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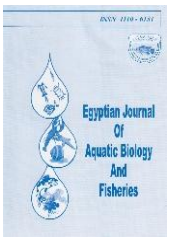

How the Hyaline Cartilage is Formed in the Siphonopoda as the Model of Higher Organisms?

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Abstract:

For several decades attempts have been made to find evidence for the origination of tissue and the mechanism of the formation of hyaline cartilage; however, no definition for the mechanism was provided to support that. Thus, the current study presented and explained the mechanism and origins of the formation process of cartilage by using three different stains and determining the corpuscle cell, which is responsible for the formation and division process as the primary unify of the cartilage formation in animals, particularly in vertebrates. The hyaline cartilage is originated from connective tissue (collagen fiber). The current work explained the condensation of collagen fibber in tissue passing to the two divided origin cells, then the nucleus begins formation at each side of cartilage in all new cells, followed by a step during which the cartilage divides the cell passing from the cartilage cell into two cells. Determination of the origin of cartilage and its mechanism is great evidence that may explain bone and cartilage diseases and facilitate their treatment. These common unknown diseases are attributed to the deficiency of information about cartilage formation and the ignorance of all previous steps.

INTRODUCTION

Many studies have tried to find the relationship between collagen fibers and the origin of hyaline cartilage, but without finding the mechanism and the cell of origin. The hyaline cartilage began to appear in phylum Annelida as a solid capsule, originating from solid epidermal connective tissue, and in echinoderms by lower condensation of collagen fibers, and then differentiated into cephalopod for different types and functions **(Bairati *et al.*, 1998; Cole *et al.*, 2004; Whittaker *et al.*, 2006; Brittberg *et al.*, 2008; Cole, 2011; Person, 2012; Owen, 2022).** The cephalopods contain different types of hyaline cartilage according to the differentiation and functions of fibroblast cell itself; in addition, they differ from other invertebrates with respect to the formation from lose tissue and the nature of origin from connective tissue **(Boyan**

***et al.*, 1999; Cole *et al.*, 2004a; Hall, 2005; Cole, 2011a; Ehrlich, 2015; Grässel *et al.*, 2016; Irawan *et al.*, 2018; Krings *et al.*, 2022; Ragheb *et al.*, 2022; Yu, 2022)**. The differential process first appears and condensation in crustaceans passes to top qualification in Mollusca as the first appearance of the cartilage in several tissues of the animal body parts as mantle tissue. The cartilage is present in radula, radular sheath, odontophore, and finally in the skin, and decreases to a great extent by developing the organisms from human to one type through the evolution of animal **(Anderson *et al.*, 2022; Ragheb, 2022)**. The skin of mollusca has unique characteristics as containing small hyaline cartilage originating from the small condensation of collagen fiber. The skin of the cephalopods differs from other Mollusca with its large condensation in addition to containing some simple cells of chondrogenesis which are lacking in mollusks animals including the nucleus of cartilage origin. The condensation process begins in the first stage of the previous description, then continues to give chondrogenic cells. The duplication process grows rapidly to give double cells and a medulla of hyaline cartilage formation. The process itself indicates the final evolution process by the appearance of the bone in fishes and continues the ossification process to the hardness of the bone in humans. The hyaline cartilage origination takes many shapes in different organisms according to the position and function of the organism in the animal kingdom. The collagen fibers in cephalopods contain interstitial tissue and a large distance between tissue cells, this characteristic appears in artificially cultured tissue and animals breathing through the skin or soft tissue. For instance, *Octopus vulgaris* traps the air taken by the animal through the skin to the skin tissue and then uses it for breathing process matching the higher terristerials animals (**Grześkowiak, 2022; Nakano *et al.*, 2022; Patte *et al.*, 2022; Wang *et al.*, 2022; Yin *et al.*, 2022; Abd Elrheem, 2023)**. The interstitial area functions as similar as the lower lung, with lower content of dissolved oxygen which is adequate for survival, and this is common in kingdom Animalia such as hagfish **(Smith *et al.*, 2010; Birk *et al.*, 2018; Eom *et al.*, 2022)**. The organization of cartilage and its origin is genetically controlled; during the embryonic stage, the origin of the process and the presence of different types of line cartilage in the cephalopods indicate the beginning of the appearance of the qualification of the skeletal system; the presence of two stomachs also indicates that these animals share the vertebrates in several characters, one of which is the advanced eye **(Abd Elrheem, 2022)**. The cephalopod is a protostome animal with all the characteristics of lower organisms such as the presence of the permanent conchiolin layer **(Koch *et al.*, 2022; Lindauer *et al.*, 2022; Orvis *et al.*, 2022; Schulreich *et al.*, 2022)**. The cartilage in organisms, especially Mollusca shows the starting appearance of the skeletal system and the ossification of cartilage that emerges in mantle cartilage muscles **(Chong, 2022; Lindauer *et al.*, 2022)**. The new paleontology scientists tried to find a link between crustacea and Mollusca on basis of aging, which is the

