Institution: University of Leeds

Unit of Assessment: UOA3 (Allied Health Professions, Dentistry, Nursing & Pharmacy)

Title of case study: Case study 2. "Filling without Drilling": Use of Self Assembling Peptides as Biomimetic Scaffolds in Treatment of Early Enamel Decay (Caries) Lesions

1. Summary of the impact

Multi-disciplinary research at Leeds has led to a step change for treatment of early tooth decay using a minimally invasive regenerative therapy, eliminating the need for surgical excavation ("Filling without Drilling"). The patented technology was licensed to a spin-out company (Credentis ag), completed "first in man" trials at Leeds [6] and received a CE-label for clinical use in Switzerland, Europe and Canada. The trials demonstrated clinical efficacy that is safe and favoured by patients. Two new products are now on the market. Credentis were recognised as one of the top start ups in Switzerland [A], won the Swiss Technology Award in 2013, have established a new UK base and have engaged a UK company as suppliers, creating new business for a UK owned industry.

2. Underpinning research

The new regenerative therapy for treatment of early enamel decay (caries) developed out of multi-disciplinary basic and applied research via a Leeds collaboration led by **Amalia Aggeli** (Department of Chemistry, formerly Royal Society University Research Fellow at Leeds, now Lecturer) and **Jennifer Kirkham** (Professor of Oral Biology, School of Dentistry).

In 1997, supported by the EPSRC and the Wellcome Trust, **Aggeli** and colleagues in the Faculty of Biological Sciences described the driving principles governing the spontaneous self-assembly of β-sheet forming peptides into fibrillar scaffold structures, including the ability to design in responsiveness to specific external triggers in order to control the assembly process [1,2]. Under specific conditions, these peptides undergo one dimensional self-assembly, forming micrometer-long, β-sheet "nanotapes". Nanotapes then stack in pairs to form ribbons which in turn further assemble to form fibrils and pairs of fibrils entwine edge-to-edge to form fibres. This assembly process is principally driven by intermolecular H bonding arising from the peptide backbone together with additional interactions between specific side chains and offered a new generation of biomaterials with potential uses across a range of applications.

Kirkham's group (funded through Kirkham's Wellcome Trust programme and associated Wellcome Trust and BBSRC project grants from 1998 onwards) utilised enamel development as a paradigm for understanding the way in which extracellular matrix proteins control crystal nucleation, deposition and tissue architecture in mammalian biomineralisation. Via a programme of gene discovery coupled with rodent models and structure-function studies, the principles underpinning the control of crystal growth in developing enamel were described, leading to the hypothesis that domains of negative charge on extracellular matrix proteins (themselves self-assembling) were responsible for crystal nucleation during enamel biomineralisation [3-4].

Kirkham and Aggeli's collaboration used knowledge gained from an understanding of the way in which mineralised tissues form, combined with an understanding of the drivers behind peptide self-assembly to address clinical challenges in mineralised tissue repair and regeneration. Peptides were selected that would be unassembled (monomeric) at pH values >7.5, providing a low viscosity, injectable fluid that would spontaneously assemble to form a 3D fibrillar scaffold under physiological conditions. In addition, peptides were designed to provide, via their amino acid side chains, domains of negative charge once assembled. The resulting 3D structures, therefore, mirror biological macromolecules found in extracellular matrices of the mammalian skeleton.

Applied, collaborative research between the two groups (funded by an EPSRC CASE award, a Leeds Teaching Hospitals Trust research award, the Leeds Wellcome-EPSRC Centre of Excellence in Medical Engineering and Geistlich Biomaterials, Switzerland) went on to test the hypotheses that rationally designed self-assembling synthetic peptides could nucleate mineral

crystals *in vitro* and *in situ* within artificial decay lesions in human teeth [5]. Taking this information together, a first-in-man clinical trial was completed in 2012 (led by **Paul Brunton**, Professor of Restorative Dentistry, School of Dentistry, Leeds) applying one of the peptides (P11-4) to early enamel decay lesions in patients. The results provided unequivocal evidence of safety and efficacy following a single treatment of the lesions with the peptide material [6].

