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Obstacles and challenges for tissue engineering and regenerative medicine: Australian nuances

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Summary

The clinical need and historical approaches to tissue engineering as applied to regenerative medicine are introduced, along with comments on activities in this field around Australia, and then the huge advances for tissue culture studies are discussed (Part A). Combinations of human stem cells and new approaches for generating bioscaffolds present great opportunities for in vitro studies of basic biology and physiology, drug testing and high throughput screening for the pharmaceutical industry, and the advanced tissue engineering of organs and devices. The future here is bright. The major obstacles arise with in vivo application of these bioengineering advances using animal models and humans (Part B), and the complexity of living tissues and the challenges of increased scale required for clinical translation to the large human situation are first discussed. While clinical success seen with implantation of acellular bioscaffolds (with population by host cells) is likely to expand for human use, the major challenge relates to (generally) low survival in vivo of (donor or autologous) cells that are expanded and grown in tissue culture before implantation into the living body. Another major challenge is revascularisation of implanted tissues/organs at the human scale. The innovative approaches and rapid advances in tissue bioengineering hold great promise for overcoming these major obstacles and extending the clinical applications of these technologies.

KEYWORDS

bioengineering, bioscaffolds, extracellular matrix, human inducible pluripotent stem cells, organ transplants, regenerative medicine, revasacularisation, skeletal muscle, tissue engineering, transplantation

1 | BACKGROUND

The goal of tissue engineering is to use biological materials and bioengineering to construct functional living replacement devices, organs and tissues, by combining cells on natural or synthetic scaffolds to replace or help regenerate damaged human tissues. The foundations and challenges of tissue engineering were discussed for this new field in 1993[1] with rapid expansion of interest demonstrated by many publications over the next decades.[2-4] Before discussing the current status of these emerging therapeutic possibilities, it is pertinent to provide some clinical background.

1.1 | Clinical situation

There is an urgent need for clinical transplantation to replace failing organs and tissues. Where tissues/cells are transplanted between different individuals, the foreign donor tissue is called an allograft and

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there can be serious problems with the host immune system rejecting foreign cells. To help overcome this major problem, the 2 people must have a closely matched immune system (the donor is often a relative of the patient) and drugs are required to suppress the host immune response (reviewed by Barker and Markmann[5]). Where the patient's own cells are used for the transplant (self) this autograft avoids the problem of immune rejection. While allografted human organs/tissues (such as kidneys, hearts, lungs, corneas) are in high demand, there is a serious lack of human living donor organs: thus many patients will never receive a vital transplant. Some tissues, eg, 1 kidney or bone marrow cells, can be donated by a living donor (often a relative), otherwise most donor tissues are obtained immediately after brain-death where permission is given by the human "organ donors" for their organs/tissues to be harvested and used for anonymous transplantation. However, severe logistical restraints means that many living donor organs cannot be matched and successfully transplanted into a patient within the very limited time available. New approaches for "perfusion preservation" of such living donor organs (and also tissue engineered synthetic organ), to allow organ banking for "organs on demand" would overcome this major problem to provide many more successful outcomes.[6] The use of cadavers as a source of human stem cells has also received attention.[7]

Xenotransplantation is a different approach to try and overcome the lack of human tissues to treat the many patients on waiting lists for transplants. Xenotransplantation uses donor organs/tissues/cells from another species (eg, monkey or pig) and potentially might provide an unlimited supply of organs and cells for clinical transplantation and for tissue engineering to replace human tissues. There have been many clinical attempts at xenotransplantation over the last 300 years (reviewed in Cooper[8]). The significant barriers to xenotransplantation are mainly being addressed by attempts to generate alternative sources of suitable histocompatible donor organs/tissues by using genetic engineering of pigs to remove the foreign antigens (in the xenograft) that provoke the human immune response. Progress in this field is illustrated by a review of pig to primate islet cell xenotransplantation.[9]

