Institution: Sheffield Hallam University		
Unit of Assessment: UOA05 - Biological Sciences		
Title of case study: Novel methods for replacing animals in pre-clinical studies		
Period when the underpinning research was undertaken: 2004 - present		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Malcolm Clench	Professor of Mass Spectrometry	1990-present
Neil Cross	Reader	2008-present
David Smith	Reader	2010-present
Christine Le Maitre	Professor of Cell Biology and Tissue Regeneration	2008-present
Laura Thickett-Cole	Senior Lecturer	2011-present
Period when the claimed impact occurred: 2016 - present		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

The development of two non-animal multi-cellular 3D models and the application of Mass Spectrometry Imaging (MSI) has provided a commercial alternative to pre-clinical testing using animal models. Research with Innovenn UK Ltd led to a modified version of the Human Skin Equivalent (Labskin) product, expanding market reach into the wound care and cosmetics sector. Development with Precision for Medicine (UK Labs) Ltd resulted in the commercial launch of a cancer model (PhaseZero® 3D Oncology OrganDot™). Uptake of MSI with 3D models by AstraZeneca and CRODA has changed their practice, produced cost savings and increased customer demand. This new model enables the 3Rs Agenda to be put into practice.

2. Underpinning research

The principles of the 3Rs (replacement, reduction and refinement) provide a framework for performing more humane animal research; these are embedded in national and international legislation and regulations on the use of animals in scientific procedures. Opinion polls of public attitudes show that support for animal research is conditional on the 3Rs being put into practice. As well as ethical considerations, animals are expensive. Reduction and replacement of animals will reduce cost and increase competitiveness for companies that adopt these procedures fulfilling industry commitment to more sustainable innovation, meeting public expectations and customer demands. There is widespread interest by pharmaceutical industries in the use of 3D cell culture models, which mimic the biological microenvironment of tissues, as a commercial alternative to research using animal models in pre-clinical drug development.

Research was undertaken at SHU in collaboration with industry using the application of mass spectrometry imaging (MSI) to 3D in vitro human tissue models as a valid approach for investigating both pre-clinical drug absorption in skin (pharmacokinetic (PK) studies) and complex interactions between the drug and the human body (pharmacodynamics (PD) studies). MSI can visualize the distribution of multiple compounds within tissues simultaneously and in a label-free manner, making MSI fast and relatively inexpensive. Two non-animal models were used; a novel tumour spheroid model developed at SHU and a commercial 3D human skin construct or Living skin Equivalent (LSE).

Clench was the first to apply MSI to PK and PD studies of drugs, metabolites, and changes to lipid profiles in a commercial LSE model. Working with CRODA, Clench demonstrated quantitative MSI as a method to determine the amount of active pharmaceutical ingredient in skin and associated responses (**R1**). Research was developed further (Cole, Smith, Cross, Clench) with Labskin (**G1**) and reported the MSI of an LSE model modified to represent a diseased state (skin condition psoriasis) (**R2**) and through a KTP with Clench and Innovenn (**G2**), examined its value as a platform for wound-healing studies (**R3**). These projects revealed that absolute quantification of drugs in the LSE model by MSI was possible (**R1**) and that pathways of wound healing in the LSE could be studied by changes on the metabolome of the LSE (**R3**) demonstrating this methodology as an alternative to testing using animal models to represent human tumours and skin.

Cross developed 3D tumour spheroid models in Alginate gel scaffolds for Multiple Myeloma and Osteosarcoma cancers and demonstrated efficacy for biopharmaceutical drugs in 3D cell cultures (**R4**). In collaboration with Precision for Medicine (**G3**) Cross developed the company's existing OrganDot 3D cell culture system from a non-tumour pancreatic Islet model, into a PhaseZero® 3D Oncology OrganDot™ model and validated this against SHU's Alginate-based 3D cell culture system. The 3D Oncology OrganDot™ was comparable in most chemotherapy regimens using cell lines, and superior to 3D cell culture models using fresh tumour material. Clench and Cross further developed the Alginate-based model to quantify drug distribution and response to therapy in Osteosarcoma. This proof-of-principle study (**R5**) (Smith, Cole, Le Maitre, Clench, Cross) showed that chemotherapy-induced changes in metabolite abundance and distribution can be determined in 3D cell culture by MSI, highlighting this method as a tool in the elucidation of chemotherapy responses as an alternative to in vivo testing. In collaboration with AstraZeneca, Clench, Cross and Cole applied advanced imaging techniques to undertake in-depth phenotyping of a novel aggregated tumour model improving understanding of the molecular activity within a 3D cell culture tumour microenvironment, highlighting this as a potential methodology for in vitro applications of biomedical research and pharmaceutical development (**R6**).

