Institution: University of Reading

Unit of Assessment: 8 Chemistry

Title of case study: Collagen Stimulating Lipopeptides for Cosmetic Applications

1. Summary of the impact

The multi-million pound skincare industry has benefitted greatly from research carried out since 2009 at Reading, demonstrating for the first time that an ingredient in some anti-ageing face creams can genuinely increase the amount of collagen produced by skin cells, thereby removing the appearance of wrinkles. The research investigated the nanostructure of the lipopeptide known as Matrixyl and how changes to its environment and composition affect its structure and activity. These findings received widespread media coverage in the UK and abroad, leading to noticeable increases in sales of Matrixyl-containing products by Procter and Gamble and enhancing the business of the UK-based SME *Forme Laboratories*, who have developed a new line of effective skincare products for menopausal women, based on the Reading results.

2. Underpinning research

Background

Ian Hamley, Diamond Professor of Physical Chemistry (2005-present), has been investigating the nanostructure of the lipopeptide C_{16}-KTTKS (Matrixyl) since 2009. Matrixyl is an ingredient found in a number of commercial face-creams and Hamley's group has demonstrated its ability to increase the amount of collagen produced by fibroblasts - cells found in connective tissues such as skin - by 70%. Collagen is the principal protein that contributes to the strength and elasticity of skin.

While Hamley's research on C_{16}-KTTKS has inherent implications for skin-care products, his primary interest is in the development of next generation biomaterials for assisting wound healing and regenerative medicine, focusing on strategies to increase production of collagen, the main structural protein in mammalian connective tissue. The peptide KTTKS has been previously shown to promote and stimulate collagen production. In 2000, a French biotechnology company (Sederma) added a 16-carbon lipid chain to KTTKS to confer enhanced stability in vivo, and thus increase its bioavailability by increasing resistance to enzymatic degradation. This *lipopeptide* or *peptide amphiphile,* (PA) C_{16}KTTKS, was registered under the trade name Matrixyl.

Investigating the nanostructure of Matrixyl

The way in which molecules of Matrixyl-like peptides aggregate depends upon the length of the lipid tail and the surrounding chemical environment, both of which can influence peptide activity. In 2009, Hamley and Castelletto (Research Fellow at Reading, 2005-present) began studying the nanostructure of Matrixyl to better understand its mode of action. Despite its widespread commercial use, no previous studies of the physico-chemical properties of Matrixyl had been undertaken. Castelletto and Hamley (at Reading) led the research, although some experiments were also carried out in specialist facilities at the Israel Institute of Technology (Technion). Techniques including atomic force microscopy and pyrene fluorescence microscopy were used to reveal the aggregated nanostructure of Matrixyl. In 2010, the team reported that, unlike compounds of similar size that form cylindrical nanostructures, Matrixyl forms flattened aggregates termed "nanotapes".[1] The molecules were stacked in a way that suggested the structure itself may be important in stimulating collagen production.

How the chemical environment influences structure

In skincare products, Matrixyl is formulated with surfactants such as emulsifiers or dispersants, and it was of fundamental interest to understand how these materials affect self-assembly of Matrixyl and ultimately its biomedical activity. Between 2011 and 2012, Hamley's group demonstrated that a non-ionic polymeric surfactant (Pluronic P-123) can be used to prepare solutions of Matrixyl without disrupting its self-assembly characteristics or altering its activity. [2] They also examined the influence of the anionic surfactant SDS on Matrixyl, finding that, due to electrostatic forces, different nanostructures are formed depending on the amount of SDS added, resulting in modification of its macroscopic characteristics such as a change in morphology from sol to gel. [3]

In 2013, the team found that changes in pH also affected self-assembly of Matrixyl. At pH2, the Matrixyl formed spherical micelles with the hydrophobic tails inside, whilst at pH3, they assembled into tape-like structures and, at pH4, they formed right-hand twisted fibrous structures. [4]

Modifying the lipid tail

The Reading group also investigated how the length of the lipid tail altered the self-assembly of Matrixyl-type peptide amphiphiles, using techniques including circular dichroism and small angle X-ray scattering, to understand how tail length affected their physico-chemical properties. They found that skin-permeability increased with longer lipid tails, with a C_{14} tail formulation estimated to be 3.8 times less permeable than a C_{16} tail and a C_{16} tail 3.8 times less permeable than a C_{18} tail. [5] Therefore it seemed possible at this stage that a PA with a longer lipid tail could have an enhanced collagen-stimulating effect due to a higher skin-permeability, with implications not only in regenerative medicine but also for the skin-cream industry.

Proving that Matrixyl stimulates collagen production

In 2013, Hamley and colleagues reported that Matrixyl stimulates collagen production in human skin and corneal cells in a concentration-dependent manner, indicating that self-assembly and collagen production are interrelated. [6] The finding was based on nanostructural analyses carried out by Roanne Jones (PhD student, 2010-2013) who was co-supervised by Hamley and Dr Che Connon (Lecturer/Associate Professor in Pharmacy, 2007–date) at Reading. This was the first independent demonstration of the stimulation of collagen production by C_{16}-KTTKS / Matrixyl.

