Institution: University of the West of England (UWE), Bristol

Unit of Assessment: 3 – Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Development of healthcare products using biofilm model systems

1. Summary of the impact

Industrial collaborators have achieved more efficient and cost-effective routes to market for their oral hygiene and wound healthcare products as a result of UWE *in vitro* biofilm models based on perfusion flow. These have been used to measure the products' efficacy.

These companies include GABA international, GlaxoSmithKline, Insense Ltd (now Archimed), and Healthcare International.

Healthcare International has developed an oral healthcare product following the use of the UWE models to identify which of several compounds were best suited for this; it has been sold and distributed by Boots International.

Practising dental professionals have been trained by UWE researchers, using insights gained from our research findings, in correct procedures to diagnose and treat breath malodour.

2. Underpinning research

Professor John Greenman's research concerns the continuous culture of microorganisms as systems to study microbial growth and physiology. Over the last decade, his work has focused on the study of pure or mixed cultures growing as biofilms. Prior to this research, the only means of studying the efficacy of oral bioactives involved the use of expensive animal models. Animal studies take time (many weeks) and are expensive whilst the biofilm model (n=6) produces useful data within 7 days.

The UWE **research team** included Greenman (Senior Lecturer 1993-1997, Reader 1997-2001, Professor 2001-present), Saliha Saad (Research Associate 2005-2010, Research Fellow 2010-present), and Robin Thorn (Research Associate 2003-2012, Research Fellow 2012-present) and Benjamin Taylor (Research Associate 2013-present).

Initially working on biofilms derived from the oral cavity, we have demonstrated the utility of the matrix perfusion flow system as a dynamic steady-state model to study many aspects of growth and behaviour of target species. A perfusion matrix system is a biofilm model that permits the setting and control of growth rate of the attached microbial cells, allowing them to achieve dynamic steady-state. In practice, all the important physicochemical conditions around the biofilm can be controlled at will by the operator (see reference [2]). Uni-factorial control of main physicochemical parameters (e.g. pH or nutrient composition) allows the researcher to obtain clear cause-effect relationships between the induced perturbation and the resulting response of the biofilm by comparison with appropriate controls run simultaneously over time [2].

Research underpinning Impact 1 (as detailed in section 4 below):

Our biofilm models have been used to study oral malodour processes and the relative efficacy of intervention using putative inhibitory agents when added either as pure compounds or as prototype product formulations (e.g. mouthwash, tongue spray and tooth-gel). Its main advantages over other systems are that it is a continuous culture model (dynamic steady state system) and biofilms can be continuously monitored through perturbations and transient states, and made to behave in a very similar manner to the behaviour of real tongue-surface oral biofilms (from which the model was inoculated). In addition to adding basic knowledge of oral, wound and environmental biofilms across a wide range of conditions, our systems allow us to measure and compare the efficacy of targeted chemical agents for various companies [2, 3].

Research underpinning Impact 2

The perfusion matrix flat-bed model [3] was devised as a way of testing flat surfaces including novel prototype wound dressings (e.g. progenitors of **Oxyzyme™ and Iodozyme™** - see section 3 below) since conventional methods (e.g. AATCC Test Method 100-1993) do not allow the glucose-

oxidase based integral layer system to function [4].

Research underpinning Impact 3

As an expert centre for performing human clinical trials on oral health products to tackle bad breath, we have tested brand product (SB12) against four leading commercial mouthwash products and demonstrated its efficacy [5].

Research underpinning Impact 4

Research led by Greenman in 2004 into oral malodour established the relationship between mean organoleptic scores from odour judges (using the human nose) to concentrations of pure target odorants representative of those found in oral malodour [1]. This established the possibility of using human judgement of odour as the basis for diagnosis and treatment of oral malodour.

3. References to the research

1. Greenman J, Duffield J, Spencer P, Rosenberg M, Corry D, Saad S, Lenton P, Majerus G, Nachnani S, and El-Maaytah M (2004) Study on the Organoleptic Intensity Scale for Measuring Oral Malodor *Journal of Dental Research* **83** (1) 81-85, http://dx.doi.org/10.1177/154405910408300116

2. Taylor B and Greenman J (2010). Modelling the effects of pH on tongue biofilm using a sorbarod biofilm perfusion system. *Journal of Breath Research* **4** (1):017107. http://dx.doi.org/10.1088/1752-7155/4/1/017107

3. Thorn RMS and Greenman J (2009) A novel *in vitro* flat-bed perfusion biofilm model for determining the potential antimicrobial efficacy of topical wound treatments. *Journal of Applied Microbiology*, **107**, (6), pp.2070-2079. ISSN 1364-5072. http://dx.doi.org/10.1111/j.1365-2672.2009.04398.x

4. Thorn R M, Greenman J and Austin A J (2005) *In vitro* method to assess the antimicrobial activity and potential efficacy of novel types of wound dressings. *Journal of Applied Microbiology* 99, 895–901. http://dx.doi.org/10.1111/j.1365-2672.2005.02671.x

5. Saad S, Greenman J, Shaw H (2010) Comparative effects of various commercially available mouth-rinse formulations on oral malodour. *Oral Diseases* **17**, 2 (2010) p.180. http://dx.doi.org/10.1111/j.1601-0825.2010.01714.x

4. Details of the impact

Impact 1: *in vitro* assessment of mouthwash formulations to aid product development

The research at UWE has informed GABA International and its parent company Colgate-Palmolive in its choice between putative mouthwash formulations, and hence its product development priorities (see source [S1]).