classification of Mollusca depending on morphology, without paying great attention to the histological studies on several species. The non-culture of the cephalopods has no reference, and the root as a word refers to mixing a group of animals with shell, such as chition and nautilus. The old nomenclature has a unique structure in their description of the animal position in evolution. The Siphonopoda indicates the presence of a siphon, so the old term is true nomenclature and right in classification. The cartilage formation process in all animals' appearance of cells is responsible for the formation of bone **(Depew, 2008; Debiais-Thibaud, 2019; Molnar *et al.*, 2022)**. Some authors identified the presence of a permanent layer of conchiolin as collagen fibers' origin; however, they considered the appearance of protostome characters to be the remains of animal creatures **(Gong *et al.*, 2012; Ruiz, 2020; Whalen *et al.*, 2022; Salas *et al.*, 2022)**. The anterior and posterior of cephalopods indicate protostomes animals with some characters. On the other hand, malacologists depended on locomotion in the backward direction; this is common in animal kingdoms then the direction indicted to Siphonopoda name and direction. The common literature on Siphonopoda reported the presence of multi-cartilage types, while animals contain monotypes in different shapes according to examination and age of animals. The medical application for unknown cartilage disease is returned to the unknown mechanism of cartilage formation; multicultural tissue could perform artificial cartilage from collagen fibers but without any knowledge about the steps **(Bay-Jensen *et al.*, 2008; Silvipriya *et al.*, 2015; Avila Rodríguez *et al.*, 2018; Irawan *et al.*, 2018; Nakano *et al.*, 2022)**. The evidence of steps seems similar to the steps of cartilage formation in the artificial tissue cultured field, with some modifications. The artificial engineering in the medical field gives sex following steps. All the steps in cephalopods indicate the presence of the evolution process by the animal itself, without chemical stimulation materials used in the artificial lab. The present study provided detailed steps to the known available structure of cells in the tissue.

MATERIALS AND METHODS

Sampling

Samples of *Octopus vulgaris* were collected from one site on the western coast of the Red Sea. This site is located 17km south of Safaga City (latitude 26° 38' N longitude 33° 59' E). The collection site is on rocky shores, and samples were collected from the intertidal zone at the time of low tide. Samples were collected by hand and were packed afterwards. One specimen was collected and put in a plastic container containing seawater. Specimens were narcotic by adding menthol crystal from El-Naser Chemicals Company in Cairo, Egypt to the water surface of the jar and

waiting until relaxation. Specimens were dissected in the field to get the studied organs, fixed and put in Blouin's solution for 24 hours for histological preparations.

Histological studies

Organs sectioned were cut off from the body and placed into Blouin's solution in seawater for 24 hours. Fixed parts were then passed to the graded series of alcohol from 30 to 100%. They were cleared in toluene three times each for 5 minutes then embedded in paraffin wax. Sectioning was made by microtome at 5- 7µm thickness. Sections were stained with the following stains:

- Harris hematoxylin and eosin (H&E) combination **(Steedman, 1950)**.
- Masson's trichrome stains for the determination of general collagen **(Brury *et al.*, 1980)**.
- Mercuric Bromophenol blue for demonstration of general proteins **(Mazia *et al.*, 1953)**.

