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ABSTRACT

Cnidarian proteins are considered useful for the development of therapeutics, as well as this they have also received the attention of biotechnology and the cosmetic industries. In 2017 the first ever sea anemone venom peptide cosmetic, named SensAmone P5, was launched by Mibelle Biochemistry. This synthetic peptide is based on the interaction of APHC1, from *Heteractis crispa*, on the pain relevant ion channel TRPV1. This peptide reduces TRPV1 signalling *in-vitro* and skin sensitivity in human volunteers. Aside from venoms, jellyfish mucus and collagen are both used in cosmetic preparations. Many legal definitions of animals do not include the invertebrates and thus it is likely that invertebrate proteins are more acceptable as an alternative to mammalian proteins. Mucins are important proteins for moisturisers and using jellyfish as the source appears to be a suitable alternative to bovine and porcine proteins which were previously used. The main structural protein that supports the soft bodied jellyfish is collagen. This collagen appears to be biocompatible with human tissues and thus has been successful as a cosmetic, as well as being used in-vitro for 3D tissue engineering scaffolds. This short communication will discuss the use of Cnidarian proteins in cosmetics.

KEYWORDS

Cnidarian, Cosmetics, Biotechnology, Venom, TRPV1, Collagen, Jellyfish, sea anemone, SensAmone P5

INTRODUCTION

Cnidarian venoms in cosmetics

Cosmetic science is driven by the human need to portray a certain image of ourselves, either for attracting potential partners, making us feel good through looking good, or the desire to hide the signs of the inevitability of ageing skin. From the outside there appears to be no shortage of ingredients to tackle all these things. However, further investigation reveals a constant strive for improvement, rapid changes in trends, and a need for novel ingredients and activities. To meet the ever-increasing demands of the consumers, cosmetic scientists are striving to find new compounds that meet these needs, testing the activities to produce the data that supports the use of the product, this is called the product claim. As well as the functional activity to support the claim, there is also the need for strong visual appeal to support the product, as the overall approach of cosmetics is to produce visual appeal. Venoms are functional proteins with a wide range of activities, but the most well-known groups of venomous animals, spiders and snakes, instil fear in many people, whereas the majority of Cnidarian species are admired for their beauty and calming movements (Figure 1). Jellyfish,

however, do instil fear in some people and for the few exceptional species that fear is justified due to the potential of fatal envenomation(1). The potent cocktail of pharmacologically active compounds in Cnidarian venoms make them useful for drug discovery research (see Powerful Proteins from Polyp Possessing Predators chapter in this book) however, cosmetic science also relies on compounds hitting targets in a similar way.

CNIDARIAN COSMETIC ACTIVES AND THEIR USE

Cnidarian venoms contain a diverse collection of active peptides which includes antibacterial activity, ion channel modulators, and enzymes. Ion channels are important targets in cosmetics for applications including antiwrinkle and sensitive skin. Enzyme targets include collagenase and proteases for skin firming(2) and tyrosinase for tanning and whitening(3). Larger proteins such as the mucins and collagen, which is present in jellyfish, are also interesting for cosmetic science and will be discussed later in this chapter along with antioxidants.

Nicotinic acetylcholine receptors (nAChR) are key ion channels involved in the conduction of nerve impulses into muscle cells to effect muscle contraction. It is well known that many venoms act on these targets to cause paralysis, including that of the Moon Jellyfish (*Aurelia aurita*)(4) as seen in figure 2. Another venom that acts on nAChRs is that of the Temple Viper (*Trimeresurus wagleri*) from which a cosmetic active peptide, Waglerin(5), has been developed that is functional in reducing wrinkles when topically applied(6). Thus, similar peptides could be developed from Cnidaria for this key target.

Other ion channels, such as voltage gated sodium channels and Transient Receptor Potential (TRP) channels are very important in the transduction of pain from a variety of stimuli. TRP channels are ligand gated ion channels predominantly located in the sensory neurones in the skin(7). Other cell types in the skin, such as keratinocytes and sebocytes, also express channels of the TRP family, which are involved in the regulation of cell proliferation and differentiation(8). The most important member of the TRP channel family is the vanilloid receptor TRPV1. This ion channel produces action potentials in sensory neurones in response to noxious heat and irritant compounds such as capsaicin (the hot compound in chilli peppers). Activation of TrpV1 by capsaicin produces pain, redness and irritation(9). Interestingly, a connection between TRPV1 activation and the expression of the collagen-digesting enzymes matrix metalloproteinases (MMPs) in the skin has been discovered. Furthermore, aged and photoaged skin express higher levels of TRPV1 directly linking skin aging and skin sensitivity(10). Thus, inhibition of TRPV1 reduces these effects and acts to sooth the skin.

