Institution: The University of Edinburgh

Unit of Assessment: UoA5: Biological Sciences

Title of case study:

11. Plant stem cell culture is used for the manufacture of biological products.

1. Summary of the impact

Impact on the economy and on commerce Using novel technology developed with UoE researchers to isolate and culture cambial meristematic cells (CMCs), Korean biotech company Unhwa Corp tripled their production of CMCs and have brought sixteen skincare products and three nutritional products to a global market. The impact of this technology on the South Korean economy has been recognised by the Korea Ministry of Knowledge Economy.

Beneficiaries: Korean Biotech company Unhwa Corp, and international consumers of their nutrition and cosmetic products.

Significance and Reach: The technology provides a platform for the cost-effective, environmentally friendly and sustainable production of plant stem cells. The business strategy and operations of Unhwa Corp changed as a direct result of the research: Unhwa invested [text removed for publication] in 2011-13 to construct a base in Jeojuni, Korea for a new production facility. Products arising from this are sold world-wide (Unhwa has subsidiaries on 5 continents) and have generated [text removed for publication] profit, with a doubling of company turnover since the key research was carried out.

Attribution: Professor Gary Loake, UoE, led the research in collaboration with Unhwa Corp, from 2006 to 2010 and ongoing.

2. Underpinning research

Plant cell culture would offer a cost-effective and environmentally-friendly option for producing many natural products. However, only plant stem cells, embedded in meristems located at the tips of shoots and roots or contained inside the vascular system, can divide and give rise to cells that ultimately undergo differentiation while simultaneously giving rise to new stem cells. Such cambial meristematic cells (CMCs) had never previously been isolated or cultured. Consequently, plant cell suspension cultures had been routinely generated through a dedifferentiation process, which results in mitotic reactivation of specialised cell types within a given organ, generating a multicellular mixture of proliferating cells. These dedifferentiated plant cells (DDCs) often exhibit poor growth properties with low and inconsistent yields of natural products, owing to deleterious genetic and epigenetic changes that occur during this process and so it is often not commercially viable to culture DDCs on an industrial scale.

To bypass this problematic dedifferentiation step, Professor Gary Loake at UoE and the Korean biotech company Unhwa Corp initiated a research collaboration in 2006 to isolate, culture and confirm a stem cell identity for innately undifferentiated CMCs. The Loake group used deep sequencing technologies to confirm the putative stem cell identity of cultured innately undifferentiated CMCs isolated by Unhwa. Massively parallel pyrosequencing was used to profile the transcriptome. This allowed use of digital gene expression tag profiling to compare gene expression in the prospective CMCs with gene expression in DDCs. The group identified marker genes and transcriptional programs that defined the genetic identity of CMCs and verified them as genuine plant stem cells. This use of innovative sequencing and bioinformatics allowed the UoE research team to molecularly fingerprint Unhwa's proprietary plant stem cells [1].

UoE further supported the stem cell identity of CMCs by confirming their morphology, their hypersensitivity to γ-irradiation and radiomimetic drugs and their ability to differentiate at high

frequency [1]. The use of suspension culture of CMCs derived from *Taxus cuspidata*, the source of the key anticancer drug, paclitaxel (Taxol), circumvented numerous obstacles routinely associated with the commercial growth of DDCs, namely shear stress, yield instability, cell aggregation, slow rate of growth and low product yield. The researchers also isolated CMCs from other plant species such as *Panax ginseng* which produces neuroprotection and antioxidative compounds [1]. CMCs from *P. ginseng* produced 23.8- and 24.1-fold more ginsenoside F2 and gypenoside XVI products, respectively, than when produced from DDCs. This indicates that the procedure has broad utility and that cultured CMCs can therefore provide a cost-effective, environmentally friendly and sustainable source of paclitaxel, ginsenosides and potentially other important natural products. This work generated a key patent [2] "Plant cell lines and methods of isolating the same" for University of Edinburgh and Unhwa, published in 2012.

This research was undertaken between 2006 and 2010. The work was a collaboration of UoE and the Korean biotechnology company, Unhwa. The key researchers involved in the project were Prof Gary Loake (Principal Investigator, UoE (1995-present) and Dr Byung-Wook Yun (UoE PDRA (1999-2012) along with colleagues Tomlinson, Elfick and others at UoE; and Dr Eun-Kyong Lee and Dr Young-Woo Jin of Unhwa Corp.

3. References to the research

1. Cultured cambial meristematic cells as a source of plant natural products.
Lee EK, Jin YW, Park JH, Yoo YM, Hong SM, Amir R, Yan Z, Kwon E, Elfick A, Tomlinson S, Halbritter F, Waibel T, Yun BW, Loake GJ. Nature Biotechnology. 28(11):1213-7, 2010. DOI:10.1038/nbt.1693.
27 Scopus citations at 11th October 2013.

2. Patent: WO/2012/052854
International Application Number: PCT/IB2011/003287
International Filing date: 24.10.2011
Publication Date: 26.04.2012
Title: PLANT CELL LINES AND METHODS OF ISOLATING THE SAME
Applicants: UNHWA CORPORATION, South Korea and UNIVERSITY OF EDINBURGH: LEE, Eun Kyong; JIN, Young Woo; PARK, Joong Hyun; OH, Il Seok; LIM, Min Jung, LOAKE, Gary John (University of Edinburgh); YUN, Byung-Wook (University of Edinburgh); WAIBEL, Thomas (University of Edinburgh)

4. Details of the impact

Numerous important and chemically diverse products are derived from plants. For example, ~30% of contemporary drugs are either natural products or can trace their origins to such compounds [a]. Unfortunately, natural products can often be difficult to exploit because they are typically found at low concentrations and the plants that produce them are not usually available on a commercial scale. Furthermore, natural products often possess complex biochemical structures and therefore even their chemical synthesis is not economically viable routinely.