Successful clinical transplantation of normal human cells (rather than organs) is classically demonstrated by bone-marrow stem cell replacement (the allogenic donor cells need to be closely matched for histocompatibility). Autografted skin cells are also widely used for epidermal replacement (skin grafts or cell suspensions from another part of the patient's own body) and tissue engineering is used to reconstruct the dermis after deep injuries such as severe burns, to increase skin regeneration and reduce scarring[10]; new biomimetics also help to inhibit scarring.[11]

In contrast, it has proven difficult to achieve the dream of implanting human pancreatic islet cells to provide an intrinsic source of insulin to treat diabetes and other serious conditions. The clinical use of allogeneic human cells is associated with the usual major problems (limited availability of donor cells and immune rejection); there is intensive research in this area (reviewed in Aghazadeh and Nostro[12]) and the use of human induced pluripotent stem cells (hiPSCs) to generate large numbers of autologous islet cells holds promise.[13]

1.2 | Personal and Australian perspective

To provide some background for this commentary, my specific expertise relates to skeletal muscle regeneration and hence some examples are provided for this tissue, with relevance to the wider field. Furthermore, as a niche world perspective it is of interest to comment on the expansion of the disciplines of tissue engineering and regenerative medicine around Australia, which is a vast country with a relatively small population (<25 million people), yet the wealth of pertinent research by an innovative scientific community exemplifies the international developments. In 1999, a Tissue Engineering Research Centre (TERC) was established at the University of Western Australia (WA) and this interdisciplinary network of researchers and clinicians evolved into the Centre for Cell Therapy and Regenerative Medicine in 2012 in WA. An unusual aspect of the WA research community is the contribution of Symbiotica, a leading global laboratory/studio for Art and Science that engages with diverse disciplines including tissue engineering to creatively explore unusual aspects, ethical issues and non-biological applications.[14] The Australiasian Society for Biomaterials and Tissue Engineering was formed in 1989, and researchers with strong interests in the extracellular matrix through the Matrix Biology Society of Australia and New Zealand (established 1997) expanded the early tissue engineering network across the continent and the wider region.[15] There are now many dedicated Australian institutes and research centres, with some examples being the Australian Institute for Regenerative Medicine Institute (ARMI) established in 2009 at Monash University, the Australian Institute for Bioengineering and Nanotechnology at the University of Queensland, Biomaterials, Tissue Engineering and Regenerative Medicine at the University of New South Wales, the Tissue Engineering group at the University of Melbourne and the Materials Science and Engineering Division of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and these complement the global situation. Expansion of international networks in the region are demonstrated by conferences such as the Tissue Engineering and Regenerative Medicine International Society-Asia Pacific (TERMIS-AP) and establishment in 2013 of the Australian-China Centre for Tissue Engineering and Regenerative Medicine that involves many institutes in China and Australia.

The rich diversity of research across this region and New Zealand, is demonstrated by publication of a series of 16 papers in 2014 in a special journal issue entitled Regenerative Medicine: the Challenge of Translation[16]; this collection serves as a microcosm of global research to illustrates a wealth of experimental approaches. These investigations use tissue culture and disparate animal models such as axolotls, zebrafish, rodents (including genetically engineered mouse models and immunocompromised mice), deer (to study bone growth),[17] and humans. These models are combined with technological and creative approaches to investigate many tissues such as the brain, spinal cord, nerves, eyes, adipose, bone, lungs, skin, heart, skeletal muscles and limbs.

The contributions of Australian researchers to the fields of tissue engineering and regenerative medicine are further demonstrated in

the present commentary by about 41% of the references cited having one or more Australia authors.

Central issues for tissues engineering for in vivo transplantation purposes are whether to use (i) cells alone and (ii) cells attached to a bioscaffold, or (iii) a bioscaffold alone to provide an ideal environment to stimulate host cells immigration, with proliferation and differentiation to form new functional tissue.[1] These issues are first discussed for tissue culture studies.