3. References to the research

Underpinning SAP design:

1. **Aggeli A, Bell M, Boden N, Keen JN, Knowles PF, McLeish TC, Pitkeathly M, Radford SE** (1997). Responsive gels formed by the spontaneous self-assembly of peptides into polymeric beta-sheet tapes. *Nature,* **386**(6622): 259-62. PMID: 9069283
2. **Aggeli A, Bell M, Carrick LM, Fishwick CWG, Harding R, Mawer PJ, Radford SE, Strong AE, Boden N** (2003). pH as a trigger of peptide beta-sheet self-assembly and reversible switching between nematic and isotropic phases. *Journal of the American Chemical Society,* **125**(32)*:* 9619-9628. PMID: 12904028

First papers describing the principles of self assembly based upon rational design and the ability to use external triggers to switch between peptide monomers and self-assembled structures.

Principles of matrix-directed mineral interactions:

3. **Kirkham, J**, Zhang, J, **Wallwork, ML**, **Smith, DA**, **Brookes, SJ**, **Shore, RC**, **Wood, SR** and **Robinson, C** (2000). Evidence for Charge Domains on Developing Enamel Crystal Surfaces. *Journal of Dental Research,* **79**: 1943-1947. DOI: 10.1177/00220345000790120401

First ever atomic force microscopy characterisation of individual enamel crystal surfaces including evidence of the role of surface charge domains in directing protein-mineral associations.

4. **Kirkham J, Brookes SJ, Shore RC, Wood SR, Smith DA**, Zhang J, Chen HF, **Robinson C** (2002). Physico-chemical properties of crystal surfaces in matrix-mineral interactions during mammalian biomineralisation. *Current Opinion in Colloid & Interface Science,* **7**(1-2):124-132. DOI:10.1016/S1359-0294(02)00017-1

Invited review containing original unpublished data supporting new paradigm for mechanism of protein-mineral interaction and nucleation of crystal growth

Effect of peptides *in vitro*, *in situ* and in man

5. **Kirkham J, Firth A**, Vernals D, **Boden N, Robinson C, Shore RC, Brookes SJ and Aggeli A** (2007). Biomimetic self-assembling peptides promote enamel remineralisation. *Journal of Dental Research,* **86**:426-430 PMID: 17452562

Research describing in vitro repair of enamel lesions in human teeth in situ using self assembling peptides.

6. **Brunton PA**, Davies RWP, **Burke JL, Smith A, Aggeli A, Brookes SJ and Kirkham J**. Treatment of early caries lesions using biomimetic self-assembling peptides – a clinical safety trial**.** *British Dental Journal.* ePub ahead of print: DOI:10.1038/sj.bdj.2013.741

First in man clinical trial of P11-4 as treatment for early enamel lesions showing safety and proof of repair concept.

Note: Leeds researchers are in **bold**. Copies of all publications are available from the HEI on request.

New award building on the success of this research:

EPSRC co-development project (via EPSRC Medical Technologies Innovation Knowledge Centre Tranche II award with Credentis ag): **"**Filling without Drilling": Use of self assembling peptide biomimetic scaffolds in dental repair. 2012-2015 £1.02 million (Credentis contribution £570K). Grant number: EP/J01762011.

4. Details of the impact

Context: Dental decay is the most common of all diseases (prevalence is 1/10 of the population of the western world, i.e. 100 million lesions per year) yet the principles of treatment for dental decay have remained unchanged for almost 100 years. The earliest sign of tooth decay is the "white spot" lesion, visible to the clinician on the tooth surface. There is no current consensus view as to how this should be treated. Clinicians have three choices: 1) monitor the lesion to determine whether or not it is advancing (ie getting bigger), then excavate and fill; 2) apply fluoride treatments, then proceed as in (1) or 3) place a small restoration. Ultimately, all restorations will fail needing to be replaced by larger fillings. Treatment currently costs the UK £2 billion each year within the NHS alone (Office of the Govt Auditor), driving oral health inequalities. Drilling is feared by many patients, inhibiting their attendance at the dentist and so precluding opportunities for early diagnosis and treatment of decay as well as diseases such as oral cancer. Leeds' self-assembling peptide technology provides a simple and cost effective alternative to current treatments (costs approx. 50% of the most simple conventional filling), allowing the clinician to heal rather than repair dental decay. This removes the clinician's dilemma of "to treat or not to treat" and takes away the need for drilling and therefore the fear of visiting the dentist.

New IP generated

The platform technology for self-assembly design at Leeds was first patented in 1999 [B]. This underpins a number of diverse applications, including the design of biomimetic scaffolds in tissue engineering. Following Leeds research using self-assembling peptide technology to treat artificially created decay lesions in extracted human teeth, an applications patent was filed in 2002 in Europe and the US [B]. Later Leeds research comparing different peptide designs in respect of their ability to nucleate mineral crystals and to regenerate bone led to the filing of a Divisional patent in the US [B].