In 2008 a Russian laboratory reported the discovery of an analgesic peptide from the Leathery Sea Anemone, *Heteractis crispa*. This 56 amino acid protein has a Kunitz fold stabilising its secondary structure(11) and is named APHC1 (Analgesic peptide from *Heteractis crispa* 1). It potently blocks TRPV1 with an EC50 of 54 nM, producing analgesia(11) and therefore is a good candidate for soothing sensitive skin. Many Heteractis species are large, beautiful anemones such as *Heteractis magnifica* (Figure 1). Acid sensing ion channels (ASICs) are also important in pain and inflammation. The venom from the anemone *Anthopleura elegantissima* contains a peptide APETx2, which is the most potent and specific toxin to block ASIC3(12). Neither of these authors used the rational nomenclature proposed by Glenn Kings' team(13) and later revised specifically for sea anemones(14) although this is partly due to timing. Therefore, APETx2 should be referred to as π-AITX-Ael2b and APHC1 should be correctly referred to as τ-SHTX-Hcr2b (14). This gives a standardisation to the nomenclature and richer

information in the naming. The Greek letter prefix denotes the target, π for ASIC and τ for TRP, the four letter toxin code denotes family AITX for Actiniidae Toxin and SHTX for Stichodactylidae toxin, and lastly the genus letter and two letters from species confirm the taxonomy and toxin chronology. Just like chemical names encode information about structure use of rational toxin names encodes biological information on activity and taxonomy.

CASE STUDY: THE FIRST CNIDARIAN VENOM COSMETIC PEPTIDE SENSAMONE P5

The sea anemone protein τ-SHTX-Hcr2b is a perfect candidate to help reduce skin sensitivity because of its action on TRPV1 pain receptor inhibition. However, the full-length protein is unstable in a cosmetic formulation and too big to penetrate into the skin. Also, harvesting it from sea anemone venom is not feasible for cosmetic applications, synthetic production of proteins of this size is expensive, and refolding can be challenging. Therefore, a five amino acid peptide was designed through computer modelling of the active site of τ-SHTX-Hcr2b with the aim of still engaging TRPV1 with a more convenient sized peptide. This was tested in *in-vitro* patch clamp experiments, with cells expressing high levels of TRPV1. The experiment revealed a more potent TRPV1 inhibition by the peptide compared to the full-length τ-SHTX-Hcr2b protein at the same molecular concentration. The small size of the final peptide allows for simple synthetic production and thus sustainability through avoiding the need for venom extraction of many of *H. crispa* animals. Human skin can be detrimental to non-human proteins, as part of the host defence system, so chemical modification of the C-terminus was made to improve stability. The formulation added further stability and bioavailability through the incorporation into a soft sphere carrier system based on shea butter (Figure 3). The resulting novel cosmetic active was named SensAmone P5 (INCI: Pentapeptide-59 (and) Hydrogenated Lecithin (and) *Butyrospermum parkii* (Shea) Butter (and) Phenethyl Alcohol (and) Ethylhexylglycerin (and) Maltodextrin (and) Aqua/Water) and is a solution to relieve sensitive and irritated skin.

