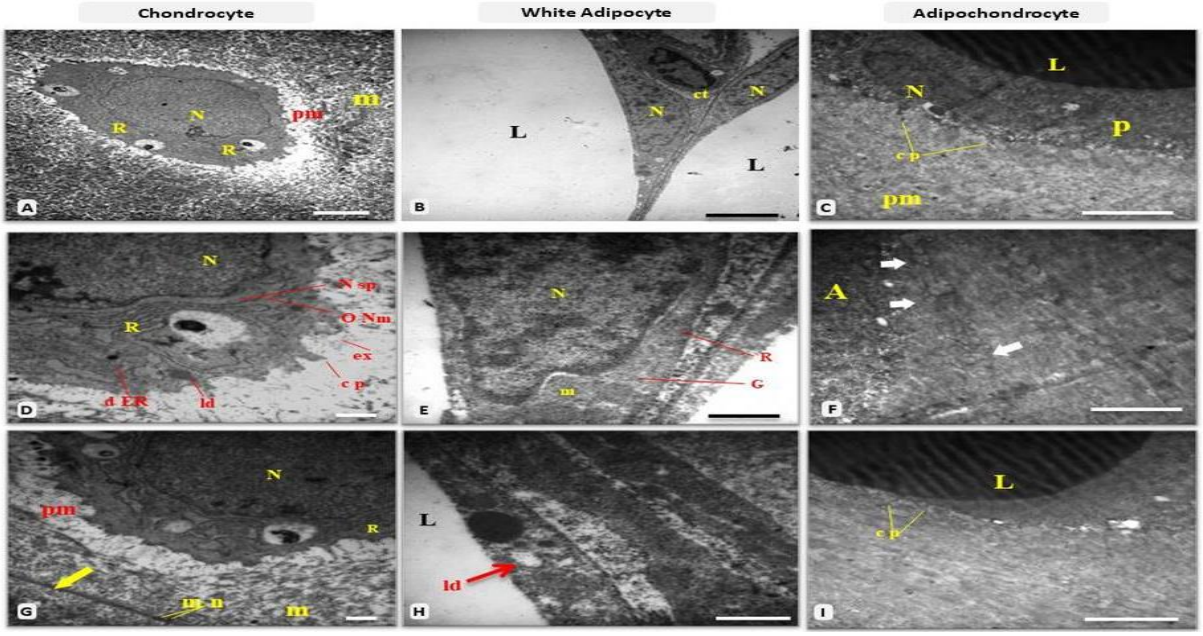

Figure 5: Chondrocytes, Adipocytes and Adipochondrocytes Ultrastructures in Adult Rabbit Articular Cartilage, Pre-Renal White Fat and Auricular Cartilage

Electron micrographs of chondrocytes in articular hyaline cartilage in knee joint (A, D, G), Adipocytes in pre-renal white fat (B, E, H) and Adipochondrocytes in auricular cartilage (C, F, I) stained with Uranyl acetate and Reynold's stains. RER (R), nucleus (N), dilated nuclear intramembrane space (N sp), outer nuclear membrane (O Nm), cytoplasmic processes (c p), cell extrusions (ex), pericellular matrix (pm), territorial matrix (m), collagen fiber (yellow arrow), mineralized nodules (m n), large lipid globule (L), cytoplasm (p), small lipid droplet (ld), mitochondria (m), connective tissue (ct), adipochondrocyte (A), golgi apparatus (G) and secretory granules (white arrows); Bars= 500 nm in (A, B, C, F and I) and 100 nm in (D, G, E and H).

Discussion

Chondrocytes are usually described as one cell population and the sole cell type comprising all subtypes of the cartilage tissue (Salinas et al., 2019, Vincent and Wann, 2019). Some studies described that chondrocytes in the auricular cartilage of mice, rats, and rabbits are unique and have a morphology similar to that of a white adipocyte, as their cytoplasm is mostly occupied by large lipid globule, and they were termed "adipochondrocytes" or lipochondrocytes" (AHMED and Abdelsabour-Khalaf, 2018, Sanzone and Reith, 1976). The current study was carried out with the aim of rediscovering adipochondrocytes and exploring the similarity of their morphology to chondrocyte and adipocyte morphology in adult white New Zealand rabbits.

