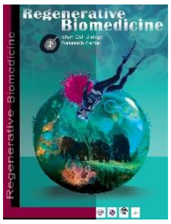

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Review Article

Regenerative Biomedicine and Cuttlefish Camouflage: Unveiling Nature's Masterpieces

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Abstract

Understanding the camouflage mechanisms in cephalopods, particularly the role of pigment-bearing organelles like chromatophores, holds significant relevance in regenerative medicine. These organelles exhibit rapid changes in color and shape for purposes such as camouflage, warning signals, and communication. The pigments within these cells play a central role in the skin coloration of these animals by scattering light. Recent research has elucidated that expanded chromatophores contribute to the creation of coloration structures. Investigations involving chromatosome proteome assays and microscopic evaluations of cuttlefish have revealed the presence of a laminar, reflective iridescent sheen generated by a biochemical component, facilitated by reflectin proteins found in the enveloping cells that cover each chromatophore. Within the chromatophores themselves, pigment molecules interact closely with the protein ω-crystallin, also present in the eye lens. These discoveries highlight the intricate interplay between pigments and color-producing structures, offering valuable insights into biophotonics. The dynamic relationship between these flexible pigment-containing organelles presents an inspiring avenue for the development of material engineering technologies encompassing simultaneous changes in color and pattern. Drawing inspiration from cephalopods' capabilities, researchers can explore innovative approaches for regenerative medicine, incorporating biophotonic interactions and adaptive coloration principles. This review compiles ongoing research on cephalopod camouflage, emphasizing its potential implications in regenerative medicine, paving the way for novel advancements in therapies, tissue engineering, and materials development.

Keywords: Regenerative medicine, Cephalopods, Camouflage, Pigment-bearing organelles, Chromatophores Ovarian Regeneration

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Introduction

The richest and most diverse color patterns in animals exist in certain organisms that have been able to create beautiful combinations of structural elements and pigmentary components for more effective manipulation of light; squid, octopus, and cuttlefish (Figure 1). These remarkable cephalopods provide inspiration and insights into the realm of regenerative biomedicine. Through their incredible camouflage techniques, cuttlefish showcase the potential of natural regeneration in the field of biomedicine. For example, they have the ability to dynamically change their appearance to rapidly display a wide range of camouflage and signaling (1, 2) (Figure 2). This rapid and dynamic adaptation involves the use of specific skin structures that alter the animal's appearance through multiple effects (3). These structures include chromatophores, pigment-containing organelles located in the uppermost dermal layer, as well as two types of non-pigmented (structural) cells (Figure 3): iridocytes, which can reflect almost any color and appear iridescent, and leucophores, which sporadically reflect the entire visible spectrum and produce bright white shine. Iridocytes are composed of proteinaceous platelets with high refractive index - reflectin - that selectively reflect light through "thin-film interference," producing various brilliant colors in the visible spectrum (4-7). Leucophores also function based on reflectin but primarily utilize microspheres for reflecting white light (8). In contrast to leucophores and iridocytes, the combination of light-interacting elements within complex chromatophores is more intricate (9).

Considering the ability of chromatophores as dynamic color filters in living tissues, the functional histology of chromatophores has received considerable attention in both basic and applied sciences. The neuro-muscular components of chromatophores comprise five types of cells: nerves, glial cells, radial muscles, sheath cells, and central large chromatophores filled with flexible sacs containing nanostructured pigment granules (11). In the longfin inshore squid (*Doryteuthis pealeii*), each chromatophore consists of 18-30 muscle fibers radially arranged around the sac and, upon neural stimulation, retract the pigment cell, resulting in a flattened disc-shaped pigmented plate (12, 13). Fully mature chromatophores are mostly packed with nanostructured granules containing pigmentary eumelanins and associated proteins that may aid in spatial organization among the granules (10, 14). This combination of pigments and proteins maintains color uniformity and richness of chromatophores even when the cell transforms into a very thin layer (2-4 granules thick) during activation (15, 16). The rapid spread of this system is due to contractions of radial muscle fibers attached to the chromatophores (approximately 125 milliseconds). Therefore, understanding the extent to which light is modulated by pigments and structural coloration within the dynamic chromatophore contributes to its photic functions and is of particular significance (3, 7).