3. References to the research

R1. Russo C, Brickelbank N, Duckett C, *Mellor S*, *Rumbelow S*, **Clench MR** (2018) Quantitative Investigation of Terbinafine Hydrochloride Absorption into a Living Skin Equivalent Model by MALDI-MSI. Anal Chem. 2018 90(16):10031-10038. https://pubs.acs.org/doi/10.1021/acs.analchem.8b02648

R2. Harvey A, **Cole LM**, Day R, *Bartlett M*, *Warwick J, Bojar R*, **Smith D**, **Cross N**, **Clench MR** (2016) MALDI-MSI for the Analysis of a 3D Tissue-engineered Psoriatic Skin Model. Proteomics.16(11-12):1718-25. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/full/10.1002/pmic.201600036

R3. Lewis EEL, *Barrett MRT*, Freeman-Parry L, *Bojar RA*, **Clench MR** (2018) Examination of the Skin Barrier Repair/Wound Healing Process using a Living Skin Equivalent Model and Matrix-Assisted Laser Desorption-Ionization-Mass Spectrometry Imaging. Int J Cosmet Sci. 40(2):148-156. https://doi.org/10.1111/ics.12446

R4. Arhoma A, Chantry AD, Haywood-Small SL, **Cross NA** (2017) SAHA-induced TRAIL-sensitisation of Multiple Myeloma Cells is Enhanced in 3D Cell Culture. Exp Cell Res. 360(2):226-235. https://doi.org/10.1016/j.yexcr.2017.09.012

R5. Palubeckaitė I, Crooks L, **Smith DP**, **Cole LM**, Heijs B, **Le Maitre C**, **Clench MR**, **Cross NA** (2020) Mass Spectrometry Imaging of Endogenous Metabolites in Response to Doxorubicin in a Novel 3D Osteosarcoma Cell Culture Model. J Mass Spectrom. 55(4) e4461. https://doi.org/10.1002/jms.4461

R6. Flint L, *Hamm G*, Ready J, *Ling S*, Duckett CJ, **Cross NA**, Cole LM, **Smith DP**, *Goodwin RJA*, **Clench MR** (2020) Characterization of an Aggregated Three-Dimensional Cell Culture Model by Multimodal Mass Spectrometry Imaging. Anal. Chem. 2020, 92, 18, 12538–12547. https://doi.org/10.1021/acs.analchem.0c02389

Additional indicators of the quality of the underpinning research

Outputs were rigorously peer-reviewed prior to publication in leading journals in the field. **R2** was awarded the Society of Cosmetic Scientists Prize for the most significant paper in Int J Cosmetic Science. References are for research that was carried out by staff employed by the university at the time of publication except for those in italics who are commercial collaborators. Outputs **R2** and **R3** were supported by grants **G1** and **G2**, respectively. Output **R6** methodology was developed through **G1**.

G1. National Centre for the Replacement Refinement and Reduction of Animals in Research: (NC/L001896/1); Imaging Technologies for the 3Rs; Labelled IMS TAG Proteins for Quantitative Mass spectrometry Imaging; 01/08/14 - 31/07/16; £268,000

G2. Innovate UK (KTP010322); Knowledge Transfer Partnership between Sheffield Hallam University and Innovenn Ltd; 15/02/16 - 03/01/18; £109,472 (£73,346 IUK and £36,126 Innovenn Ltd)

G3. Innovate UK (57424-423196); Developing Non-animal Technologies; Development of a robust and sustainable in vitro 3D model of human tumours for the identification and evaluation of anti-cancer drugs; 11/09/15 – 28/02/18; £465,971

4. Details of the impact

1. New product development for commercial application and contribution to the 3R agenda

Skin model

The increase in the incidence of chronic wounds across the globe, and the lack of innovation in wound care research and development, has created a demand for an in vitro testing platform to improve wound care testing. There was a clear market gap for testing the effectiveness of products designed to promote healing in a pre-clinical setting. Innovenn UK Ltd (operating through Labskin UK) makes a Human Skin Equivalent product known as Labskin, which is used to offer testing services on topical products, particularly those affecting skin microflora. Services are provided for skincare, healthcare, pharmaceutical manufacturers and the cosmetic industry to test and validate their product claims on full thickness human skin in a real-world environment.

Research conducted by SHU in collaboration with Innovenn (**R3**; **G2**) has led to the further development of their 3D skin models, in particular, the development of a new modified version of the Labskin product 'Woundskin' (**E1**). Woundskin provides a platform for the testing of wound healing products and other medical devices without having to use animals, fulfilling company commitment to the 3Rs being put into practice. The Managing Director of Innovenn confirms the success of this partnership, '*We worked together on a particularly successful project on wound healing that led to a prize winning paper that generated lots of good publicity for the company (as well as sales). The success of these projects was a factor in the investment in the company made by Integumen in 2019 […].Prof Clench's work has helped to generate business for us particularly in the wound care and cosmetics sectors*' (**E2**). Integumen PLC is a group of life sciences companies with specific domain expertise to lever product and business development; the Chairman's statement (2019) confirms the Labskin models as one of the highlights of 2019. Revenues for the group were up 371% with contracts for Labskin clinical services (**E3**). Through the work of Clench Labskin are engaging with new academic customers. Prof Cooper from the University of Birmingham confirms that as a result of introductions through the SHU-Innovenn

KTP project “we were invited (by Labskin) to take part in the trial of the company’s Wound skin product and subsequently adopted their 3D skin model” (**E4**).