3. References to the research (Citations retrieved from *Scifinder* on 24/10/13)

Publications have been internally reviewed and assessed as of at least 2* quality. Outputs marked as * are suggested to assessed quality of research:

[1] Castelletto, V., Hamley, I.W., Perez, J., Abezgauz, L. & Danino, D. (2010) Fibrillar superstructure from extended nanotapes formed by a collagen-stimulating peptide. *Chem.l Commun.,* 46 (48): 9185 – 9187. DOI: 10.1039/c0cc03793a. (Cited 18 times).

[2] Dehsorkhi, A., Castelletto, V., Hamley, I.W. & Lindner, P. (2012) Influence of a non-ionic amphiphile copolymer on the self-assembly of a peptide amphiphile that forms nanotapes. *Soft Matter*, 8 (33): 8608-8615. DOI: 10.1039/c2sm25990g.

[3] *Castelleto, V., Hamley, I.W., Adamcik, J., Mezzenga, R. & Gummel, J. (2012) Modulating self-assembly of a nanotape-forming peptide amphiphile with an oppositely charged surfactant. *Soft Matter*, 8 (1): 217-226. DOI: 10.1039/c1sm06677c. (Cited 20 times).

[4] Dehsorkhi, A., Castelletto, V., Hamley, I.W., Adamcik, J. & Mezzenga, R. (2013) The effect of pH on the self-assembly of a collagen derived peptide amphiphile. *Soft Matter*, 9 (26):6033-6036. DOI: 10.1039/c3sm51029h.

[5] Palladino, P., Castelletto, V., Dehsorkhi, A., Stetsenko, D. & Hamley, I.W. (2012) Conformation and Self-association of peptide amphiphiles based on the KTTKS Collagen sequence. *Langmuir* 28 (33): 12209 – 12215. DOI: 10.1021/la302123h. (Cited 4 times).

[6] *Jones, R.R., Castelletto, V., Connon, C.J. & Hamley, I.W. (2013) Collagen stimulating effect of peptide amphiphile C_{16}-KTTKS on human fibroblasts. *Mol. Pharmaceutics,* 10 (3): 1063-1069. DOI: 10.1021/mp300549d. (Cited 1 time).

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4. Details of the impact

This very recent work has informed the public about the scientific basis of the reported activity of peptide-based skincare treatments, with high levels of media coverage stimulating public interest and awareness of the underpinning science. This is important since many previous cosmetic skin care claims have been based on absent or insubstantial scientific evidence. The work has already had substantial impact on industry – the UK-based SME *Forme Laboratories* has, for example, launched a new skincare product for menopausal women, "Stratum C", based on this work. *Procter and Gamble* has experienced increased sales of "Olay Regenerist", an existing product range containing the key KTTKS peptide, and other companies in the cosmetics field have sought

collaboration with Prof. Hamley. Between 2010 and 2013, the research conducted at Reading received considerable media attention in popular science magazines, national newspapers, national radio, industry publications and women's magazines. The media linked this rigorous, independent science with existing products in the multi-million pound skincare market, bringing genuine evidence of product efficacy to fact-hungry consumers. This not only increased public awareness of the science, but has had a major impact on the entire skincare industry.

Informing the public through media

Hamley's research publications [1 – 6] generated a series of media publications that reached millions of people in the UK and across the world. Examples include:

- "The nano-secret of youthful skin", *New Scientist* (21 November 2010) [Online audience of over 2.3 million unique users]
- Macrae, F. & Kisiel, R., "Anti-wrinkle creams that really work...but only with a magic ingredient", *Daily Mail* (6 March 2013) [42 million UK unique browsers/month; 129 million unique browsers worldwide]
- "Scientists find 'miracle ingredient' in anti-wrinkle creams", *Huffington Post UK* (7 March 2013) [4.8 million unique users]
- "Research proves Matrixyl CAN make you look younger!", *Woman Magazine* [over 600,000 people weekly – print, online and social media]
- "Key to tissue growth may be in anti-wrinkle cream", *Phys.org* (1 November 2010) [>1.5 million unique monthly users]
- "Scientists claim to discover "anti-wrinkle secret", *ITV News* (6 Mar 2013) [1.5 million unique monthly users]
- "Anti-wrinkle creams actually work!", *The Times of India* (20 Mar 2013) [average daily readership over 7.6 million]
- "There's the rub with anti-wrinkle creams", *The Australian Weekend Magazine* (6 Apr 2013) [weekly readership ~700,000]
- "Research suggests a specific peptide has enhance anti-ageing properties", *CosmeticsDesign-Europe.com* (7 March 2013) [highest-read European news website in the cosmetics industry]
- BBC Radio 4 – *You and Yours*, 21 Jun 2013

