Chapter 15
Capsular Bioelastomers of Whelks

The eggs of the whelks are laid enclosed in protective capsules which, after the hatching of the eggs, are frequently found cast up by the sea along the shoreline. These capsules have a horny sclerotized appearance and are semitransparent and brownish in colour.
S. Hunt, Nature, 1966

Abstract Diverse whelks which represent a group of marine caenogastropod snails localize their embryos in unique biocomposite-based egg capsules. These multilaminate and capsular proteins-containing constructs are highly resilient. Capsular protein possesses comprehensive flexibility with ability to fast recovery which is proved by the decrease in the magnitude of elastic modulus (seeming damage) which starts at 3–5% strain. Take into consideration of the mechanical reaction to strain, this material is dissimilar to typical elastomeric proteins such as elastin or collagen. It is suggested that capsular elastomers possess high biomimetic potential for design and development of novel artificial hybrid materials.

In majority of marine invertebrates, the eggs, which are developed in the benthos, are deposited with characteristic surrounding structure such as multilaminated capsule or gelatin-based belts and masses (Fretter and Graham 1994). The formulation of these encapsulating structures, which is typical for the gastropod molluscs and polychaetes, allows developing of their embryos in benthos (Pechenick 1979).

Elastic capsules function as protectors of embryos against predation, microorganisms, desiccation, osmotic stress as well as UV irradiation (Ojeda and Chaparro 2004). In some species they can be very large in size. Thus, the eggs of *Busycon* are yielded in the form of a 1 m in length, helical strand usually called the mermaid necklaces, which can have almost 160 capsules (Fig. 15.1). They are subjected to the breakers with velocities reached even 10 ms^{-1} along the banks of the North America east coast (Miserez et al. 2009). Eggs capsules are acellular, with the structure and morphology closely connected with their functional roles. To carry out appropriate interpretation of the evolutionary aspect, function, origin of the structural patterns of the capsule as well as for material science and biomimetics

H. Ehrlich, *Marine Biological Materials of Invertebrate Origin*,
Biologically-Inspired Systems 13, https://doi.org/10.1007/978-3-319-92483-0_15

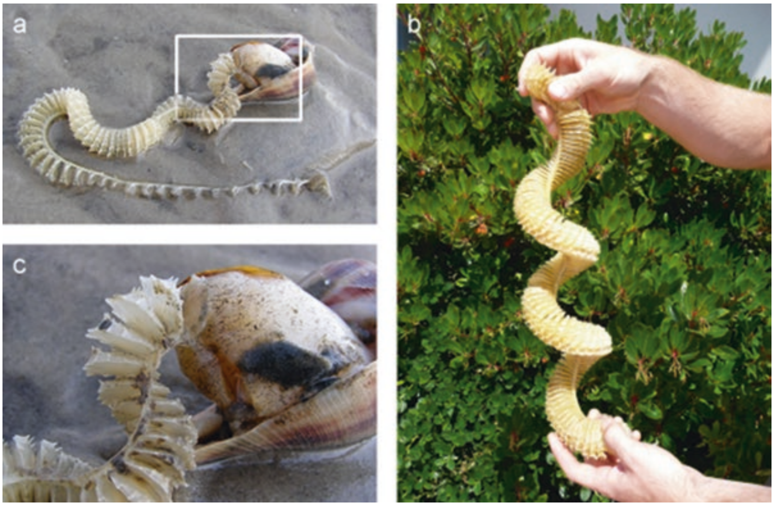

Fig. 15.1 Whelks are able to synthesise unique egg capsules (**a**, **c**) with interesting mechanical features (**b**). (Image courtesy Ali Miserez)

applications, the knowledge regarding the capsule wall's chemical structure is essential.