In a double-blind placebo-controlled clinical study, 31 volunteers (female, average age 47 years) with sensitive skin applied cream with 2% SensAmone P5 or the corresponding placebo on each side of their face. The current perception threshold (CPT) was measured using a Neurometer (Neurotron Inc., USA) two hours following a single application and compared to the initial CPT before application. The Neurometer applies an electrical stimulus to the skin at different frequencies (250 Hz and 5 Hz) in order to target different sensory nerve cells. The CPT is then determined as the amount of electrical stimulus needed for it to be felt by the volunteer. As a result, the higher the CPT value, the more electrical stimulus is needed in order to be felt by the volunteer, the less reactive the skin is. A single application of 2% SensAmone P5 significantly increased the CPT of the skin and so reduced skin reactivity (Figure 4). In addition to the short-term skin soothing effect of SensAmone P5, a long-term effect on the reduction of skin sensitivity was also investigated. For this, a double-blind placebo-controlled clinical study was carried out in which 31 volunteers (female, average age 47 years) with sensitive skin applied a cream with 2% SensAmone P5 or the corresponding placebo on each side of their face, twice daily for a period of 28 days. To measure skin sensitivity, a lactic acid stinging test was performed by applying an aqueous 5% lactic acid solution on the nasolabial fold and assessing the stinging, burning and itching sensations on a four point scale at one minute intervals for a total period of nine minutes. Treatment with SensAmone P5 significantly reduced skin sensitivity by more than 26%. Therefore, SensAmone P5 was launched in 2017 help to protect sensitive skin from overreacting to environmental stimuli and is paving the way for other potential functional cosmetic ingredients from Cnidaria.

NON-VENOM PROTEINS IN COSMETICS

In cosmetics you cannot go far without some mention of collagen, either as injectable fillers, topical creams(15), and even as oral supplements(16). This does not come as a great surprise as collagen is one of the major structural components in the skin of many animals, including humans. Slowing and hiding the signs of skin ageing is a major market and many of these features are due to changes in collagen, such as reduced content and disorganisation(17). Typical sources of collagen come from food waste such as bovine and porcine skin and tendons, and fish skin and scales. However, there is a growing interest in sustainable harvesting of jellyfish collagen, especially as anthropogenic changes in ocean chemistry have led to many blooms which can also lead to damages to tourism(18), fish farms(19), and even power stations through impingement on inlets(20). Also, there is reduced interest in mammal collagen sources due to diseases such as Bovine Spongiform Encephalopathy (BSE) and Foot-and-mouth, as well as cultural attitudes to mammalian products. Collagen structure is a long alpha helix containing glycine at every third amino acid in a GXX motif. Crosslinking of these chains gives them greater stability and reduced solubility. Many authors of cosmetic articles claim that jellyfish collagen is similar to human collagen, but with this shared motif across all animals it's not surprising as they are 30% identical, before considering the other two amino acid positions. Scyphomedusae are particularly rich in collagen, with a dry weight up to of 35% from *Rhopilema asamushi* and 46% from *Stomolophus meleagris*(21). This collagen does have low immunogenicity and is biocompatible with mammalian tissues(22), making it useful. One downside of aquatic collagen sources such as fish and Cnidaria is a low denaturation temperature, reported between 26ºC (23) and 28ºC (24), presumably connected to the water temperatures where they evolved. Intact collagen is less useful than collagen peptides produced through hydrolysis of the crosslinks by pepsin, this renders the collagen soluble and thus more amenable to use. *Rhopilema esculentum* collagen has been shown to protect mouse skin from UV damage and photo ageing as both intact collagen and peptides (hydrolysate)(25). The concept of oral collagen to improve skin tone and moisture appears futile due to the normal digestion of proteins, however there are reports of the di- and tripeptide fragments being absorbed into the blood stream, including one from a double blinded placebo controlled trial(16). An oral nutraceutical called Celergen® (Celergen Ltd) from fish collagen has been shown to improve sebum production and skin elasticity (26). Another protein prevalent in Cnidaria is mucin, these large glycoproteins have evolved in marine animals as protective layers and in terrestrial animals as a way of maintaining moisture. It may be surprising to think of mucus in cosmetics, however even terrestrial snail mucus is used(27). The ability of mucin to bind large amounts of water is used in cosmetic products to maintain skin hydration and to protect the skin surface. Mucin proteins, like collagens, are ubiquitous throughout the animal kingdom and thus there are many potential sources, however the blooms of Cnidaria are likely to be the richest sources.