UoE offered a unique skill set in plant biotechnology that attracted Unhwa to initiate a research collaboration. Unhwa Corp is a research-orientated company venturing into the higher value-added industry of developing botanical drugs and manufacturing new biomaterials for pharmaceutical, nutrition and the cosmetic business. It was established in 2005, is based in Jeonju, Korea and employs 125 people. Unhwa Corp has local subsidiaries in Asia, China, Japan, USA, Europe, Latin America and Africa.

Unhwa and the Loake group worked together to develop the technology to isolate and culture CMCs from *T. cuspidata* and thus circumvented obstacles routinely associated with the commercial growth of DDCs. We also cultured CMCs from *P. ginseng*, the products of which show multiple bioactivities.[1,2] Therefore, the research has provided a cost-effective, environmentally friendly and sustainable source of naturally produced pharmacological and other important natural

products. The collaboration allowed Unhwa to confirm and validate the proprietary CMCs that are now trademarked as Ddobyul®. The commercial advantage to Unhwa was significant since the collaborative research programme leading to paper [1] both confirmed the validity of the new cost-effective approach to isolating stem cells and provided the platform on which their unique selling proposition of products derived from stem cells could be marketed [b, c]

Unhwa Corp's business strategy and operations has changed as a direct result of the research. They immediately tripled the production of CMCs in 2011 from one ton of plant stem cells per month to three tons per month. They invested [text removed for publication] in 2011 to purchase a base in Jeojuni, Korea for a new production facility to meet increased demand for their products. They invested a further [text removed for publication] in July 2013 for this facility which will allow further increased production. Initially, the production facility will house 120 x 250L bioreactors with plans to increase this four-fold with an eventual 66.5 ton production capacity.

The company strategy for the new production facility is to increase the production of CMCs from a variety of plant species for the biosynthesis of a variety of natural products and other therapeutic agents. The development of this technology with UoE has therefore made a significant contribution to Unhwa's strategic plan and to the sustainable production of key, well-established therapeutics. The research was reported on the news of all six major Korean television channels and drove a wave of significant new financial investment into Unhwa Corp through the purchase of Unhwa shares, supporting further scientific-related jobs in Jeonju, Korea.

The Lee et al. paper [1] was widely reported in the scientific and popular press, drawing attention to the opportunities for commercial and healthcare benefits. The global broadcasting channel, Discovery, featured the technology in three episodes of their primetime show "How do they do it?" from May to August 2011 [d]; Loake featured in the programme. Unhwa indicate that this exposure has helped to increase the credibility of their technologies and thus underscore their business success; the UoE paper is heavily featured on company websites [b, c, e]. Unhwa Corp was awarded the 2011 Technology Award by the Korea Ministry of Knowledge Economy and Korea Institute for Advancement of Technology (KIAT) for the technology. This is awarded to new, progressive technologies in any industrial field that have succeeded in commercialization and have a great ripple effect on domestic industry. They were also awarded a New Excellent Technology (NET) certificate by the Korean Ministry of Health and Welfare in 2011 and Green Certification from KIAT [b]. This is a national certification system certifying green technology that satisfies the global standard of green growth and economy.

Unhwa sell their plant stem cells under the trademark Ddobyul®. Unhwa has brought sixteen skin care products to a global market in more than 20 countries, all with these plant stem cells as the main ingredient. These products are sold in stores (Korea, Japan, Hong Kong) and online with country-specific websites, e.g. Korean, USA, Japanese and Russian sites. Unhwa also produce many products developed under private label for other brand distributors, including over 20 kinds of cosmetics products and 5 kinds of nutrition products for more than 4 major clients. Products based on their proprietary *P. ginseng*, *T*. cuspidata and *Solanum lycopersicum* stem cells retail at between US $33 and $209. Unhwa also sell three nutrition products including Ddobyul® Wild Gingseng, selling for up to $297, and is utilizing Ginseng Stem Cells as a form of nutrition ingredient sold as product to other commercial clients. Unhwa estimate a net profit related to these products since 2011 of [text removed for publication]; the company turnover doubled between 2009/10 and 2011/12.

Loake became Science Advisor to Unhwa's future research strategy and a further research agreement was signed in June 2011 to a value of [text removed for publication] to study "Control of natural product biosynthesis: a systems approach", a significant investment in UK academic research by a foreign industry.

5. Sources to corroborate the impact

The Tiny URLs provide a link to archived web content, which should be accessed if the original

web content is no longer available.

a) Drug natural origins quoted in: Schmidt, Ribnicky, Lipsky & Raskin, *Revisiting the ancient concept of botanical therapeutics*. Nature Chemical Biology 3, 360 - 366 (2007). doi:10.1038/nchembio0707-360 [copy available on request]

b) Unhwa international website, featuring UoE Nature Biotech paper and information on Korean government awards/certifications: http://www.unhwabio.com/ or http://tinyurl.com/oh9z36n

c) Corroboration of all business statements can be provided by Unhwa Corp CEO

d) Unhwa, Prof Loake and plant stem cells were featured on the Discovery Channel's, "How do they do it" science series in May 2011. It was shown in 31 countries. http://www.unhwa.com/eng/html/sub441.html or http://tinyurl.com/p6zwyd5

e) Unhwa USA website, which shows how the linkage between UoE research and Unhwa products is presented to American consumers http://www.unhwausa.com/ or http://tinyurl.com/ppzdpm4