As assumed in the literature (Gathercole 1969; Rapoport 2003), the formation of the egg capsules can be described as below. A pliable soft sack (precapsule) is full of embryos and made of proteinaceous material. This sack is formed before leaving the genital tract by the nidamental (capsule) gland mesodermal secretory cells. It is supposed that, the precapsule in the pedal pore underwent physical shaping processes and enzymatically catalysed post-translational modifications that conceive the final chemical and morphological properties of the rigid rubbery capsule that can be observed *in situ*. According to Rappoport and Shadwick, "*gradations in snail size within a species produce egg capsules that are scaled versions of similar morphology, while differently shaped pedal glands finally form unique egg capsule morphologies*" (Rapoport and Shadwick 2007).

Thus, it was suggested (Tamarin and Carriker 1967) that the formation of these capsules is an example of the exocrine secretory activity, and lead to obtain stable structure strictly related to the species – that is unusual in comparison to typical amorphic secretion. In the snail *Urosalpinx cinerea,* the vase-shaped egg capsule comprise of four laminas, which three are anisotropic and have various transversal arrangements in comparison to the longitudinal plain, and one of them is isotropic. From the structural point of view, the birefringent matter clearly indicates a fine periodicity of variable dark (240 Å) and light (290 Å) regions, in each five subzone striae (Fig. 15.2a). The isotropic lamella together with the basal core comprises of distracted, 50 Å in width fibers, that can merge to form periodic fibrils (Tamarin and Carriker 1967). Goldsmith and co-workers in their investigation named the striated filaments as "pre-capsulin" and "capsulin" what was a consequence of the different

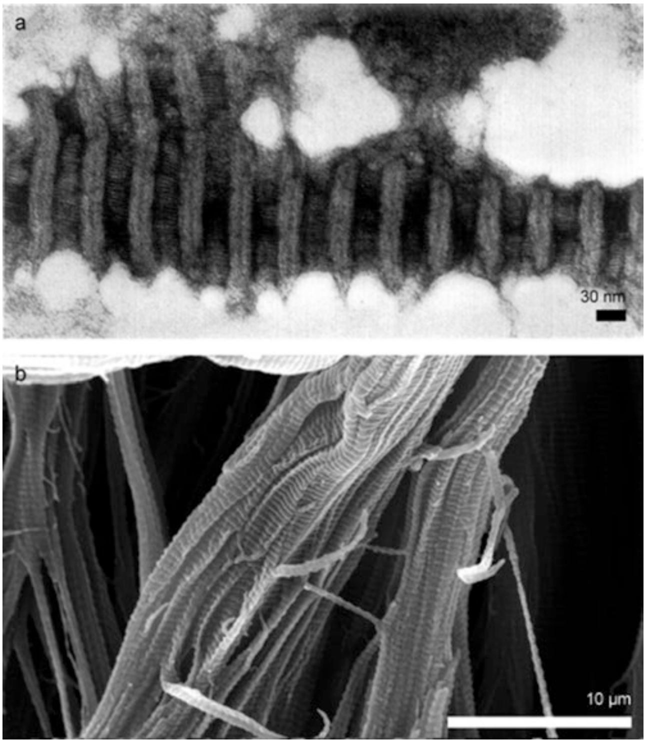

Fig. 15.2 Ribbon-like fibres of the whelks. (**a**) TEM image of stripped *Urosalpinx cinerea* ribbons stained with uranyl acetate, x 158,000. (Adapted from Flower et al. 1969). (**b**) Fibres within the egg capsule of *Busycon canaliculum* visualized using SEM. (Image courtesy Ali Miserez)

solubility of the individual pretranslationally modified matrix and the final product which is sclerotized (Goldsmith et al. 1978). It was hypothesized that both density as well as the cross-linking mode of the egg capsule biomaterial can influence its mechanical properties (Rapoport 2003). Firm fibre-like protein from the egg capsules of the *Buccinum undatum* has been characterized as resistant to both enzymatic (bactertial proteinases, chymotripsin, trypsin, pepsin) as well as to chemical (4 N NaOH, 0.5 M NaCl, 45%, 4% trichlor acetic acid at 60 °C, phenol at 90 °C,) treatment (Hunt 1966).