Our investigation demonstrated that the chondrocytes in the articular cartilage covering the femur bone in the knee joint were situated inside lacunae that were enclosed by the ECM. The chondrocyte in the transitional zone were oval to spherical cells with large oval to spherical nuclei. Chondrocyte cytoplasm contained a few small lipid droplets that appeared as empty spaces in the paraffin section and as small black droplets in the osmium tetroxide-stained specimens. Some studies have illustrated how those stratification zones of articular cartilage relate to stages of cell differentiation since chondrocytes in the most superficial zone are small, flattened, and immature cells, and the transitional zone includes proliferating spherical chondrocytes (Gadjanski et al., 2012, Matsiko et al., 2013, Newman, 1998). Proteoglycans and collagen fiber type II secreted by chondrocytes were the main components of the ECM, which was partitioned into a pericellular matrix, a territorial matrix, and an interterritorial matrix. Proteoglycans and ECM were stained positively with Crossman's trichrome stain. The pericellular matrix reacted positively with alcian blue and positively with PAS, and the interterritorial matrix gave a positive with PAS and a weak positive with alcian blue. Also, collagen fibers were stained red by the Verhoeff-Van Gieson (VVG) stain. The ECM of hyaline cartilage was described as being rich in proteoglycans and type II collagen fibers expressed by chondrocytes, giving a positive reaction with Trichrome stains, PAS, Alcian blue, and Bismarck brown stains (Deņisova et al., 2022, Endres et al., 2012, Gaytan et al., 2020, Nanduri et al., 2014). The ultrastructure of the chondrocyte exposed that its cytoplasm contained a large nucleus bounded by a well-defined nuclear envelope with a dilated nuclear intramembranous space and an outer nuclear membrane studded with ribosomes. The cytoplasm of the chondrocyte had a well-developed RER with dilated ends of cisternae, cell processes, and cell extrusions extended into the ECM that had a finely fibrillar appearance and contained collagen type II fiber. Other research has described the ultrastructure of the chondrocyte as having an euchromatic nucleus, and the cytoplasm contains abundant RER, mitochondria, lipid droplets, and glycogen granules (Fioravanti et al., 2010, Keenan et al., 2019, Marlovits et al., 2003, Raabe et al., 2010, Ungur et al., 2022, Yabe et al., 2004).

The current research illustrated that the white adipocyte in the pre-renal white fat was composed of a single large lipid globule that occupies almost the entire cell cytoplasm, with the nucleus rejected at the periphery of the cell. The lipid globule

looked like a large empty space surrounded by a rim of cytoplasm, giving it the characteristic "signet ring" appearance of the adipocytes. As well, the lipid globule appeared as a large black droplet almost filling the cell with osmium tetroxide stained specimens and a semithin section as a large black or gray single droplet filling the entire cell, leaving a thin rim of cytoplasm stained with toluidine blue. Moreover, the white adipocyte presented a positive reaction with PAS and gave a weak reaction with alcian blue stain. The extracellular loose c.t. reacted positively with Crossman's Trichrome and Verhoeff-Van Gieson (VVG) stains. The ultrastructure of the white adipocyte revealed that the thin rim of cytoplasm surrounding the single large lipid globule contained most cytoplasmic organelles such as Golgi apparatus, mitochondria and a few cisternae of RER, minute lipid droplets and a small flattened nucleus was pushed to the periphery of the cell surface. Early research illuminates that the thin film of cytoplasm contains most cytoplasmic organelles, including mitochondria, a small Golgi apparatus, a few cisternae of RER, and free polyribosomes. (Geltinger et al., 2020). The mitochondria are small and elongated, with randomly oriented cristae (Frontini and Cinti, 2010). The cytoplasm is bound by a plasma membrane, and the nucleus is small and pushed to the periphery, where it appears as a blip on the surface of the cell (Ojha et al., 2014). The thin submembranous layer of cytoplasm enclosing the lipid droplet comprises cisternae of smooth ER (SER) and pinocytotic vesicles. TEM reveals a great abundance of caveolae in the cell membranes of most adipocytes, especially immature cells, and numerous minute lipid droplets beside the large droplet (L Mescher, 2018).

