Tissue engineering using pluripotent stem cells: multidisciplinary approaches to accelerate bench-to-bedside transition

“Protocols enabling the derivation of a huge range of cell types have been developed including corneal cells, cardiomyocytes, osteoblasts and neurons.”

Keywords: biomaterials • chemistry • collaboration • embryonic stem cells • extracellular matrix • induced pluripotent stem cells • materials science • multidisciplinary • regenerative medicine • tissue engineering

First draft submitted: 15 July 2016; **Accepted for publication:** 18 July 2016; **Published online:** 3 August 2016

Key achievements

Human pluripotent stem cells (hPSCs) offer huge potential within the field of tissue engineering since they can theoretically be differentiated into any cell type and therefore be used to replace any degenerated or diseased tissue. Importantly, it offers the possibility to replace regenerative and nonregenerative tissue including cardiac, retinal and CNS tissue. The first human embryonic stem cell lines were reported in 1998 [1], but human embryonic stem cell line-derived cells are allogenic and therefore could potentially evoke an immune reaction, and are also associated with ethical problems. Technology to produce human-induced pluripotent stem cells (hiPSCs) was first reported in 2007 [2], offering the exciting potential to generate autologous tissue.

Key advancements have been made toward the clinical application of hPSC-derived cells/tissues including the development of feeder-free and defined culture systems, the use of defined extracellular matrix (ECM) substrates (e.g., laminin subtypes) and non-integrative methods of hiPSC production. Protocols enabling the derivation of a huge range of cell types have been developed including corneal cells, cardiomyocytes, osteoblasts and neurons [3]. Furthermore, multicellular 3D organoids resembling developmental tissue have been achieved, including mini brains, guts and retina [4]. The ability to genetically modify hPSCs due to the

development of zinc-finger nucleases, transcription activator-like effector nucleases and more recently clustered regularly interspaced short palindromic repeats technology, also offers the possibility to transplant disease mutation-free tissue.

Since 2010, hPSC-derived retinal cells and oligodendrocyte progenitors have been transplanted in patients with retinal diseases (age-related macular degeneration and Stargardt disease) and spinal cord injury, respectively, and trials are recruiting patients with Type I diabetes and severe heart failure [4]. Encouragingly, no tumor formation has been seen in the patients transplanted. In parallel, Japan is leading on the creation of iPSC banks to provide HLA-matched tissue for 90% of their population [5].

Why do we need scaffolds?

Existing protocols often require long culture periods and/or use of multiple growth factors (GFs), meaning tissue production is expensive. While it has been possible to make primitive tissue from hiPSCs *in vitro*, scaffolds may enhance the outcome, allowing faster, cheaper and mature tissue generation.

Within native tissue, cells are supported by the ECM, which provides cell–matrix anchorage, mediated by ECM component-specific integrin-binding. This also mediates mechanotransduction, allowing the cells to respond to ECM stiffness. Both integrin activation

Nicola C Hunt

Institute of Genetic Medicine, Newcastle University, International Centre for Life, Central Parkway, Newcastle upon Tyne, NE1 3BZ, UK

Majlinda Lako

Author for correspondence:
Institute of Genetic Medicine, Newcastle University, International Centre for Life, Central Parkway, Newcastle upon Tyne, NE1 3BZ, UK
Tel.: +44 191 241 8688
majlinda.lako@ncl.ac.uk

and mechanotransduction mediate downstream signaling pathways (e.g., MAPK/ERKs, PI3K/AKT and TGF- β /BMP), and influence differentiation and tissue morphogenesis. The ECM also binds GFs mediating their action and availability, and reducing their half life. Furthermore, cell-ECM binding can promote GF-receptor activation allowing cells to respond to GFs [6].

ECM replication using biomaterials may enhance tissue formation from hPSCs, as has been extensively shown with multipotent stem cells [7]. This may reduce the need for constant replenishment of GFs, and reduce culture times, thus reducing costs. Some initial research has shown that encapsulation of hPSCs in Matrigel hydrogel droplets enhanced the formation of cerebral tissue, allowing accelerated differentiation and the formation of a more continuous neuroepithelia (rather than neural rosettes), surrounding a fluid-filled cavity reminiscent of a ventricle [8]. Addition of Matrigel to media has also been shown to enhance the retinal differentiation of mouse PSCs [9]. However, Matrigel, derived from mouse tumor cells, is a complex mixture of over 1500 components including not only ECM molecules but also GFs, and is known to vary in composition between batches [10]. For reproducible differentiations and transplantable tissues, it is necessary to replace Matrigel with more defined, clinically acceptable materials. To this end, addition of specific ECM molecules to both 2D and 3D cultures, has been investigated, for example in generation of retinal tissue [9,11].