The Daily Mail and ITV News both quoted the Fashion and Beauty Editor of the Press Association as saying: *'Anti-ageing creams frequently boast about being packed full of peptides, but aren't specific as to which one. Now the secret's out and there's some scientific evidence for its collagen-boosting properties, women will be rushing to find out if it's in their anti-ageing potion. It's likely that brands with products that do contain Matrixyl will start shouting about it too if the "miracle" peptide becomes the new buzz word in beauty. There was a stampede at Boots in 2007 for No 7 Protect & Perfect Beauty Serum after a BBC2 Horizon programme scientifically backed the* [Matrixyl containing] *lotion.....'.* [a]

Media attention leads to increased sales of existing skin products

The UK skincare market was valued at more than £1.7 billion in 2011 and is predicted to be worth more than £1.9 billion by the end of 2016. Facial care is worth 58.8% of the total skincare market value [Datamonitor – "Skincare in the UK to 2016" published 22/11/2012]. Consumers are becoming more appearance conscious, with a 7% increase between 2008 and 2011 in the number of global consumers attaching importance to "looking good" [Datamonitor – "The Future of Skincare: Consumption trends and product preferences", published 07/10/2011]. Within such a competitive and high-value industry, every market advantage is of huge benefit to manufacturers.

As a result of the media attention, several women's magazines ran articles that promoted skin products containing the 'miracle ingredient', Matrixyl, including *Cosmopolitan* [b] and *Woman* [c] , with the latter referring directly to the research at Reading.[6]

One of the products mentioned is the Olay brand, a global market leader [d], produced by Procter and Gamble (P&G). Following the research publication [6] and the associated media coverage, P&G benefitted from a significant boost in sales of this product in the UK. Exact sales figures are

confidential, but a spokesperson from the Procter and Gamble London Innovation Centre [d,e] said there was a "*noticeable increase in sales*", as quoted below :

"The Molecular Pharmaceutics paper and subsequent mention in the Daily Mail (Anti-wrinkle creams that really work") led to a high level of media interest and actual coverage across print and online publications and social media platforms such as Twitter. The specific mention of an Olay Regenerist product containing Matrixyl led to a noticeable increase in sales of this product."

"The fact that the original Molecular Pharmaceutics paper appeared in a peer-reviewed science publication certainly gave great credibility to the following media coverage, although none of the mentioned cosmetic products had been part of the research. Media and consumers alike are hungry for independent, believable information and we have seen direct correlations between media coverage and sales numbers in this particular case as well in previous examples. This is way [sic] *research partnerships and scientific credentialling are key strategies for Olay and other P&G businesses."*

Industry invests in research and development and new products

The research has also impacted on small and medium enterprises working in the skin care area. Forme Laboratories was recently established by a team of entrepreneurs. Hamley's research influenced their approach to developing a new range of skin care products, Stratum C, which are scientifically formulated for the skin of menopausal women using Matrixyl. Hamley is featured on the company website, explaining the science behind their products [f]. The CEO of Forme Laboratories [g] stated that Hamley's research findings [6] stimulated their investment in the development of new products and further research:

"We became aware of the work of Professor Hamley and the University of Reading on peptides and collagen production through the online coverage. This contributed to the development of a new product called StratumC aimed directly at menopausal women to stimulate collagen growth during a period of compromised cellular function. We hope to use the basic science as a foundation for further research and to optimise the product with the help of Professor Hamley and the University."

Stratum C products are manufactured in the US, and are available online from the UK-based Forme Laboratories, with a full treatment regime for three months costing £120.

5. Sources to corroborate the impact

[a] MacRae, F. & Kisiel, R. (5 March 2013) "Anti-wrinkle creams that really work....but only with a magic ingredient", *Daily Mail* <http://www.dailymail.co.uk/health/article-2288955/Anti-wrinkle-creams-really-work--magic-ingredient.html>

[b] (2013) "Cosmo's 10 best wonder creams", *Cosmopolitan* <http://www.cosmopolitan.co.uk/beauty-hair/news/trends/beauty-products/the-best-day-night-creams-wonder-creams?click=main_sr#fbIndex1> Evidence that the world's number one woman's magazine was promoting products containing Matrixyl, with direct mention of this ingredient and reference to supporting science behind it.

[c] (2013) "Research proves Matrixyl CAN make you look younger!", *Woman* <http://www.womanmagazine.co.uk/beauty/hot-new-beauty-buys/research-reading-university-matrixyl-anti-ageing-olay-no7ou-look-younger/>. Promotes Matrixyl-containing products and refers directly to research by the University of Reading.

[d] P&G (2 January 2013) "Olay Regenerist", *P&G connect + develop stories* <http://pgconnectdevelop.netcrafters.com/home/stories/other-case-studies/20130102-olay-regenerist.html>. Evidence that this matrixyl-containing product is a leading product.

[e] Scientific Communications, Global Olay and EMEA Skin Care, Procter and Gamble London Innovation Centre.

[f] "How Stratum C works", *Stratum C*, *The Science* <http://www.stratumc.com/the-science/> Links Hamley's research directly to this new product line of skincare for menopausal women.

[g] Director, Forme Laboratories – Contact details provided.