Cancer Model

The demonstration of the biopharmaceutical drug efficacy in 3D cancer cell cultures using alginate scaffold by Cross led to a collaboration with Asterand Bioscience (**G3**) to develop a non-animal replacement for 'patient-derived Xenograft’ (PDX) models used in personalised treatment. Asterand Bioscience was subsequently acquired by BioIVT, a leading global provider of biological products to the life sciences and pharmaceutical companies. Through this collaboration Asterand/BioIVT’s existing OrganDotTM platform was successfully developed into a commercially available PhaseZero® 3D Oncology OrganDotTM model, which was launched by BioIVT in 2019 (**E5**). This Oncology model was the only model tested that could reliably grow primary lung cancer cells direct from patients to achieve this aim; BioIVT expanded their OrganDotTM range to include a normal lung model based on the findings from the collaborative project with SHU.

In May 2020 Asterand/BioIVT was acquired by Precision for Medicine (UK Labs) Ltd; the Senior Vice President of the company confirms that “our Innovate UK funded project led to the commercial release of the organ Dot model in 2019…our work with Dr Cross has helped raise our profile as an organisation that is able to support oncology R&D with human in-vitro based pharmacological relevant models supporting NC3R principles” (**E6**). Public support for animal research is conditional on the 3Rs being put into practice. The use of human-based solutions to accelerate the identification/validation of drug targets and enhance the selection of drug candidates with increased likelihood of clinical success contributes to the 3R agenda. For example, if a single dose of chemotherapy is tested in vivo using a mouse PDX system, 8-12 tumour-bearing mice per dose are required. Because up to four in vivo transfers of tumour material are needed to produce enough tumour-bearing mice, up to 50 mice in total are needed to test the chemotherapy for a single patient. No mice are needed if the Oncology OrganDotTM system is used.

2. Knowledge transfer and change in industrial practice

In the pharmaceutical industry cost is a key driver and, in many respects, shapes the capital, revenue and resource decisions made during the drug discovery process. Combining the use of a 3D solid tumour model with mass spectrometry imaging (MSI), developed by Clench, is providing a label-free methodology for drug discovery studies and is a viable non-animal alternative to animal testing. Collaboration with industry partners has enabled efficient knowledge transfer of this methodology, with direct benefits to these businesses through raised awareness, changes to their practice, and produced cost savings.

Cross and Clench developed the Alginate-based 'aggregoid' macro-3D cell culture model to quantify drug distribution and response to therapy in Osteosarcoma when used in conjunction with mass spectrometry imaging (**R5**). This model has been employed by AstraZeneca (a global pharmaceutical company) in combination with MSI through a collaborative PhD studentship with SHU on the use of aggregoids for the study of biotherapeutics in non-small cell lung cancer (NSCLC) (**R6**). The group’s Director confirms that this work ‘has raised awareness of the potential MSI has when used in combination with 3D models, which is an area of increasing interest and demand for us’ (**E7**). As a science-led organisation AstraZeneca places particular value on collaborations (**E7**) and the transfer of knowledge from academic to industry has been facilitated by members of AstraZeneca completing part-time doctorates at SHU.

CRODA, an international speciality chemical company, has supported 3 PhD students at SHU who have spent time at both UK and US sites to transfer methodologies to the company. The collaboration has developed the MSI methodology with their 3D models of skin, gastrointestinal tract, seeds and plants. Outcomes from the research have been published in joint papers and the projects been featured in CRODA publications, seminars and for staff training purposes. This has led to raised awareness within CRODA of MSI's potential as a methodology and there is an increasing interest/demand for its use within the company with resulting business efficiencies and a competitive advantage. CRODA's Research & Technology Director confirms that 'using MSI saves the company time and money in studying compound distribution in both plant and animal tissue' (**E8**).

5. Sources to corroborate the impact

E1. Innovenn/SHU Novel Wound Assessment Video shortlisted as part of Global JoVE Film your Research Contest. Produced from the KTP collaboration: http://www.innovenn.co.uk/wp-content/uploads/2018/07/AN008-Wounding-Healing.pdf

E2. Testimonial Dr Fin Murray, Managing Director, Innovenn UK Ltd/Labskin

E3. Integumen's Chairman's statement on Final Results for the year ended 31st December 2019 (post-year-end highlights): https://www.investegate.co.uk/integumen-plc--skin-/rns/final-results/202006150700088851P/

E4. Testimonial Prof Cooper, School of Biosciences, University of Birmingham

E5. BioIVT PhazeZero brochure for Oncology Research

E6. Testimonial, Amanda Woodrooffe, Senior Vice President, Precision for Medicine (UK Labs) Ltd

E7. Testimonial, Prof Richard Goodwin, Director Head of Imaging and AI, Astra Zeneca UK

E8. Testimonial Stephen Mellor, Research and Technology Director, CRODA Europe Ltd