Figure 1. Cephalopods, including octopuses (A), squid (B), and cuttlefish (C), possess the ability of camouflage

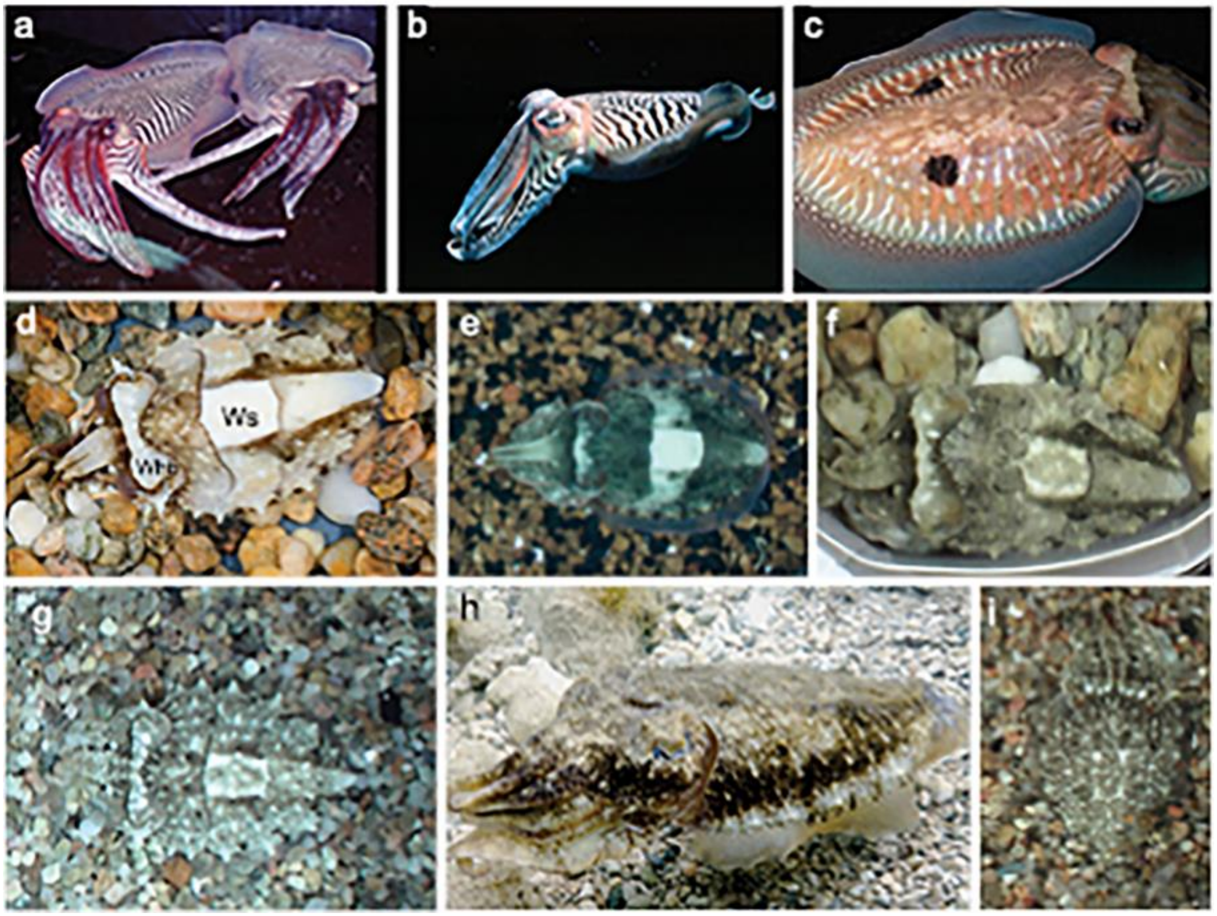

Figure 2. The ability of cuttlefish in color change is utilized for communication (a), response to the environment (b and c), and camouflage (d to i).