The alpha configuration of this protein has been proved (Rudall 1968; Flower et al. 1969) as well as in some features is similar to the proteins of the connective tissue origin of other invertebrates and vertebrates (Price and Hunt 1973). Studied on the chemistry of selected egg capsules showed that predominantly protein with minor amounts of lipid and carbohydrate is the main structural component (e.g., Hunt 1966). Amino acids as glutamic acid, aspartic acid, leucine, and lysine represent the dominant phase of the protein (Rapoport 2003).

Like elastin and some other structural proteins, the *B. undatum* capsule protein possesses yellow colour and under UV light displays a strong blue-white fluorescence in native state and after solubilization. Price and Hunt (1974) evaluated the possible role of the fluorophore-chromophore during the capsule protein cross-linking and that possesses aldehyde functional groups. It seems that di- or trityrosines are not to be present in the egg capsules of the whelks.

Comparative data on basic mechanical and biochemical egg capsules features obtained from different whelk snails species, such as the Kellet's whelk snail, *Kelletia kelletii*, characteristic to the West Coast of USA the *Busycon canaliculatum* channelled whelk snail usually found in the East Coast, are to found in corresponding papers (Rapoport and Shadwick 2000, 2001, 2002, 2007).

Research evaluated the qualified response of the material to stretching. The most interesting was the measuring of the energy diffused during the returning of material to its previous shape. Shadwick and Rappoport, stated that in all examined snails species, the material of capsule was technically identical, which proves that, during evolution this material has not changed and therefore, enhances survivorship of the snail.

Based on Rapoport and Shadwick (2002, 2007), capsular protein possesses comprehensive flexibility with an ability to fast recovery which is proved by the decrease in the magnitude of elastic modulus (seeming damage) which starts at 3–5% strain. Take into consideration the mechanical reaction to strain, this material is dissimilar to typical proteins such as elastin or collagen. However, the qualitative affinities of keratin, another common structural protein, in strain/stress behaviour are more than accidental in relation to structure and mechanical quantification. This suggests that the matrix organization and alpha-helical structure may resemble these two proteins. In fact, the protein in egg capsule can be tightly connected to keratins of vertebrate origin like intermediate filaments. Using the SEM microscopy, Rapoport and Shadwick (2007) confirm the fibrous hierarchical arrangement of *K. kelletii* and *B. canaliculatum* capsules. It was implied that the primary step in formation of such arrangement is responsible for binding of the fibrous components together. It is based on some still undetermined mechanism of sclerotization occurring in the ventral pedal gland. These researchers proposed a model (Fig. 15.3) for the mechanical and structural properties of whelk egg capsule biopolymer based on decomposing the mechanical behaviour of this biomaterial (WECB) under diverse chemical and physical treatments.

Recently, it was proposed (Fischer et al. 2013) that hysteresis during cyclic loading within this pseudoelastic biomaterial is based on a mismatch between the rate at which the phase transition occurs and the speed of the corresponding mechanical driving force. Some precursor proteins that make up this material have been reported too (Wasko et al. 2014). Thus, the behaviour of welk capsular protein is extraordinary in the recovery speed as well as the value of the dissipated energy, and is similar to the crystallization induced by strain in some polymeric fibres and deformations of superelastic character related to the transitions of the diffusionless phase known for shape-memory alloys (Corbett 2006; Harrington et al. 2012). Consequently, few attempts have been undertaken to develop constitutive models for this class of biomaterials (Kazakevičiutè-Makovska and Steeb 2011).