“Matrigel, derived from mouse tumor cells, is a complex mixture of over 1500 components...”

It is, however, unlikely that addition of ECM components alone will be sufficient to effectively engineer tissues. Although the biochemical composition of the substrate (e.g., adhesion ligands) is important, it is likely that other factors including microtopology of the scaffold including pore size and interconnectivity, ridges and grooves, fibre sizes, as well as mechanical properties, are also critical. This has been extensively shown for example, with mesenchymal stem cells [12–14], and to some extent with PSCs [15,16]. Some more defined biomaterials have been investigated to enhance hPSC differentiation, including 3D poly(ethylene glycol)-fibrinogen hydrogels for the generation of cardiac tissue [17], and hyaluronan and alginate hydrogel scaffolds for retinal differentiation [HUNT NC, HALLAM D, KARIMI A *ET AL.* 3D CULTURE OF HUMAN PLURIPOTENT STEM CELLS IN ALGINATE HYDROGEL IMPROVES RETINAL TISSUE DEVELOPMENT (2016), SUBMITTED].

A need for multidisciplinary teams

A multidisciplinary toolbox is likely necessary to successfully engineer tissues from hPSCs for transplan-

tation purposes in the laboratory. It is unlikely that a single person will possess all the required skills and therefore the formation of a multidisciplinary team, with good communication and cooperation, is likely necessary. The increasingly collaborative nature of studies in the field is becoming apparent, partly through the increased authors, and there range of departments.

First, a good understanding and experience in hiPSCs generation and culture is required. Generation of hiPSCs is routinely performed in a number of laboratories now, but for successful generation, culture and subsequent differentiation, extensive experience is required to ensure proper *ex vivo* expansion and characterization as well as maintenance of genomic stability. It is also vital to involve surgeons, to ensure an understanding of their requirements and limitations (e.g., the host environment, incision sizes, equipment available, ease of handling), so that tissue substitutes which would be acceptable for clinical use are developed. It is also imperative to have a good understanding of good manufacturing practice (GMP) process design, to ensure suitability of both the scaffolds and the overall protocol to future GMP production to allow clinical translation. One needs sight of the regulatory issues at both national (Medicines and Healthcare Products Regulatory Agency [MHRA], Clinical Trial Authorisation [CTA]) and international level (EMA, US FDA).

To effectively engineer tissues, it is also imperative to have a good understanding of the normal biology and development of the target tissue, including the expression profiles, the ECM, cell-ECM, ECM-ECM (e.g., HA binding of proteoglycans) and GF-ECM interactions, so that the ECM is effectively replicated with the artificial scaffold. As mentioned earlier, mechanotransduction as well as chemical composition influences cell differentiation and tissue morphogenesis. Sometimes, the mechanical properties of the tissue, and especially specific niches at the nano- or micro-scale, are not known, and characterization requires specialized techniques such as: atomic force microscopy, as for example, has been demonstrated in the characterization of retina [18]; small angle x-ray scattering as has been demonstrated in musculoskeletal tissue including tendon [19]; or scanning acoustic microscopy combined with high-resolution synchrotron radiation microcomputed tomography, as for example, has been demonstrated in musculoskeletal tissues, including tendon [19]. These techniques can also be useful in the characterization of biomaterials, along with bulk property measurement techniques such as rheology and tensile testing.

In development of scaffolds, chemistry can be a useful attribute. Although several biomaterials are com-

mercially available (for example, hydrogels derived from materials of plant or animal origin, synthetic peptides/polymers or ECM molecules), chemically modifying materials could be beneficial. For example, GFs and cell adhesion ligands can be chemical bound, or specific cleavage sites can be incorporated to enable tailored host-mediated degradation [20]. Furthermore, a good understanding of chemistry allows for characterization of ECM and materials, including degradation products, for example, through infrared and Raman spectroscopy as well as mass spectroscopy techniques.

Involving engineers can help address the problems associated with reproducible manufacture and scale-up. For example, recently we have seen a surge in the number of publications using 3D printing of both the scaffold and the cells. Several different types of machine exist, but numerous parameters must be changed to achieve accurate printing of multiple components, and to ensure maintained cell viability. Finally, if we are to create engineered tissue for clinical application, animal studies will be necessary. It is important to have a good understanding of the animal models, and the disease models where applicable, so that meaningful results are obtained that indicate

whether clinical success will likely be seen with the engineered tissues.

