

Taking a 3Rs approach to wound biology

Phil Stephens, Professor of Cell Biology and Dean of International for the College of Biomedical and Life Sciences at Cardiff University, and President of the European Tissue Repair Society, developed an interest in soft tissue wound healing while studying for his PhD in biochemistry at the University of Leeds. Subsequently he moved to Cardiff where, along with a clinical colleague, he co-founded the Wound Biology Group with a focus on soft tissue wounds inside the mouth, which heal particularly well. He discusses his research into this subject, and the use of alternatives to animal models, such as GrowDex®.

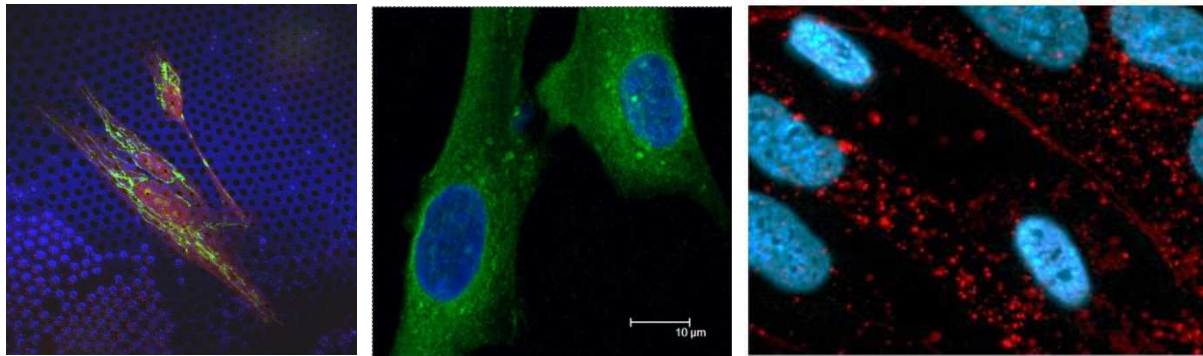


Professor **Phil Stephens'** research group is focused on understanding the mechanisms involved in the repair and regeneration of oral and dermal tissues in health and disease, to help develop therapeutic strategies that will promote wound repair. Compared to those on normal skin, wounds in the mouth heal extremely well; inflammation is minimal, healing is faster, and there is little, if any, scarring. The group also has an interest in non-healing, chronic wounds – such as venous leg and diabetic foot ulcers – which are often associated with the older population.

Fibroblasts are the key cells that synthesise normal tissue matrix, and they play an important role in wound healing. During the healing process, fibroblasts produce new extracellular matrix and help to build up fresh soft tissue. However, the matrix laid down during healing is not arranged correctly, which results in scar tissue. This doesn't happen in wounds in the mouth – they heal rapidly without scarring – so it is hoped that understanding the

mechanisms behind this will pave the way to the development of novel therapies to help repair and regenerate damaged or diseased tissues.

Phil explained: “We first looked at the age of the cells in the wound and found significant differences between oral and skin tissues. Cells in oral wounds are much younger, which is demonstrated by their longer telomeres. In contrast, cells in chronic, non-healing wounds have quite short telomeres, as oxidative stress causes the cells to divide too quickly. On the back of this initial research, our interest switched from fibroblasts to stem/progenitor cells. We've since discovered and characterised these cells inside the mouth (cheek) tissues and find that they grow for a long time, can make lots of different tissues, and are extremely immunosuppressive and antibacterial, which is logical as the oral cavity cannot function properly if bacterial load and inflammation are not under control. The latest phase of our research is focused on exosomes – nanoparticles secreted from cells that are the current hot topic in wound healing – secreted by the oral progenitor cells. We were able to demonstrate in the lab that they have anti-scarring potential; oral exosomes put onto skin (scarring) cells caused these cells to behave in a similar way to oral (non-scarring) fibroblasts. The exosomes have not yet been fully characterised – proteomics studies are planned – especially in relation to the anti-scarring potential, however, we know that haptoglobin and osteoprotegerin are involved in the antibacterial activity. The next step will be to investigate the potential of exosomes to improve healing of chronic wounds.”



On the left: Fibroblasts cultured on a synthetic material stained to reveal their actin cytoskeleton (green). In the middle: Oral progenitor lamina propria-progenitor cells stained for the presence of a pluripotency marker (green). On the right: Oral progenitor lamina propria-progenitor cell exosomes (red) can be taken up by skin fibroblasts (blue nuclei). Image courtesy Phil Stephens, Lindsay Davies, Rob Knight.

3D studies very important

As his research continues, Phil, like all his fellow researchers today, must justify the experimental use of animals when submitting funding applications and show that viable alternatives are not available. New technologies are being developed – often with the support of organisations such as NC3Rs (www.nc3rs.org.uk), a UK research funder dedicated to replacing, refining and reducing the use of animals in experimental research (3Rs) – and the options are becoming more complex. Phil said: “With all our investigations, we always begin with in vitro studies of our human disease-specific or non-disease-specific cell lines, but now we'd like to explore three dimensional, rather than two dimensional, models of wound healing as this is more typical of what happens in the body. The simplicity of in vitro models makes them ideal for screening purposes, allowing us to identify differences between cells. However, cell behaviour is very different under 2D and 3D conditions in terms of gene expression and phenotype, making 3D studies very important. Traditionally, 3D studies have been performed using animal collagen, which is reproducible but also expensive, and there is also now a lot of interest in organoids. Another alternative as a 3D matrix is animal-free GrowDex hydrogel, which I came across about 18 months ago through a contact in the industry.”

GrowDex has great potential from the point of view of replacing, refining and reducing the use of animals in experimental research. “I work quite closely with the NC3Rs, which has been supportive of our research, funding work to develop cell lines from chronic wounds and set up some reporter lines. We've run some successful experiments using GrowDex and find that cells behave in a similar manner in both this matrix and collagen; we plan to follow this up with a biomarker project, to gain further information. GrowDex is easy to use and reproducibly manufactured, giving batch-to-batch consistency. Imaging is simple and GrowDex can be shipped at ambient temperature without degrading. It's a really great product.”

“Currently, all our work is cell line-based but, to translate therapies through into patient care, we are legally obliged to perform some animal experiments to establish safety and efficacy. It is the complexity that you get with an animal model that is so important in determining whether your drug, construct, or cells are suitable to go into humans. Novel systems – organ-on-a-chip, lab-on-a-chip, multi-organ and microfluidic systems – are coming through that offer a degree of complexity that is closer to that of an animal. However, laboratories can be resistant to taking these technologies on board. For that reason, the NC3Rs recently launched a Skills and Knowledge Transfer funding scheme to support wider adoption of existing alternative models, tools and approaches in academia and industry.

While alternative models, such as GrowDex, may not completely replace animal experiments, they will drastically reduce the number of animal experiments that must be undertaken before a product can be brought to market, and that can only be a good thing,” Phil concluded