There are a number of species of Cnidaria that contain peptides with claimed antioxidant activity, which may be potentially useful to the cosmetics industry as many cosmetics contain antioxidants as active ingredients. Gelatin from the edible jellyfish *Rhopilema esculentum* has found to be a useful source of polypeptide antioxidants (26). Of the three polypeptides extracted SCP2 and SCP3 were found to be the most potent in *in vitro* antioxidant tests. The aqueous soluble extract of the large jellyfish *Rhizostoma luteum* also has antioxidant properties(26) and are thought to be the highest so far reported in a jellyfish. The Barrel Jellyfish *Rhizostoma pulmo* and the Mediterranean or Fried Egg Jellyfish *Cotylorhiza tuberculata* also have been reported to have high levels of antioxidants whereas the Moon Jellyfish, *Aurelia* sp., has been found to contain lower levels (28).

FUTURE PERSPECTIVE

The diversity of Cnidarian species and their beauty will continue to attract cosmetic scientist and their customers to this rich source of active ingredients from the deep and surface waters of our planet. The amazing colours of the hard corals and the soothing motion of the soft bodied animals will also continue to allure the marketing teams. However, it is the rich source of novel proteins and peptides providing the active ingredients that will drive this search. As more is discovered about the diversity of Cnidarian peptides from their venoms and elsewhere, new targets will be discovered, and thus new active ingredients developed.

CONCLUSION

Cnidarians contain a rich source of molecules which have be utilised in many ways in the cosmetics industry from products for sensitive skin, to antioxidants which are found in a wide range of products. Cosmetic scientists have demonstrated the use of proteins and other molecules from these magnificent creatures and thus opened the door to further work. There are many challenges and opportunities to utilise these magnificent creatures, but to do so we must protect them and their underwater world. Microplastics and sunscreens have hit the headlines as problems for the marine ecosystems and thus products from this watery habitat may well be more ecological solutions for future products.

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Conflict of interest declaration

ST declares that he designed the TRPV1 blocking pentapeptide at Venomtech that was developed into SensAmone P5 for sale by Mibelle Biochemistry. FW declares that she works for Mibelle Group Biochemistry that developed, produces and sells SensAmone P5.

Figures

Figure 1: Captive *Heteractis magnifica* showing the flower like beauty of anemones even under the blue lights that allow photosynthesis in the zooxanthellae and thus allows effective captive maintenance. Photo by Phillip Robinson

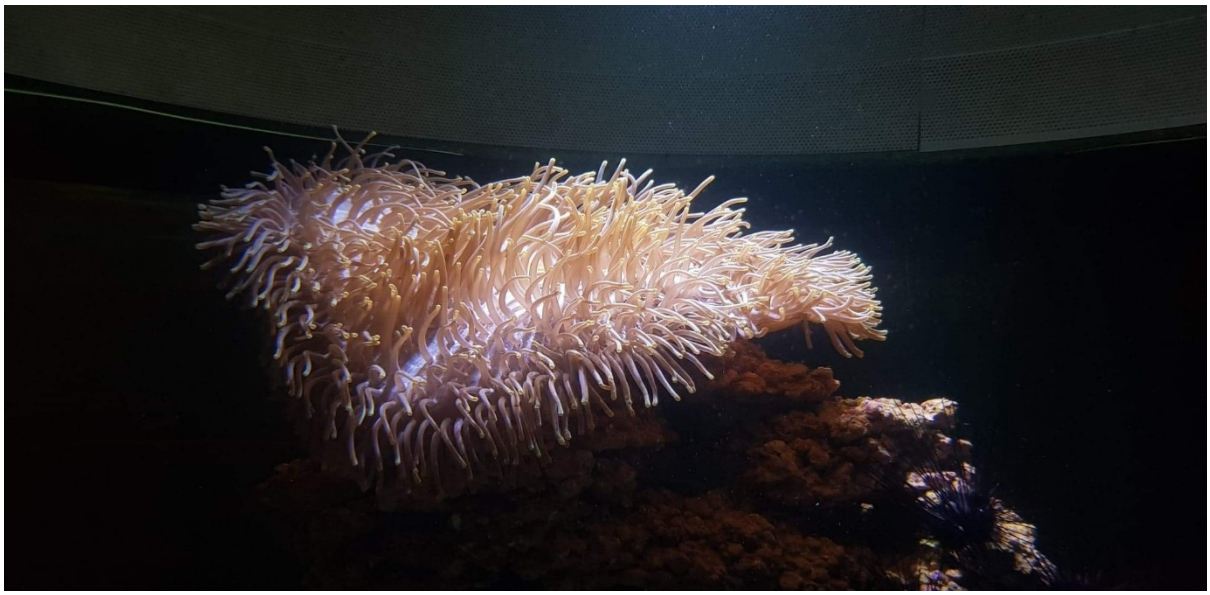

Figure 2: The Ethereal moon jellyfish *Aurelia aurita* is quite a common captive Cnidaria but still requires specialist conditions for effective captive maintenance. Photo by Phillip Robinson