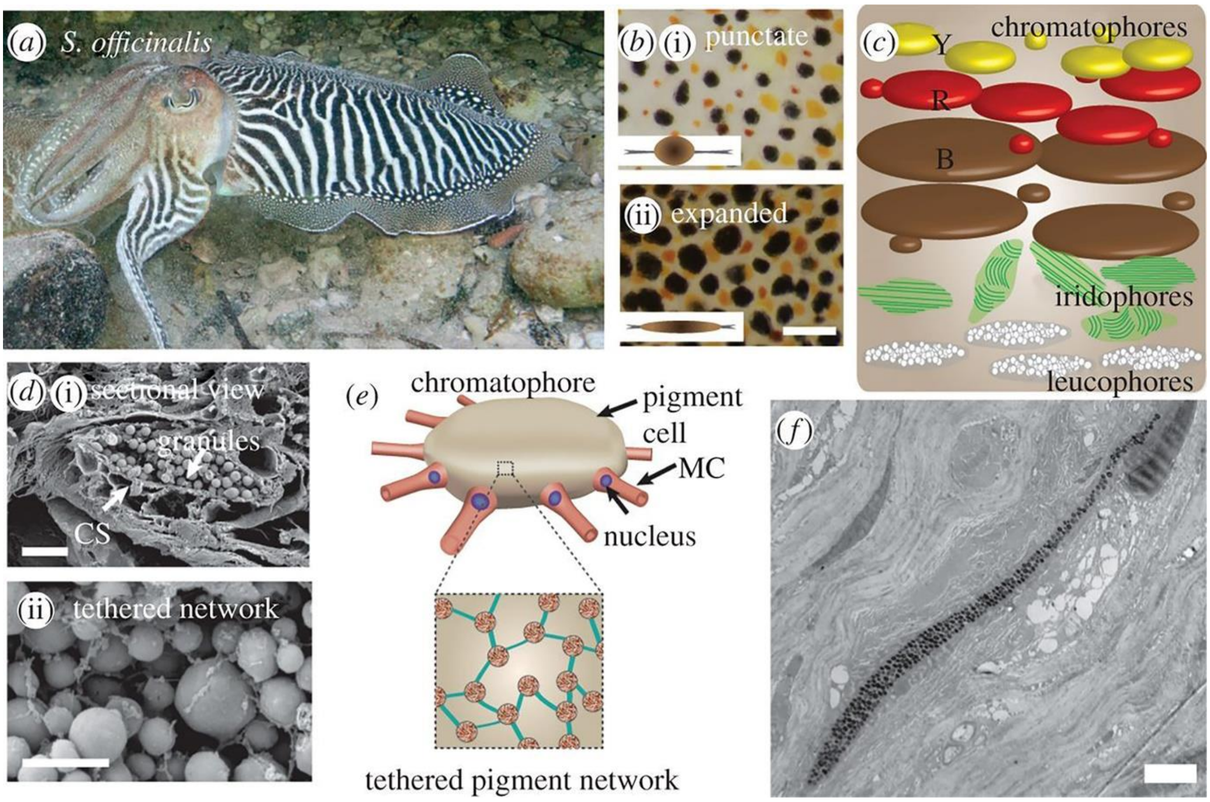

Figure 3. The process of cutaneous pigmentation in cuttlefish. (a) Adult cuttlefish, (b) when activated, chromatophores transform from a resting state (i) to an expanded state (ii) in response to visual cues. Scale bar is 1 mm. (c) Schematic representation of the optical units in the cuttlefish skin. Layering is important for light modulation during function. (d) Scanning electron microscopy image of a cross-section of a chromatophore, scale bar is 2 µm. (ii) Intracellularly connected pigment granules within the chromatophore. Scale bar is 1 µm. (e) Schematic representation of pigment granules in the chromatophore, tethered to pigment cells by radial muscle cells. (f) Transmission electron microscopy image of an expanded chromatophore, showing its thickness equivalent to just one granule. Scale bar is 4 micrometers. Source with permission: (10).

Molecular structure of pigments in the cuttlefish

Although the combination of pigments present in the cuttlefish has been confirmed to be a mixture of pterins and carboxylated pterins (17), proteins within the chromatophore sacs, especially those that may coordinate with the pigments and associate with them to assist in color filtering during their function, have also been recently identified (18). Several proteins inside or near the chromatophores, including S-crystallin (10), reflectin (10), and R-opsin (19, 20), have been identified.

Crystallins are a diverse group of proteins found in animal lenses. An isoform called S-crystallin has been found in the eye and skin of cuttlefish (21). It has also been shown that this crystallin prevents spherical aberration by forming clumped crystallinoid patches with different refractive indices and dispersions in the lens of the cuttlefish (22). Another crystallin isoform present in their skin is called gamma-crystallin, which structurally resembles aldehyde dehydrogenase, although it is enzymatically inactive (21, 23, 24). This gamma-crystallin isoform is predominant in the bioluminescent light organ of the cuttlefish (25). Although S- and gamma-crystallins differ in their composition, they possess high refractive indices, optical transparency, and water solubility, which play a primary functional role in the eyes and skin of animals (21, 23, 26). The abundance of gamma-crystallin and its association with pterins indicate a dual role of this protein, one in the nanostructure of granules and the other in the stabilization or sequestration of pterins within the granules (18).