This current investigation revealed that the adipochondrocytes in the auricular cartilage shared both characteristic morphological features of the chondrocytes in the articular cartilage covering the fumer bone in the knee joint and the adipocytes in pre-renal white fat in the white New Zealand rabbit. The adipochondrocytes were housed in lacunae, which were separated from each other by a sparse ECM. The adipochondrocytes are active chondrocytes that synthesize collagen, elastic fibers, GAGs, and proteoglycan, as confirmed by Crossman's Trichrome, Verhoeff-Van Gieson (VVG) alcian blue, PAS and PAS-alcian blue staining, and by TEM examination. The outer perichondrium, which is made of connective fibers and vessels, the inner perichondrium containing cartilage stem progenitor cells, the transitional layer comprising chondroblasts, and the mature cartilage layer having the chondrocytes located in lacunae and surrounded by a high intensity staining of alcian blue, safranin O, and toluidine blue GAG content of the territorial matrix with Crossman's Trichrome-stained collagen fibers are all described as components of the human auricular cartilage (Liao et al., 2019, Zucchelli et al., 2020). The adipochondrocytes contained electron-dense cytoplasm with a dark peripheral nucleus, secretory granules, and the plasma membrane showed many cellular processes extended into a proteoglycan-rich territorial matrix, and that structure was similar to the chondrocytes in the articular cartilage covering the fumer bone in the knee joint. Early studies described the adipochondrocytes in the auricular cartilage of mice, rats, and rabbits as

hypertrophic white adipocyte-like chondrocytes having large lipid globule surrounded by electron-dense cytoplasm, which has Golgi apparatus with many secretory granules, developed RER, and cellular processes (AHMED and Abdelsabour-Khalaf, 2018, Sanzone and Reith, 1976). Also, these characteristic features are described in chondrocytes in the articular cartilage of different species (Keenan et al., 2019, Marlovits et al., 2003, Raabe et al., 2010, Ungur et al., 2022, Yabe et al., 2004). The amorphous materials around the lipid globule shown in this study were seen in the auricular cartilage of rabbits (AHMED and Abdelsabour-Khalaf, 2018) and paralleled those seen in the auricular cartilage of rats, where they were described as microfilaments (Kostović-Knežević et al., 1981).

In conclusion, the adipochondrocyte was a subtype of chondrocytes population that occupied the elastic cartilage of the white New Zealand rabbits, where they shared the morphological characteristics of both chondrocytes and adipocytes. Also, the adipochondrocytes appeared to be involved in the creation of the ECM and synthesis of both collagen type II and elastic fibers. These results should be considered when improving cartilage tissue engineering models, and more research is needed to further understand the molecular and functional characteristics of adipochondrocytes and to understand the importance of its content of lipid.

References

ABD-ELHAFEEZ, H. H., ABDO, W., KAMAL, B. M. & SOLIMAN, S. A. 2020a. Fish telocytes and their relation to rodlet cells in ruby-red-fin shark (rainbow shark) Epalzeorhynchos frenatum (Teleostei: Cyprinidae). Scientific Reports, 10, 18907.

ABD-ELHAFEEZ, H. H., ABOU-ELHAMD, A. S., ABDO, W. & SOLIMAN, S. A. 2020b. Migratory activities and stemness properties of rodlet cells. Microscopy and Microanalysis, 26, 1035-1052.

AHMED, Y. & ABDELSABOUR-KHALAF, M. 2018. Adipochondrocytes in rabbit auricular cartilage. Assiut Veterinary Medical Journal, 64, 105-109.

AHMED, Y. A. G. 2007. Analysis of physiological death in equine chondrocytes, University of Melbourne, School of Veterinary Science.

AMERMAN, E. C. 2021. Human anatomy & physiology, EBONY HOLT.

BANCROFT, J. D. & STEVENS, A. 1990. Theory and Practice of Histological Techniques, Churchill Livingstone.

BISSELL, T. & STEELE, L. 2010. Human Anatomy & Physiology. Human Anatomy and Physiology, 8th ed.; Pearson Benjamin Cummings: San Francisco, CA, USA.

BRADAMANTE, Z., KOSTOVIĆ-KNEZEVIC, L., LEVAK-SVAJGER, B. & SVAJGER, A. 1991. Differentiation of the secondary elastic cartilage in the external ear of the rat. Int J Dev Biol, 35, 311-20.

CARBALLO, C. B., NAKAGAWA, Y., SEKIYA, I. & RODEO, S. A. 2017. Basic science of articular cartilage. Clinics in sports medicine, 36, 413-425.

CASALE, J. & CRANE, J. S. 2019. Biochemistry, glycosaminoglycans.