RESULTS

The body plane of *Octopus vulgaris* indicates the cephalopod member with eight arms as common colloids, with special patterns as solitary of first suckers and invaginated Beak (Fig. 1).

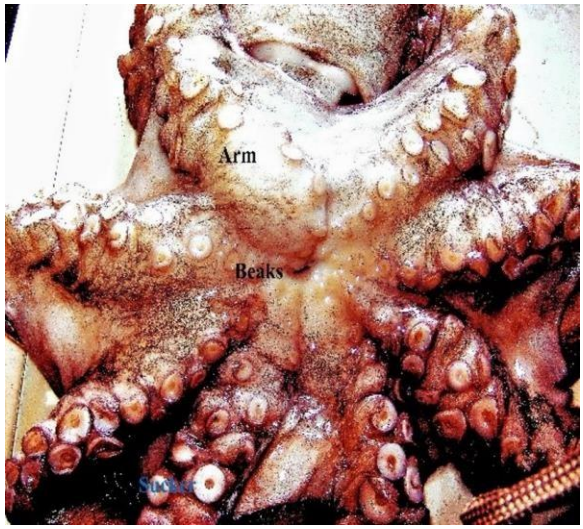


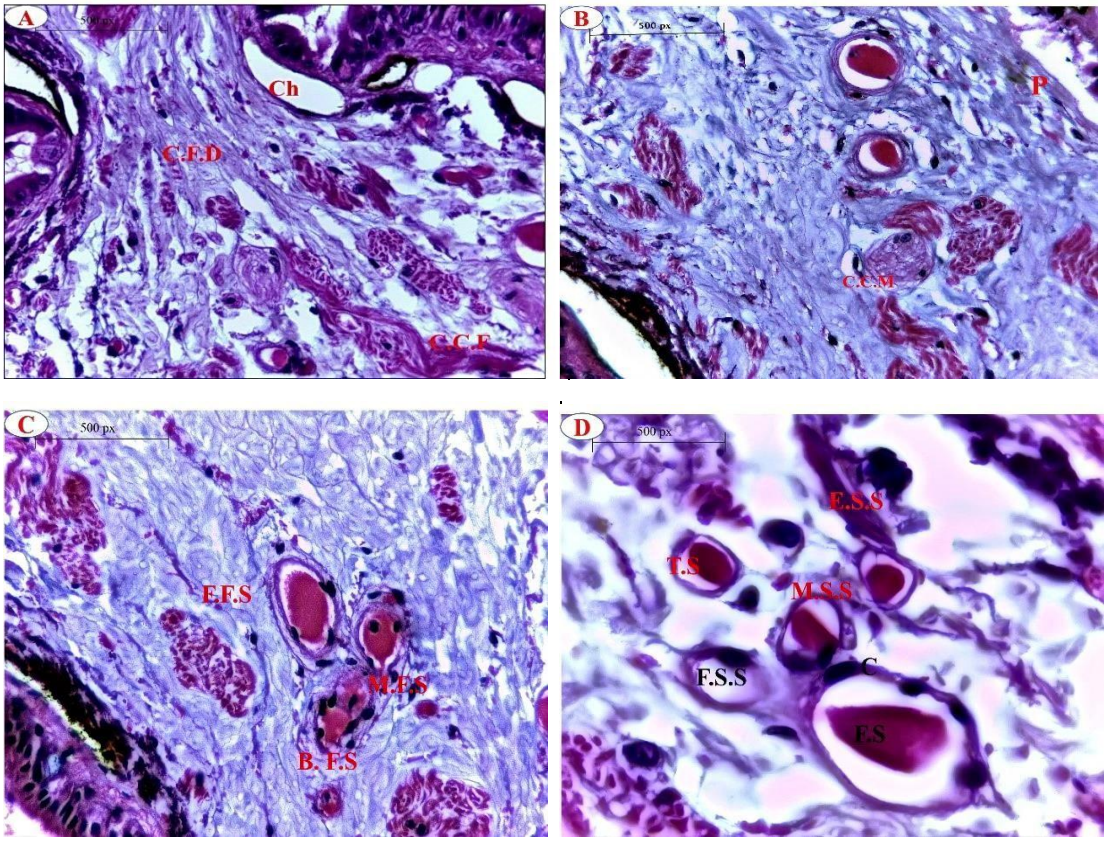
Fig. 1. The body plan of *Octopus vulgaris* **(Cuvier, 1797)**. (Ventral view).