Reflectin is a cuttlefish-specific structural protein that forms either nano-particles or interconnected strips (27-29) to scatter and reflect light, creating iridescent or white coloration (30, 31). Rhodopsin, a light-sensitive protein, plays a role in light transmission and also in the light-mediated function of the skin, such as the chromatic response ability of the chromatophore system (octopuses), even when detached from the body (6, 19, 20). Evidence of additional optical features of the cuttlefish skin, including intense color reflection from chromatophores, has also been demonstrated (18). These organs constitute a highly regulated system that creates dynamic coloration. It will be further addressed how cuttlefish can produce structural coloration in the absence of iridophore layers and how light can be manipulated to produce both pigmentary and structural coloration with photon sets beyond what was previously known.

The Cellular Structure of Pigment Cells in Squid

Chromatophores, which were believed to be exclusively absorptive and pigmentary, are also structurally reflective based on the angles of reflected light (18). This characteristic was revealed by the identification of reflectin in the vicinity of chromatophores and crystallin within chromatophores (18) (Figure 4). Reflectin, known as the main component responsible for producing static or adjustable iridescent colors through neural network control in iridophores and creating exceptionally bright white colors in leucophores in the subcutaneous layers below the chromatophores, had been identified (6, 8). On the other hand, isoform S-crystallin exists in the eyes of squid as a colloidal gel with a refractive index gradient that enables the fish lens to cope with spherical aberration (24), while gamma-crystallin isoform found in the photophore of squid reduces oxidative stress in the photic organ (25). Using a specific chromatophore proteome assay in squid, the presence of these proteins in cutaneous chromatophores, especially in the vicinity of the pigment sac (gamma-crystallin) and surrounding areas (reflectin), was examined and confirmed (18) (Figure 5).

The pigment elements and coloration structure in squid differ from other species. In species such as butterflies, spiders, reptiles, and birds (32, 33), these elements can be observed in the same tissue, either in cellular layers or discrete assemblies, or if mixed in a tissue like butterfly scales, they produce only a single visual effect (33). For example, different skin colors in geckos are produced by iridophore cells scattered among melanophores and erythrophores; however, this combination only creates fixed color patterns (34). In contrast, dynamic coloration in the panther chameleon is created by two distinct layers of cells, one with iridophore characteristics that modulate brightness and a second layer formed by either iridophores or pigment cells used for color control (35). The spatial separation of color production sources in cellular layers is observed on a large scale in the skin of squid, where the uppermost layer contains chromatophores and the lowermost layer contains iridophores. However, unlike many other systems, the chromatic and structural color components in squid are fully integrated at the molecular and cellular level, producing both high chromatic diversity and conspicuous brightness (Figure 6).

Since enveloping cells completely surround pigment granules within chromatophores, light entering the expanded chromatophore passes through enveloping cells and encounters reflectins before exiting vertically through pigment granules, creating light scattering and doubling their effects (Figure 7) (18). In other cases, light can enter chromatophores from below (the skin is often transparent) to pass through enveloping cells on both sides of the pigment sac. In such cases, this structural coloration can enhance the brightness of pigment by amplifying the light, as observed in biomimetic analogs of chromatophores (16).

The Pigment Cells in Squid: Application in Regenerative Medicine

In the field of regenerative biomedicine, the application of cuttlefish-inspired mechanisms and principles holds promising potential for various human applications. Here are a few examples:

Cuttlefish skin possesses remarkable properties that allow for rapid color and pattern changes. Understanding the composition and structure of their skin could inspire the development of advanced biomaterials and scaffolds for tissue engineering. Mimicking the cuttlefish's ability to adapt and respond to environmental cues could lead to the creation of smart materials that support tissue growth, facilitate cell adhesion, and promote regeneration.

Cuttlefish camouflage techniques involve the precise control of pigment distribution and tissue remodeling. Applying similar mechanisms in wound healing may lead to innovative approaches for promoting efficient healing and reducing scar formation. By studying the molecular and cellular processes underlying cuttlefish camouflage, researchers can develop strategies to modulate wound healing responses in humans, potentially improving outcomes and minimizing scar tissue formation.

Cuttlefish demonstrate extraordinary control over their skin coloration and patterning. Translating this ability into the field of human skin grafts and transplantation could have significant implications. Researchers can explore methods to manipulate and regulate skin pigmentation, aiding in the matching of skin grafts to the recipient's natural skin tone and reducing the risk of rejection.