Diffusion of oxygen within egg capsules seems to be crucial from biological view. Thus, the egg capsules which mainly should save the embryos from predators,

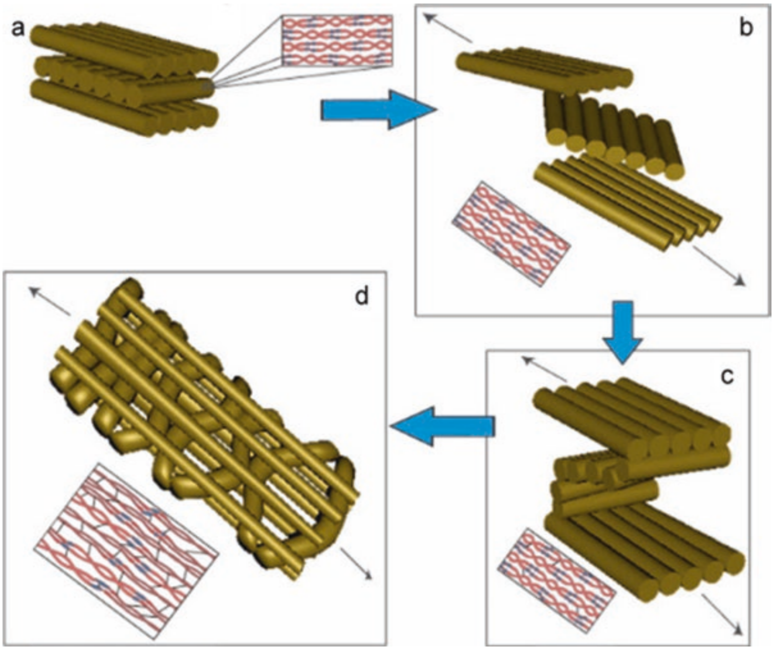

Fig. 15.3 Model of WECB mechanics through maturation: (**a**) generalized structure; (**b**) pre-ventral pedal gland (**c**) ventral pedal gland, and (**d**) post-ventral pedal gland. See detailed information in the text. (Adapted from Rapoport and Shadwick 2007)

also may pose physiological issues because these capsules provide a barrier to the gases diffusion (Gutowska and Melzner 2009). It was showed that the coefficients of oxygen diffusion in the egg capsules of marine animal are usually only 10–20% that of pure H_2O (Brante 2006). During molluscs development, the rate of the oxygen consumption increases greatly (Cronin and Seymour 2000; Brante 2006). Therefore, to enhance the oxygen fluxes, in many molluscan the eggs bulge during the development increases the surface areas, and reduces the thicknesses of their walls (Cronin and Seymour 2000), and thus enhances the oxygen conductance. Additionally, inside the capsules fulfilled with fluid embryos are able to produce some currents to preclude the development of pO_2 gradients (Cronin and Seymour 2000). Molluscs species which do not have active brooding behaviour (Gutowska and Melzner 2009), their eggs or capsules can be located in such niches where exchange of water and oxygen is high.

Additionally, the construction of the capsule has to provide the possibility to survive, grow and escape of the encapsulated embryos. To get the detailed information about fulfilments of these different requirements may be achieved the evaluation of composition and physicochemical features both of capsules and gas exchange during onthogenesis.

15.1 Conclusion

It was showed that the capsules of the molluscs egg (especially *Busycon* species) may be used as a source of novel bioelastomeric material with greater extensibility and fast recovery than it was supposed. Better understanding of naturally occurring impact-absorbing properties and reversible extensibility (>100%) for the insulation of molluscan damage-prone tissues remains to be a crucial point. The understanding of corresponding mechanisms can be applied to formation of new encapsulants for tissue implants, pharmaceuticals, the cells transplantation or other tissues in various medical researches. Interestingly, these naturally existing encapsulation strategies are not a point of interest of engineers, however they are well organized.

References

Brante A (2006) An alternative mechanism to reduce intracapsular hypoxia in ovicapsules of *Fusitriton oregonensis* (Gastropoda). Mar Biol (Berl) 149:269–274

Corbett CM (2006) The mystery of the whelk egg capsule protein – electrospinning, mechanical testing and being outsmarted by an invertebrate. PhD thesis. The University of British Columbia

Cronin ER, Seymour RS (2000) Respiration of the eggs of the giant cuttlefish *Sepia apama*. Mar Biol (Berl) 136:863–870

Fischer FD, Harrington MJ, Fratzl P (2013) Thermodynamic modelling of a phase transformation in protein filaments with mechanical function. New J Phys 15:2–15