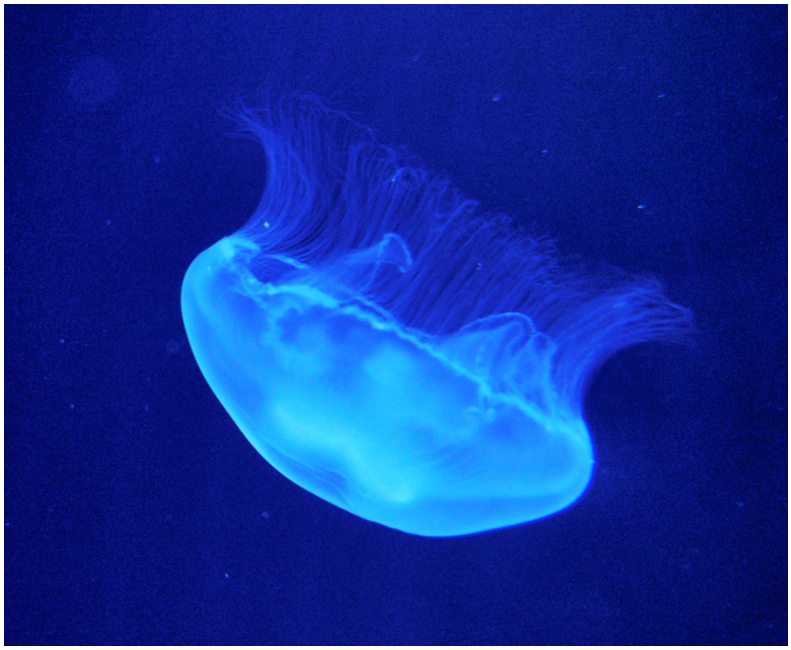

Figure 3: The sequence of SensAmone P5 pentapeptide. In order to stabilise the peptide against degradation, increased skin penetration and to enable controlled release, the pentapeptide was incorporated into a soft sphere carrier system based on shea butter. Image produced by Mibelle Biochemistry.

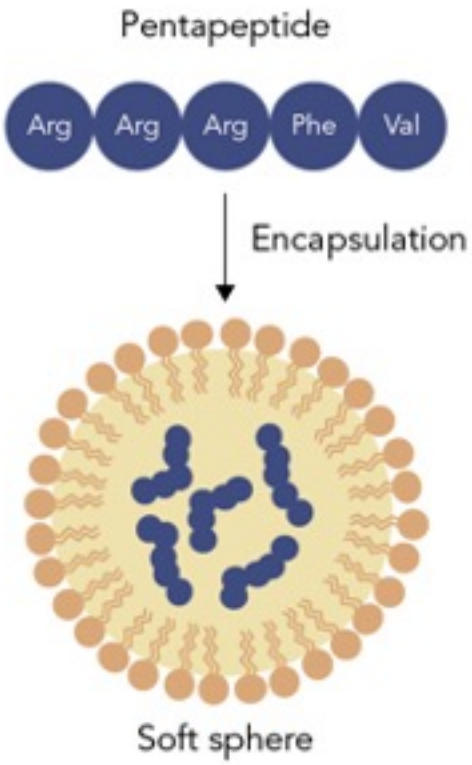

Figure 4: Current perception threshold (CPT value)
Application of an electrical stimulus to the skin at different frequencies to target different nerve populations: The higher the CPT value, the less reactive is the skin. **250 Hz:** small myelinated nerve fibers that transmit fast pain, temperature and pressure sensation. **5 Hz:** small unmyelinated nerve fibers that transmit dull pain and temperature, responsible for itching. SensAmone P5 significantly reduces the reactivity of the skin 2 hours after a single application. Image produced by Mibelle Biochemistry.