Conclusion

Immense progress has been achieved since the first derivation of PSCs in 1998. The very fact that these cells are already being tested in Phase I/II clinical trials demonstrates not only their immense utility, but also a great scientific determination for accelerating transition from bench-to-bedside and providing tissue that is relevant to the patients. Creation of properly funded large scientific consortiums embedding knowledge from multiple scientific and regulatory areas is an important step on the road to clinical trials and provision of so much needed therapies in multiple medical disciplines.

Financial & competing interests disclosure

This work was funded by the ERC Consolidator award (#614620) and the RPF Innovation award (#GR584). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

- Thomson J, Itskovitz-Eldor J, Shapiro S *et al.* Embryonic stem cell lines derived from human blastocysts. *Science* 282(5391), 1145–1147 (1998).
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872 (2007).
- Robinton DA, Daley GQ. The promise of induced pluripotent stem cells in research and therapy. *Nature* 481(7381), 295–305 (2012).
- Kimbrel EA, Lanza R. Current status of pluripotent stem cells: moving the first therapies to the clinic. *Nat. Rev. Drug Discov.* 14, 681–692 (2015).
- Turner M, Leslie S, Martin NG *et al.* Toward the development of a global induced pluripotent stem cell library. *Cell Stem Cell* 13(4), 382–384 (2013).
- Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. *Dev. Biol.* 341(1), 126–140 (2010).
- Murphy WL, McDevitt TC, Engler AJ. Materials as stem cell regulators. *Nat. Mater.* 13(6), 547–557 (2014).
- Lancaster MA, Renner M, Martin C-A *et al.* Cerebral organoids model human brain development and microcephaly. *Mov. Disord.* 501, 373–378 (2013).
- Eiraku M, Takata N, Ishibashi H *et al.* Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 472(7341), 51–56 (2011).
- Hughes CS, Postovit LM, Lajoie GA. Matrigel: a complex protein mixture required for optimal growth of cell culture. *Proteomics* 10(9), 1886–1890 (2010).
- Sorkio A, Hongisto H, Kaarniranta K, Uusitalo H, Juuti-Uusitalo K, Skottman H. Structure and barrier properties of human embryonic stem cell-derived retinal pigment epithelial cells are affected by extracellular matrix protein coating. *Tissue Eng. Part A* 20(3–4), 1–13 (2014).
- Guilak F, Cohen DM, Estes BT, Gimple JM, Liedtke W, Chen CS. Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell* 5(1), 17–26 (2009).
- Dalby MJ, Gadegaard N, Oreffo ROC. Harnessing nanotopography and integrin–matrix interactions to influence stem cell fate. *Nat. Mater.* 13(6), 558–569 (2014).
- Baker BM, Chen CS. Deconstructing the third dimension – how 3D culture microenvironments alter cellular cues. *J. Cell Sci.* 125(13), 3015–3024 (2012).
- Keung AJ, Asuri P, Kumar S, Schaffer DV. Soft microenvironments promote the early neurogenic differentiation but not self-renewal of human pluripotent stem cells. *Integr. Biol.* 4(9), 1049–1058 (2012).
- Wang B, Qin P, Zhao H *et al.* Substrate stiffness orchestrates epithelial cellular heterogeneity with controlled proliferative pattern via E-cadherin/ β -catenin mechanotransduction. *Acta Biomater.* (2016) (In Press).
- Kerscher P, Turnbull IC, Hodge AJ *et al.* Direct hydrogel encapsulation of pluripotent stem cells enables ontomimetic

- differentiation and growth of engineered human heart tissues. *Biomaterials* 83, 383–395 (2016).
- 18 Franze K, Francke M, Günter K *et al.* Spatial mapping of the mechanical properties of the living retina using scanning force microscopy. *Soft Matter* 7(7), 3147–3154 (2011).
- 19 Tiburtius S, Schrof S, Molnár F *et al.* On the elastic properties of mineralized turkey leg tendon tissue: multiscale model and experiment. *Biomech. Model. Mechanobiol.* 13(5), 1003–1023 (2014).
- 20 Vats K, Benoit DSW. Dynamic manipulation of hydrogels to control cell behavior: a review. *Tissue Eng. Part B Rev.* 19(6), 455–469 (2013).