DEŅISOVA, A., PILMANE, M. & FEDIRKO, P. 2022.

Glycosaminoglycan, Antimicrobial Defence Molecule and Cytokine Appearance in Tracheal Hyaline Cartilage of Healthy Humans. Journal of Functional Morphology and Kinesiology, 7, 55.

ENDRES, M., NEUMANN, K., ZHOU, B., FREYMANN, U., PRETZEL, D., STOFFEL, M., KINNE, R. W. & KAPS, C. 2012. An ovine in vitro model for chondrocyte-based scaffold-assisted cartilage grafts. Journal of orthopaedic surgery and research, 7, 1-14.

EYRE, D. 2002. Collagen of articular cartilage. Arthritis Res, 4, 30-5.

FIORAVANTI, A., COLLODEL, G., PETRAGLIA, A., NERUCCI, F., MORETTI, E. & GALEAZZI, M. 2010. Effect of hydrostatic pressure of various magnitudes on osteoarthritic chondrocytes exposed to IL-1β. Indian Journal of Medical Research, 132, 209-217.

FRONTINI, A. & CINTI, S. 2010. Distribution and development of brown adipocytes in the murine and human adipose organ. Cell metabolism, 11, 253-256.

GADJANSKI, I., SPILLER, K. & VUNJAK-NOVAKOVIC, G. 2012. Time-dependent processes in stem cell-based tissue engineering of articular cartilage. Stem Cell Reviews and Reports, 8, 863-881.

GARTNER, L. P. 2020. Textbook of histology e-book, Elsevier Health Sciences.

GATES, L., ADLER, R. R. & ELANGBAM, C. S. 2016. Osmium tetroxide post-fixation and periodic acid-Schiff dual-staining technique to demonstrate intracellular lipid and glycogen in the mouse liver section–a novel method for co-visualization of intracellular contents in paraffin-embedded tissue. Journal of Histotechnology, 39, 2-7.

GAYTAN, F., MORALES, C., REYMUNDO, C. & TENA-SEMPERE, M. 2020. A novel RGB-trichrome staining method for routine histological analysis of musculoskeletal tissues. Scientific reports, 10, 16659.

GELTINGER, F., SCHARTEL, L., WIEDERSTEIN, M., TEVINI, J., AIGNER, E., FELDER, T. K. & RINNERTHALER, M. 2020. Friend or foe: lipid droplets as organelles for protein and lipid storage in cellular stress response, aging and disease. Molecules, 25, 5053.

HUBER, M., TRATTNIG, S. & LINTNER, F. 2000. Anatomy, biochemistry, and physiology of articular cartilage. Investigative radiology, 35, 573-580.

KEENAN, C. M., BECKETT, A. J., SUTHERLAND, H., RANGANATH, L. R., JARVIS, J. C., PRIOR, I. A. & GALLAGHER, J. A. 2019. Concentric lamellae–novel microanatomical structures in the articular calcified cartilage of mice. Scientific Reports, 9, 11188.

KOSTOVIĆ-KNEŽEVIĆ, L., BRADAMANTE, Ž. & ŠVAJGER, A. 1981. Ultrastructure of elastic cartilage in the rat external ear. Cell and Tissue Research, 218, 149-160.

L MESCHER, A. 2018. Junqueira's Basic Histology Text and Atlas, FIFTEENTH. McGraw-Hill Education.

LIAO, J., CHEN, Y., CHEN, J., HE, B., QIAN, L., XU, J., WANG, A., LI, Q., XIE, H. & ZHOU, J. 2019.

Auricle shaping using 3D printing and autologous diced cartilage. The Laryngoscope, 129, 2467-2474.

MALLINGER, R. & BÖCK, P. 1985. Differentiation of extracellular matrix in the cellular cartilage ("Zellknorpel") of the mouse pinna. Anatomy and embryology, 172, 69-74.

MARLOVITS, S., TICHY, B., TRUPPE, M., GRUBER, D. & VÉCSEI, V. 2003. Chondrogenesis of aged human articular cartilage in a scaffold-free bioreactor. Tissue engineering, 9, 1215-1226.

MARTINI, F. H., NATH, J. L. & BARTHOLOMEW, E. F. 2011. Fundamentals of Anatomy & Physiology, Pearson Education.

MATSIKO, A., LEVINGSTONE, T. J. & O'BRIEN, F. J. 2013. Advanced strategies for articular cartilage defect repair. Materials, 6, 637-668.