Cuttlefish employ complex biophotonic mechanisms for camouflage. This understanding can contribute to advancements in biomedical imaging technologies and the development of biosensors. Utilizing the principles of light scattering and reflectivity exhibited by cuttlefish skin, researchers can develop novel imaging techniques and biosensors for non-invasive diagnostics, monitoring cellular activities, and detecting biomarkers.

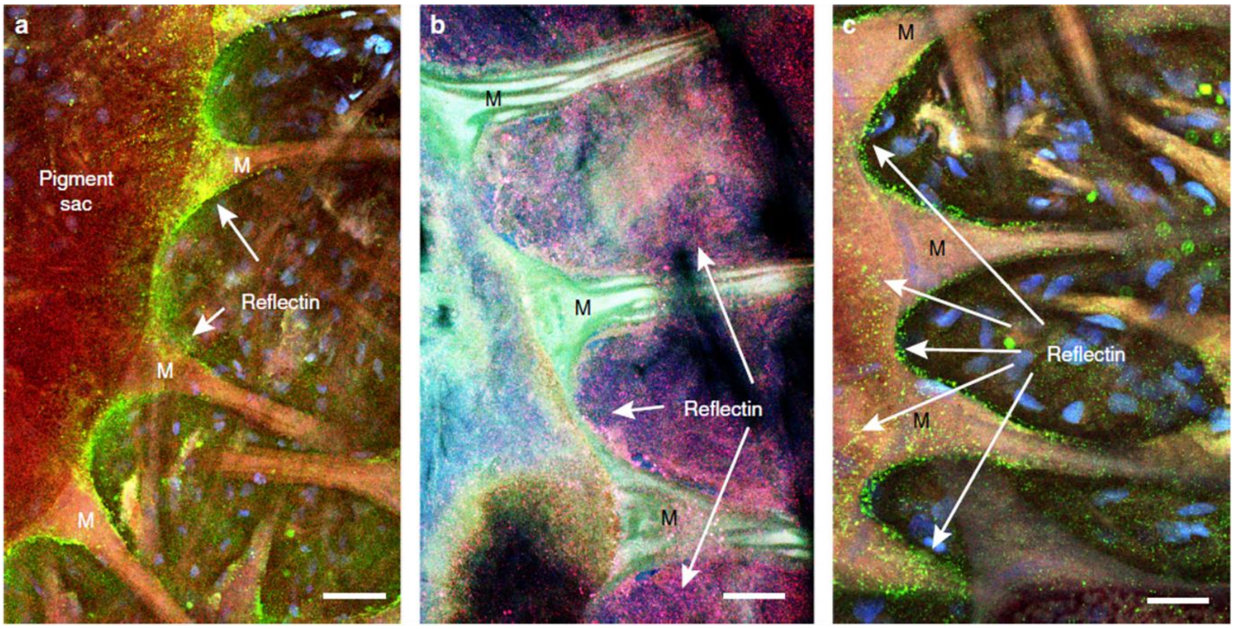

Figure 4. The anatomical location of reflectin in the vicinity of enveloping cells. Reflectin is present around the periphery of chromatophores and in the regions where they connect with radial muscles. The scale bar represents 50 µm. Source with permission: (18).