Flower NE, Geddes AJ, Rudall KM (1969) Ultrastructure of the fibrous protein from the egg capsules of the whelk *Buccinum undatum*. J Ultrastruct Res 26(3–4):262–273. https://doi.org/10.1016/S0022-5320(69)80006-7

Fretter V, Graham A (1994) British prosobranch molluscs. Their functional anatomy and ecology. Ray Society, London

Gathercole L (1969) Studies on the protein of the egg capsule of whelks. PhD Thesis. University of Leeds

Goldsmith LA, Hanigan HM, Thorpe JM et al (1978) Nidamental gland precursor of the egg capsule protein of the gastropod mollusc *Busycon carica*. Comp Biochem Physiol 59B:133–138

Gutowska MA, Melzner F (2009) Abiotic conditions in cephalopod (*Sepia officinalis*) eggs: embryonic development at low pH and high pCO_2. Mar Biol 156:515–519

Harrington MJ, Wasko SS, Masic A et al (2012) Pseudoelastic behaviour of a natural material is achieved via reversible changes in protein backbone conformation. J R Soc Interface 9(76):2911–2922

Hunt S (1966) Carbohydrate and amino-acid composition of the egg capsule of the whelk *Buccinum undatum* L. Nature 210:436–437

Kazakevičiutė-Makovska R, Steeb H (2011) Superelasticity and self-healing of proteinaceous biomaterials. Procedia Eng 10:2597–25602

Miserez A, Wasko SS, Carpenter CF et al (2009) Non-entropic and reversible long-range deformation of an encapsulating bioelastomer. Nat Mater 8:910–916

Ojeda JA, Chaparro OR (2004) Morphological, gravimetric, and biochemical changes in *Crepidula fecunda* (Gastropoda: Calyptraeidae) egg capsule walls during embryonic development. Mar Biol 144:263–269

Pechenick JA (1979) Role of encapsulation in invertebrate life histories. Am Nat 114:859–870

Price NR, Hunt S (1973) Studies of the cross linking regions of whelk egg capsule proteins. Biochem Soc Trans 1:158–159

Price NR, Hunt S (1974) Fluorescent chromophore components from the egg capsules of the gastropod mollusc *Buccinum undatum* (L.), and their relation to fluorescent compounds in other structural proteins. Comp Biochem Physiol 47B:601–616

Rapoport HS (2003) Biomechanics, biochemistry, and molecular biology of a molluscan scleroprotein elastomer: whelk egg capsules. PhD thesis. University of California, San Diego

Rapoport HS, Shadwick RE (2000) Investigations into the selfhealing behavior of whelk egg capsule biomaterial, genus *Busycon*. Comp Biochem Physiol 126B(Suppl.1):S81

Rapoport HS, Shadwick RE (2001) A keratin-like gastropod biomaterial used to clarify the mechanical models of keratin. Am Zool 41:1563

Rapoport HS, Shadwick RE (2002) Mechanical characterization of an unusual elastic biomaterial from the egg capsules of marine snails (*Busycon* spp.). Biomacromolecules 3:42–50

Rapoport HS, Shadwick RE (2007) Reversibly labile, sclerotization-induced elastic properties in a keratin analog from marine snails: whelk egg capsule biopolymer (WECB). J Exp Biol 210:12–26

Rudall KM (1968) Intracellular fibrous proteins and the keratins. In: Florkin M, Stotz EH (eds) Comprehensive biochemistry, vol. 26B. Elsevier, Amsterdam

Tamarin A, Carriker M (1967) The egg capsule of the Muricid gastropod *Urosalpinx cinerea*: an integrated study of the wall by ordinary light, polarized light, and electron microscopy. J Ultrastruct Res 21:26–40

Wasko SS, Tay G, Schwaighofer A et al (2014) Structural proteins from whelk egg capsule with long range elasticity associated with a solid-state phase transition. Biomacromolecules 15(1):30–42