The Cephalopoda skin has unique characteristics such as mucus (M), which covers the whole skin for protection from dryness and parasites, and condensed

collagen fibers bundle at the end of the lower animal cartilage and near chromophores to achieve the best control with chromophores disc. All the cephalopod's cartilages, including the mantle, radular cartilage, odontophore, radular sheath are originated from connective tissue, evidenced with the presence of these structures near the mouth, except the cartilage of the mantle. The cartilage of the mantle is more ossificated for protecting animals from the backward locomotion side; the function of hyaline cartilage is to support the loose tissue for protection. The cartilage originating from the collagen fibers of condensed connective tissue is the beginning of the differentiated and complete shape of cartilage from connective tissue. The ossification begins with condensation, followed by mid-decomposition of cells, then two cells migrate to each side of the chondrocyte cell, and finally the hyaline cartilage is formed. The present study was performed with three types of stains to identify the stage, confirm the structure of each stage and determine the origin of corpuscle divided cell appearance.

The cephalopod's cartilage originates and differentiates through the condensation of filaments of connective tissue collagen fibers from the dermis of cephalopodan animals, forming a different character from the remains of invertebrates via a special unknown mechanism. The stimulated cell division of the cartilage with an unknown mechanism is unidentified in invertebrates; whereas in vertebrates, it is called the corpuscle cell. The corpuscle cells are not stained using H & E as a higher vertebrate; thus, they were supported with a short description in literature in the way all unknown cells' structures are dwelled (Fig. 2A). The examination of the body of the cell and the wall containing condensed nucleus cells, but not stained by Masson’s trichrome or bromophenol blue is an evidence of corpuscle cells (Fig. 2H, I). The examination of the light microscope showed the presence of cells responsible for the condensation process corpuscle as a character recorded in Cephalopoda animals. The corpuscle cell migrates to the side of the cell without nucleus division, and the content of the cell diminishes losing the wall of basal cells, and hence the content of the condensation cell becomes clear (Fig. 2H, I). The migration of the nucleus on each side of the chondrocyte cell and the lacuna originating from the cell itself form the shape of a cartilage cell as a vertebrate. For the invertebrates, the corpuscle cell is the origin of the condensation filaments cell through condensing the whole filament via an unknown mechanism. The examination of the filament condensed in the bundle revealed that, the second stages are formed and the pericardium layers (p) stimulate the new formation of chondrocyte cells (Fig. 2B). The third stage begins with the final cells' division (C.F.D), passing through connective tissue into the cell of chondrocytes as the first stage (F.S) formation (Fig. 2D). The condensation of collagen fiber (C.C.F.R) and condensed collagen fibers (C.C.F) is removed to form the double nucleus, and all stages of the formation of cartilage process are stopped, forming the chondrocyte cell which was lost by being