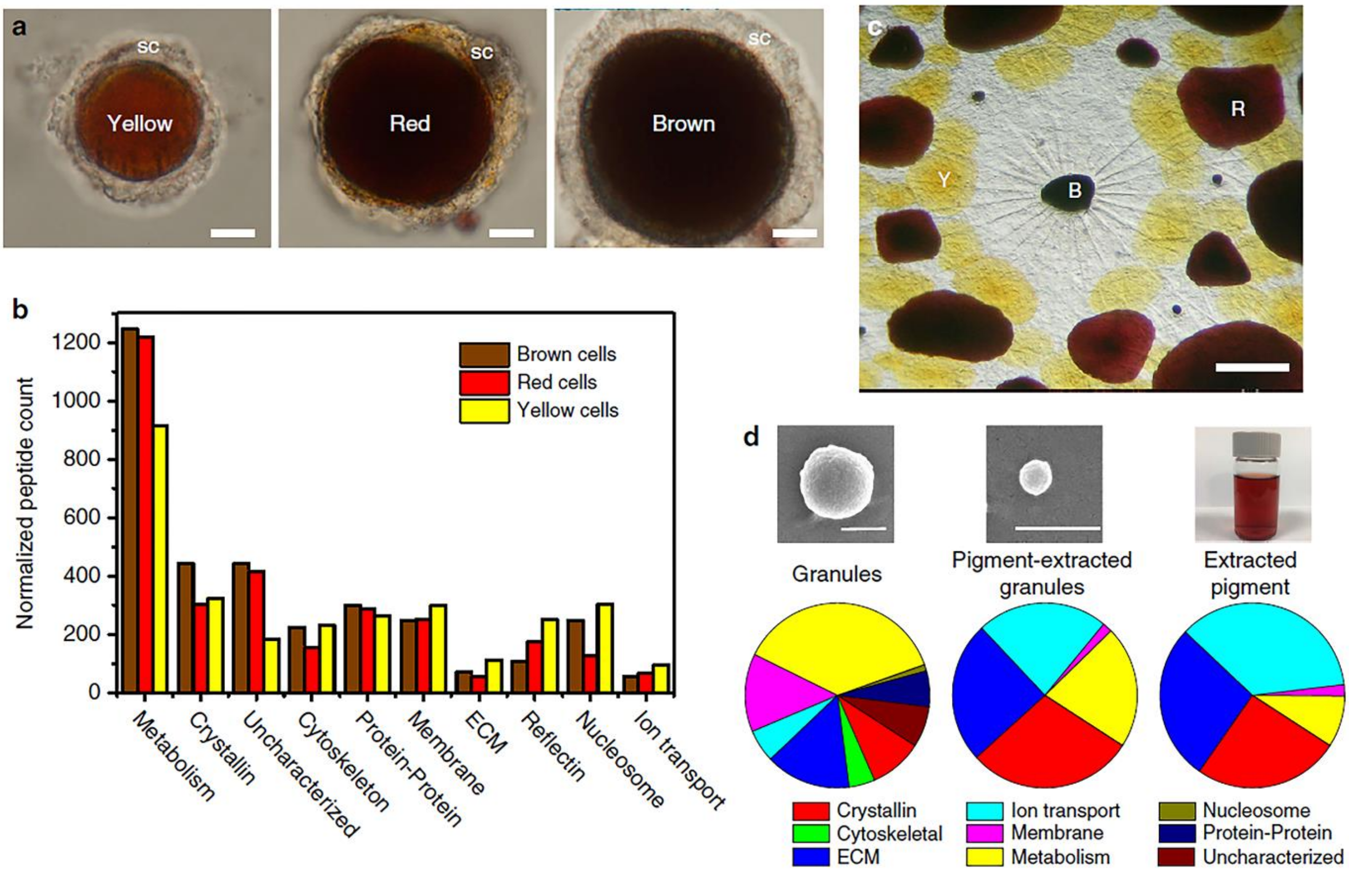

Figure 5. Chromatophore proteins. (a) Three types of chromatophores found in the skin of the cuttlefish, containing yellow, red, and brown pigments. The scale bar measures 5/6 micrometers. (b) Proteins of the yellow, red, and brown pigment cells. (c) Typical histological arrangement of chromatophores in the cuttlefish skin; pay attention to the radial muscles surrounding the brown chromatophore. The scale bar is 500 µm. (d) Granules extracted from pigments and extracted pigment proteins. The scale bar represents 500 nanometers, and the protein level in each pigment. Source with permission: (18).