Impacts on commerce:

A spin-out company ("Credentis AG", Switzerland), in which the University of Leeds is major stakeholder, was created in 2010 [C] when the license to exploit Leeds' IP on self-assembling peptides in the dental domain was granted to the company [D]. The company has now completed two successful investment rounds with a value of 4.5 million ChF [D] and has created a new business opportunity for a UK company, Optident, who will distribute and supply the products within the UK market from September 2013 [E]. Credentis is recognised as one of the top Swiss spin outs [A]. The company won the prestigious Swiss Technology Award in November 2013. In 2012, Credentis opened a UK office in Leeds, reflecting the continuing close collaboration with researchers at the University. This includes a new £1.02 million collaborative award via the Leeds EPSRC Medical Technologies Innovation Knowledge Centre to develop second generation peptides for further dental applications, increasing the Credentis product range. Leeds researchers (**Kirkham, Aggeli**) are developing new technology and minimising commercial risk via provision of access to a full and validated pipeline screening facility, including (i) rational peptide design, including self-assembling conditions, (ii) *in silico* modelling, (iii) rheological testing, (iv) screening for capacity to induce mineralisation de novo, (v) cytotoxicity testing (vi) *in situ* (ex-vivo) testing, (vii) clinical trials and (viii) stability testing, packaging and process development.

Two new products: Curodont Repair™ and later Curodont Protect™ based on the same P11-4 Curalox® technology containing peptide P11-4, developed and patented by Leeds researchers, have now entered the market in Switzerland and Germany [D]. Curalox® was granted a CE label in 2012 [D] for clinical use as a class IIa medical device and has obtained approval for use in Canada. Large scale production of the first product, Curodont Repair™ provides a fully GMP-compliant product (100,000 patient treatments in the first run) [D]. Leeds researchers contributed towards this by conducting first in man safety trials for P11-4 [6], demonstrating a clear clinical improvement after treatment of class 5 lesions with the peptide. In addition, patient acceptability was shown to be very high.

Overseas industry has invested in research and development for these peptides (including P11-4) via a collaborative research project supported by Geistlich Biomaterials (Switzerland; £55K) [F] as well as support from Credentis AG. The former sought to test the hypothesis that self-assembling peptides would promote bone regeneration. Geistlich focus on biomaterials for maxillo-facial bone repair and the Leeds peptides have proved to be highly efficient for bone regeneration in an animal model [F]. Further funding to use P11-4 in combination with Bio-Oss in sinus lift procedures (to improve healing times and outcomes for maxillary dental implants) has been secured via the EPSRC Medical Technologies IKC (£190K) to fund proof-of-concept in an animal model.

Impact on health and welfare: A new clinical intervention (a medical device for restoration of early enamel caries and a preventive treatment for acid erosion) has been developed, trialled with patients and a definite positive outcome demonstrated [6]. Leeds researchers were involved in all stages of product design and testing from bench to chairside. The product is seen by the profession to fill the previously unfilled gap between prevention and surgical intervention ("Filling") [G].

5. Sources to corroborate the impact

A. Credentis in top 50 Swiss start ups: Institut für Jungunternehmer http://www.startup.ch/index.cfm?CFID=241786181&CFTOKEN=45081364&page=129572&profilesEntry=1 (accessed 27/10/13).
B. Patents portfolio including Platform technology patent; Applications patent EU /US and US continuation patent (GB 0216286.5; EP 20030763994; US 2006/0154852 USPA 20100234304A1).
C. Details of the company can be found at: http://www.credentis.com/en/home/ (accessed 27/10/13).
D. Portfolio of corroborative evidence relating to Credentis AG, including statement from Credentis CEO, details re. investment, details of CE regulatory approval for Curodont products, license agreement between University of Leeds and Credentis and Credentis brochure referencing Leeds research.
E. Details of the company and contacts are at: http://www.optident.co.uk/about_us.aspx; corroborative statement from company confirming arrangements with Credentis to supply products to UK market (accessed 27/10/13).
F. Collaborative research agreement between University of Leeds and Geistlich Biomaterials including final report of animal study.
G. Brochure containing quotes from independent dental practitioners who have used Curadont™.

Copies of all corroboration are available on request from the HEI