stained with Bromophenol stain (Fig. 2H, I). In the third stage, the nucleus is formed to perform the semi-divided cell, which is condensed to form the cell without a nucleus, and the nucleus may be degraded as vertebrate bone formation (Fig. 2H, I). The previous description looks like the formation of artificial cartilage, thus the third stage is absent in natural formation. The artificial stages lack to animal stimulation mechanism; consequently, the number of stages in the process is not an important evidence. The fourth stage begins with elongated condensation materials of convoluted filaments of collagen fibers, without any detail for the contents of cells' natural materials. The invertebrates seem similar to vertebrates in the formation of cartilage; the process that takes place may be genetic as vertebrates since it takes place during the growth of the animal life cycle. The presence of corpuscle in this process proved that it is the ossification process but in the early stage; it was stained by Masson's trichrome and Bromophenol blue only. This indicates protein content with dense content as common corpuscles in invertebrates, with similarity in shape but it seems to lose the sheath as primitives' corpuscles. The stain of corpuscles with Masson's trichrome returned to the presence of collagen fibers cells formation. The cell of condensed filament takes a disc shape with condensed content which may be filaments of collagen fibers. The condensed cell developed into the second stage (S.S) and condensation of the corpuscle cell and homogenous cell walls structure it inside the main cell. The stages are collected in three pictures, which were stained using H&E, Masson's trichrome, and Bromophenol blue (Fig. 2C, I). However, the corpuscle cells as previously mentioned were not stained by H&E, thus the first stages did not appear. In the final stage (F.S), the corpuscle cell lost its shape, and the process stops with the formation of the cartilage in the final third stage, and convoluted fibers condensation (C. C. F) is lost in the architecture of cells, as shown in Fig. (2F, G, H, I). The cells became unstable thus the nucleus were diveded into two nuclei, each of which migrates to each side of the chondrocyte cell forming the cell, which is considered the cartilage cell mother. The third stage described by elongated condensation may be performed to form the new elastic fibers. In invertebrates, the formation is simple in manner but complex in understanding by the presence of unknown structures (S.C) and unknown function cells (E.C.F), as shown in Fig. (2G, I). The third stage during which a dense condensation takes place to form a disc of unknown structure is the collecting of collagen filaments inside the first step.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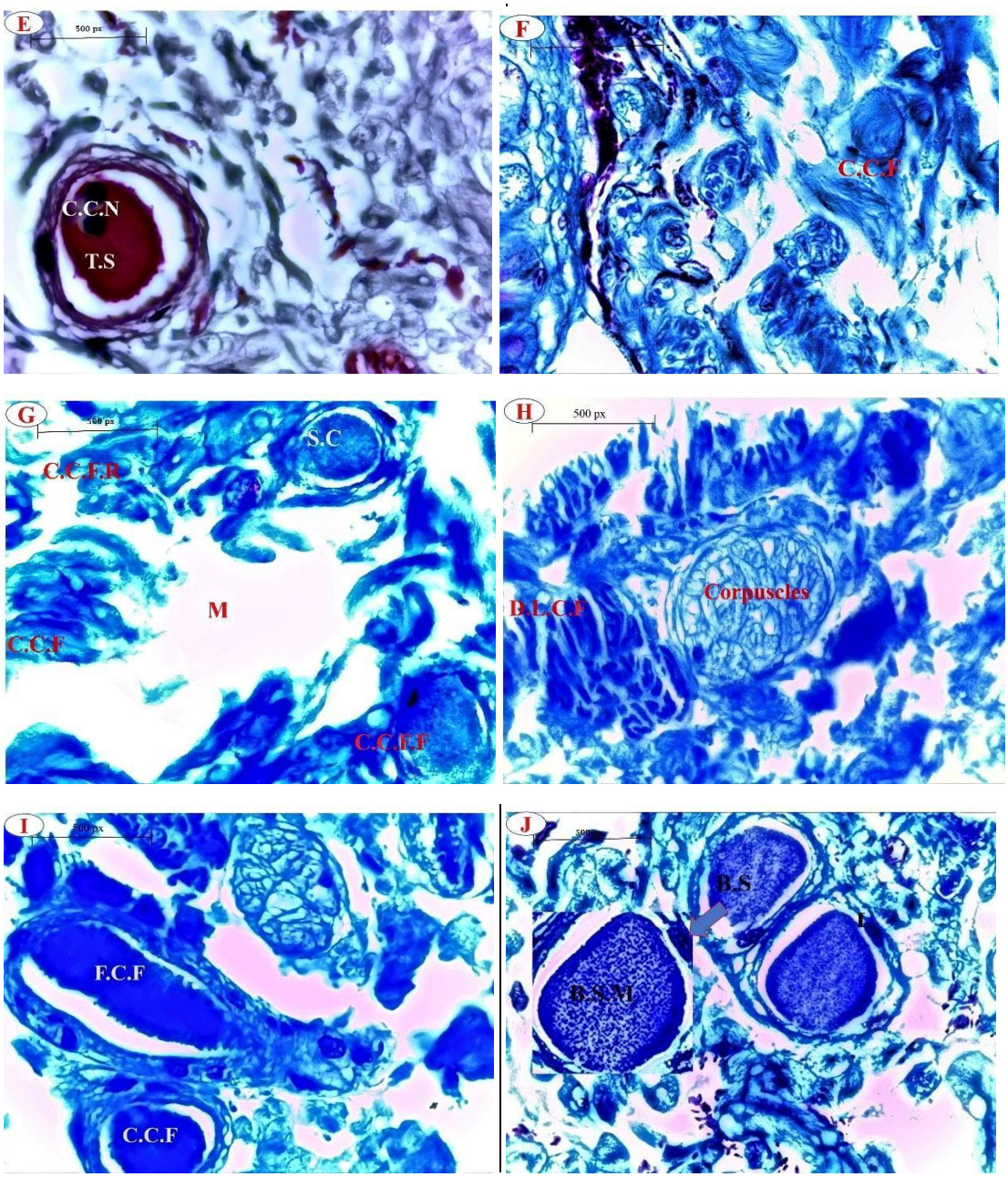