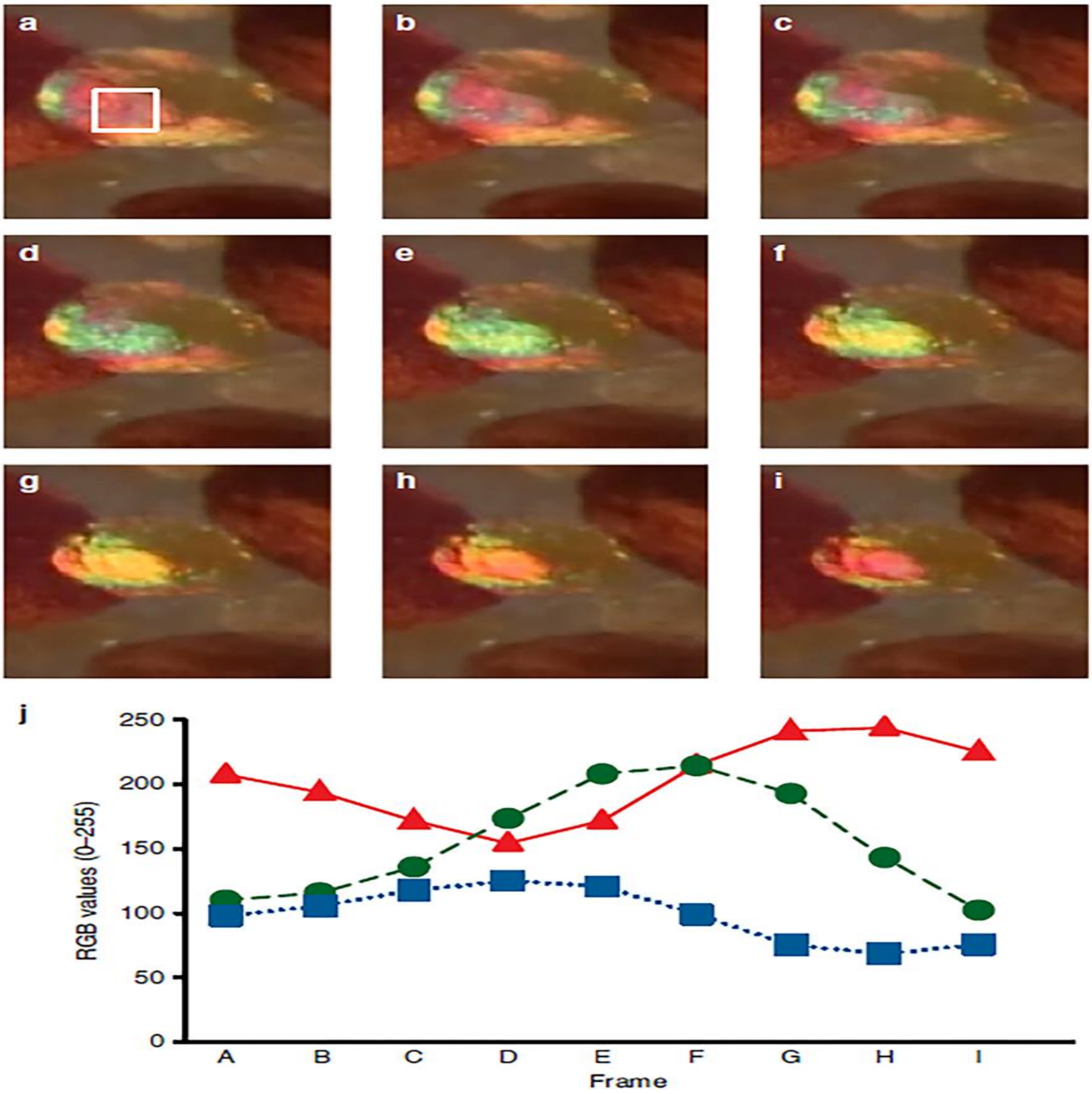

Figure 6. The visible color changes of a chromatophore over time were observed from sequential frames of a film, with each frame representing a duration of 0.03 seconds. Additionally, the quiescence of a yellow chromatophore in the bottom right corner was observed. During the stimulation cycle, distinct values of red, green, and blue (RGB) colors were generated. Using ImageJ software, the RGB values were measured in the selected region, and a graph (j) was plotted. Source with permission: (18).