As a result of the success of our model for assessing the potency or efficacy of inhibitory, biostatic or biocidal agents in oral healthcare products, GSK Consumer Healthcare now use UWE's model in-house.

The research findings have been used by Givaudan Fragrances Corporation, which provides flavours for oral care products as a core part of its business. Its Vice President, Technology (North America) says that, since 2008, UWE research has enabled them "to identify commercially successful technical solutions to deliver longer-lasting breath freshness from our flavours. These flavours are now in the marketplace in various consumer product formats (toothpaste, mouthwash and breath mints). Several of our multi-national and national clients use our Breath Freshening Flavour Technology in brands across the globe and used by millions of consumers every day. The bar has been raised in breath freshness and much can be directly attributed to the scientific and collaborative studies of Professor Greenman's research." He adds that UWE's *in vitro* model "has enabled us to increase speed to market through reduction in our flavour development time". The resulting flavour technology has contributed to flavour sales in the period 2008-2013 that were

"certainly in the $100,000's and in turn, they were incorporated into major oral healthcare brands with sales in excess of $10 million (conservative estimate)." Although confidentiality agreements with their consumer-brand clients limit what he can reveal, he confirms that Givaudan's flavours "are present in many major 'everyday' consumer products that are used by people across the world for oral hygiene and breath freshness." [S2]

Impact 2: improved industrial testing of novel wound dressing and oral healthcare products or formulations

The perfusion matrix flat-bed model was a novel test method used for the assessment of novel **Oxyzyme™ and Iodozyme™** prototype wound dressings for Insense Ltd, a client company. Without it, they would have had to rely on animal models to show efficacy against microbes, a more expensive route to market. Their Chief Scientific Advisor confirms that the research gave them "compelling evidence of the superior efficacy of our products." This was "of great importance in the promotion of our products and in building a basis (together with clinical performance and cost effectiveness) for their acceptance and adoption by healthcare professionals and purchasing authorities. The products have been accepted on the National Formulary and are in use in various clinical centres in the UK and The Netherlands. They have achieved an impressive record of clinical efficacy, and the company is now developing the next generation of products working on the same basic principles." [S3]

The company cites our research in its promotional material to justify its claims about this product, reproducing its quantitative findings [S4]. The flat-bed model has now been used more extensively to compare "biocidal/bioactive" surfaces in general and testing of novel combinations of treatments by Philips International, for purposes of incorporation into new products for oral healthcare (see source 4). This company adopted UWE's new flatbed models "in house", thus enabling them to repeat critical experiments (first performed on their behalf at UWE) prior to final selection of best candidate formulations for further evaluation.

Impact 3: clinical trials leading to commercialisation of oral health products

On behalf of Healthcare International (industrial clients), UWE has performed human clinical trials on oral health products (with appropriate ethical approval) for products to eliminate, for up to 12 hours, bad breath in human subjects. (The results were published in reference 4 above.)

A test product SB12 has been shown to be highly efficacious, thus supporting further development of product (scale-up manufacture and commercialisation). The formulation has been rebranded as CB12 and is distributed and sold by Boots International (marketed at http://www.boots.com/en/CB12/About-CB12/).

Impact 4: improved diagnosis of breath malodour; training and engagement of practitioners from the UK, Europe and the US

Building on the research findings, our group at UWE has trained organoleptic odour judges in the only such courses in the UK and EU. Delegates have learned how to assess breath malodour using scoring systems known as organoleptic and hedonic scales, and to relate these scores to individual volatile components present in human breath as well as breath samples from human subjects. Since 2008, five such 5-day courses have been conducted with up to 12 trainees per year (from Europe and US), including academics, clinicians, dental nurses and hygienists, enabling them to more effectively diagnose and treat causes of breath malodour in their patients. Since 2010, the courses have been sponsored by GABA international [S1].

Professor Greenman's workshops for dentists and health professionals across the EU and US have enabled many to improve their patient care by understanding the microbial aetiology of bad breath and the nature of the organoleptic scale for measuring malodour.

5. Sources to corroborate the impact

Testimonials listed below are available from UWE, Bristol.

S1. Joint **testimonial** Head of Product Development and Project Managers, GABA International

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S2. **Testimonial** from Vice President, Technology (North America), Givaudan Fragrances Corporation, NJ, USA

S3. **Testimonial** from Chief Scientific Advisor, Archimed (formerly Insense).

S4. Related to Impact 2, improved industrial testing of novel wound dressing and oral healthcare formulations: promotional documentation from company **Archimed** (formerly known as Insense), Colworth Science Park, Sharnbrook, Bedford MK44 1LQ, UK - http://www.archimed.co.uk/images/stories/smartscience1.pdf. The company cites UWE data in bottom four graphs on page.2 to justify its claims about the product.