Fig. 2. The cartilage formation stages showing: (Ch) Chromophore; (B.S) Basal stage; (B.S.M) Basal stage medulla; (C.C.E.F) Collagen cells elastic fibers; (C.C.F) Collagen condensation fibers; (C.C.F.R) Collagen cells fibers reduction; (C.C.R) Collagen condensation returned; (C.F.D) Cell fibers degradation; (C.C.N) Collagen cell's nucleus; (E.C.F) Elastic condensation fibers; (D.E.C.F) Degradation of elastic cells fibers; (D.L.C.F) Degradation of longitudinal cells fibers; (F.S) Fourth stage; (F.F.S) Final five stages; (M.S.S) Middle second stage, (P) pericardium, and (T.S) Third stage.

DISCUSSION

The cartilage is present in the cephalopods, and the type is hyaline cartilage, which is found in several body organs, such as mantle skin and radular sheath, radula, odontophore and mantle **(Cole *et al*., 2004; Cole *et al*., 2009; Cole *et al*., 2011a, b; Hang *et al*., 2022; Yu *et al*., 2022a, b)**. The formation of cartilage from collage fibers of dense connective tissue in artificial and cultured tissue coincide with the findings of the present study **(Boyan *et al*., 1999; Hall, 2005; Irawan *et al*., 2018; Gillis, 2019; Rolian, 2021)**. The stages of formation are similar to the formation of cartilage with little modification in the description of the process; this modification returned to new mechanism evidence **(Depew, 2008; Debiais-Thibaud, 2019; Allen *et al*., 2022; Liu, 2022a, 2023b; Molnar *et al*., 2022)**. The biological studies in the future will give highly advanced evidence of stages; the medical field can achieve progress for unknown cartilage diseases, which may be attributed to the lack of knowledge on the unknown steps **(Garcia-Enriquez *et al*., 2010; Luo *et al*., 2015; Krishnan *et al*., 2018; Yin *et al*., 2022; Yu *et al*., 2022)**. The stages of formation in the cephalopods resemble the origin of bone in semi-invertebrates and vertebrates because the *Octopus vulgaris* is considered an advanced and higher invertebrate **(Liu, 2019; Liu *et al*., 2021; Allen *et al*., 2022)**. The unknown cells appearing in cartilage formation are due to new knowledge about method formation for the cartilage in invertebrates, and the authors write short notes on the cartilage formation in vertebrates since they do not know about the formation of cells and specialized collagen fibers. The collagen fibers in higher animals differ primarily in the condensation process; the primary echinoderm appears as a capsule. This is related to the cartilage which is not differentiated into several types as the cephalopods.

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