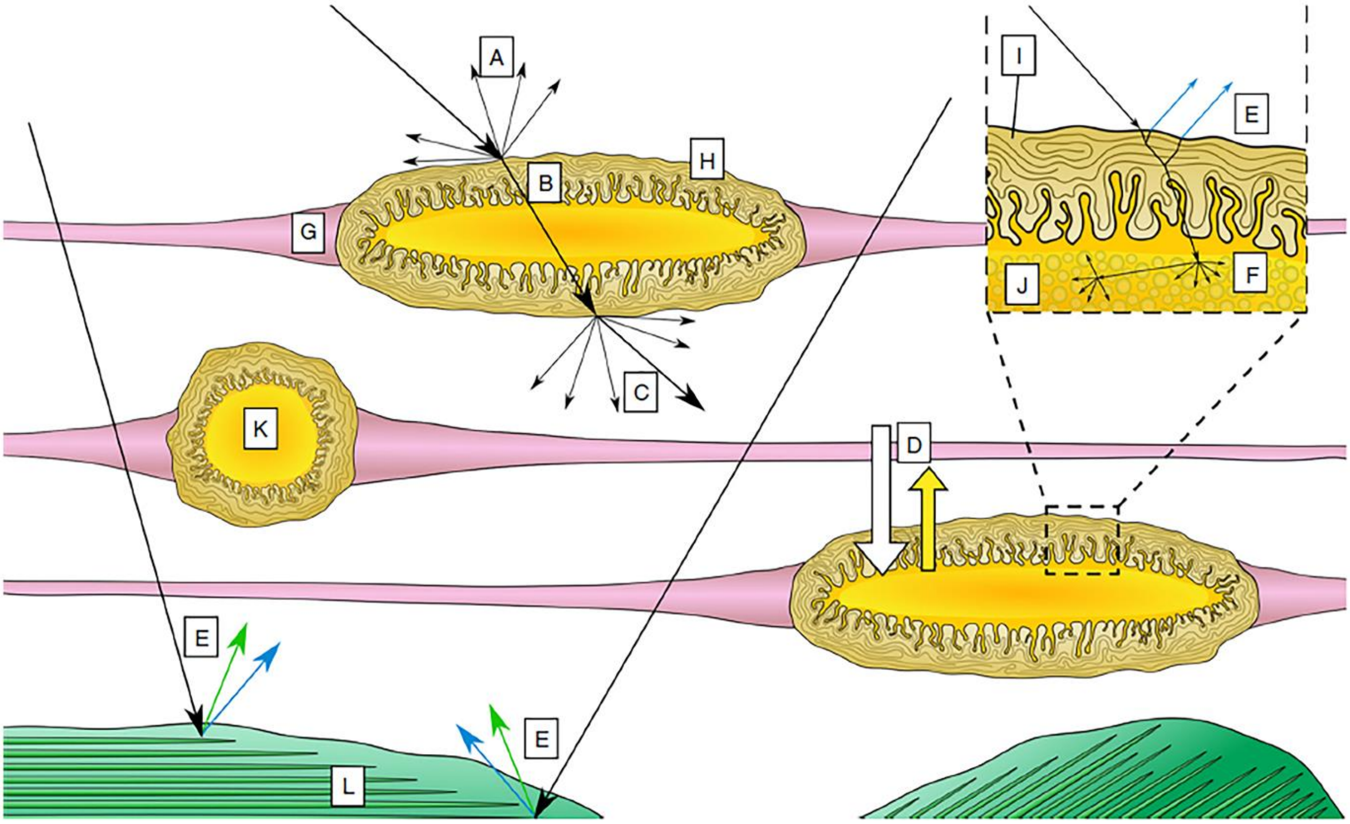

Figure 7. The light effects inside and around chromatophore structures include (A) backward scattering, (B) refraction, (C) forward scattering, (D) absorption of non-yellow wavelengths, (E) multilayer interference, and (F) diffuse scattering. (G) Represents radial muscle fibers. (H) Represents envelope cells. (I) Represents cytoplasm of envelope cells. (J) Represents individual granules. (K) Depicts a collection of granules within the yellow chromatophore. (L) Is an iridophore located in deeper sections of the dermis. Source with permission: (18).

While these potential applications are still in the research and exploration stages, the remarkable abilities of cuttlefish in the realm of regenerative biomedicine offer exciting possibilities for advancing human healthcare and therapeutic interventions. Continued studies and interdisciplinary collaborations will be crucial in unlocking the full potential of cuttlefish-inspired regenerative approaches.

Conclusions

The mesmerizing cuttlefish, with their captivating biophotonic camouflage, serve as extraordinary ambassadors of regenerative biomedicine. Through their intricate cellular mechanisms, they provide insights into the potential for harnessing the power of regeneration in the field of biomedicine. Exploring the interplay between nature's masterpieces and regenerative biomedicine technologies paves the way for exciting advancements in the quest for healing and restoration. Combining compositional, computational, and optical analysis of composite fish chromatophores provides insight into the substructural and chemical nature of these organs, from molecules to whole organelles. While chromatophores were initially thought to be solely pigmentary organs (3, 11, 13, 36), recent findings (18) indicate an additional photonic mechanism aided by structural coloration, which can create specific visual cues in prey or predators that are not easily detectable. Overall, these findings, coupled with the awareness of approximately 15 additional isoforms of reflectin in chromatophores whose functions remain unexplored, highlight the need to further understand these complex systems, including a better grasp of the molecular mechanisms governing this dynamic biophotonic phenomenon, which could pave the way for future design of engineered materials with dynamic optical capabilities.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

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