MOTA DE SÁ, P., RICHARD, A. J., HANG, H. & STEPHENS, J. M. 2017. Transcriptional Regulation of Adipogenesis. Compr Physiol, 7, 635-674.

NAHIAN, A. & SAPRA, A. 2022. Histology, Chondrocytes. StatPearls [Internet]. StatPearls Publishing.

NANDURI, V., TATTIKOTA, S. M., SRIRAMAGIRI, V. R. R., KANTIPUDI, S. & PANDE, G. 2014. Reconstruction of hyaline cartilage deep layer properties in 3-dimensional cultures of human articular chondrocytes. Orthopaedic Journal of Sports Medicine, 2, 2325967114539122.

NAUMANN, A., DENNIS, J. E., AWADALLAH, A., CARRINO, D. A., MANSOUR, J. M., KASTENBAUER, E. & CAPLAN, A. I. 2002. Immunochemical and mechanical characterization of cartilage subtypes in rabbit. Journal of Histochemistry & Cytochemistry, 50, 1049-1058.

NEWMAN, A. P. 1998. Articular cartilage repair. The American journal of sports medicine, 26, 309-324.

OJHA, S., BUDGE, H. & SYMONDS, M. 2014. Adipocytes in normal tissue biology.

PRYDZ, K. 2015. Determinants of glycosaminoglycan (GAG) structure. Biomolecules, 5, 2003-2022.

RAABE, O., REICH, C., WENISCH, S., HILD, A., BURG-RODERFELD, M., SIEBERT, H.-C. & ARNHOLD, S. 2010. Hydrolyzed fish collagen induced chondrogenic differentiation of equine adipose tissue-derived stromal cells. Histochemistry and cell biology, 134, 545-554.

SALINAS, D., MUMEY, B. M. & JUNE, R. K. 2019. Physiological dynamic compression regulates central energy metabolism in primary human chondrocytes. Biomechanics and modeling in mechanobiology, 18, 69-77.

SANZONE, C. F. & REITH, E. J. 1976. The development of the elastic cartilage of the mouse pinna. American Journal of Anatomy, 146, 31-71.

SOPHIA FOX, A. J., BEDI, A. & RODEO, S. A. 2009. The basic science of articular cartilage: structure, composition, and function. Sports health, 1, 461-468.

STANDRING, S. 2021. Gray's anatomy e-book: the anatomical basis of clinical practice, Elsevier Health Sciences.

TENG, Y., LI, X., CHEN, Y., CAI, H., CAO, W., CHEN, X., SUN, Y.,

LIANG, J., FAN, Y. & ZHANG, X. 2017. Extracellular matrix powder from cultured cartilage-like tissue as cell carrier for cartilage repair. Journal of Materials Chemistry B, 5, 3283-3292.

UNGUR, R. A., MURESAN, A., OLTEANU, D. E., FLOREA, A., CIORTEA, V. M., IRSAY, L., BORDA, I. M., CODEA, R. A., OBER, C. A. & BÂLICI, Ș. 2022. Ultrasound Protects Human Chondrocytes from Biochemical and Ultrastructural Changes Induced by Oxidative Stress. Applied Sciences, 12, 2334.

VINCENT, T. L. & WANN, A. K. 2019. Mechanoadaptation: articular cartilage through thick and thin. The Journal of physiology, 597, 1271-1281.

WACHSMUTH, L., SÖDER, S., FAN, Z., FINGER, F. & AIGNER, T. 2006. Immunolocalization of matrix proteins in different human cartilage subtypes. Histology and histopathology.

WALTHER, T. C. & FARESE JR, R. V. 2012. Lipid droplets and cellular lipid metabolism. Annual review of biochemistry, 81, 687-714.

YABE, K., SATOH, H., ISHII, Y., JINDO, T., SUGAWARA, T., FURUHAMA, K., GORYO, M. & OKADA, K. 2004. Early pathophysiologic feature of arthropathy in juvenile dogs induced by ofloxacin, a quinolone antimicrobial agent. Veterinary pathology, 41, 673-681.

ZUCCHELLI, E., BIRCHALL, M., BULSTRODE, N. W. & FERRETTI, P. 2020. Modeling normal and pathological ear cartilage in vitro using somatic stem cells in three-dimensional culture. Frontiers in Cell and Developmental Biology, 8, 666.