2 | PART A: MAJOR ADVANCES IN TISSUE CULTURE FOR BIOENGINEERING: FOCUS ON CELLS AND SUBSTRATES

2.1 | Sources of human stem cells to form diverse tissues

A stem cell is defined as a cell that can divide to self-renew, as well as give rise to precursors of diverse lineages (plasticity). The extraction of human adult stem cells from fresh tissues for tissue engineering and regeneration therapies is limited by supply (especially for autologous cells) and by proliferation in tissue culture to expand the number of precursor cells. Hence enormous interest resulted from widespread reports of stem cell plasticity in tissue culture; for example, mesenchymal stem cells (MSCs) give rise to various cell lineages, such as chondrocytes, osteoblasts and myoblasts. There were many reports of such plasticity, with multi- or pluripotent stem cells being isolated from interstitial and circulating cells and other sources, as reviewed for differences in regenerative capacity of skeletal and cardiac muscles[18] with limited capacity for stem cell renewal of mammalian cardiac muscle continuing to be emphasised.[19] MSCs remain of much interest for generation of cells for transplantation, as demonstrated for human bone marrow-derived MSCs (hBMSCs) used for cartilage repair, where microencapsulation enhanced their survival and efficacy.[20] Unfortunately, some speculation about the potential of stem cell therapies, including MSCs, for clinical applications has been inflated, unrealistic and misleading raising serious ethical and regulatory issues related to inappropriate marketing of unproven stem cell treatments.[21-23]

A major breakthrough occurred about 20 years ago when human embryonic stem cells (hESCs), isolated from the inner cell mass of a blastocyst (produced through in vitro fertilization, with spare embryos donated for research purposes), were able to be directed into many lineages in tissue culture and were confirmed to be pluripotent with the unique ability to differentiate into cells of all tissues in the body.[24] This generated enormous interest as a potential new source of human stem cells. In tissue culture, hESCs have been used to study molecular regulation of differentiation and tissue formation for many types of human cells with other diverse uses including drug screening. While hESCs also presented the possibility of generating many allogeneic donor cells for clinical transplantation, the fact that these cells were derived from "spare" human embryos raises major ethical issues.

A variation on this process was to remove the nucleus from an oocyte and to substitute a nucleus taken from a mature mammalian cell into the cytoplasm of the enucleated oocyte: this somatic cell nuclear transfer (SCNT) reprogrammed the mature nucleus back to a pluripotent state resembling an ESC. The restoration of totipotency to a single mature somatic nucleus was dramatically demonstrated for mammals when SCNT was used to form all tissues of a living sheep called Dolly in 1996[25]; subsequently SCNT has been used to generate cloned animals of many mammalian species. The importance for human studies is that SCNT can take the nucleus from a mature cell of any human and make it pluripotent, thus allowing the generation of many cell lineage from different humans, for healthy and diseased individuals including patients with a specific genetic defect. The SCNT approach expanded tissue culture investigations into the molecular regulation of diseased human cells, diagnosis and drug screening for specific diseases. These patient-specific pluripotent cells are also of interest for potential autologous transplantation back into the same patient. However, there remain controversies with such approaches, due to concerns about the developmental fidelity of the hSCNT cells (compared with "normal ESCs") with a complete ban on human cloning, plus ethical issues associated with manipulation of human oocytes.[26]

The really significant advance (that avoided these difficult ethical issues) was the development of induced pluripotent stem cells (iPSCs), where treatment of a mature mouse fibroblast with a cocktail of about 4 genes resulted in reprogramming of the mature somatic nucleus back to a condition very similar to an ESC. The field moved rapidly and human iPSCs were generated by 2 groups in 2007.[27,28] Now hiPSCs have been derived from many diverse cell types emphasising the plasticity of somatic nuclei. The hiPSCs are suitable for many in vitro studies as indicated above with expanding use of organoids for regenerative medicine,[29] drug screening and personalised medicine.[30] However ethical concerns associated with the use of cultured human stem cells and organoids need to be considered.[31,32] Autologous human cells are highly desirable for transplantation purposes, since use of the patient's own cells avoids the major in vivo problem of immune rejection (discussed further in Part B). These are extraordinary advances that extend biomedical research towards clinical translation.[33]

2.2 | Improved culture conditions

Routine tissue culture uses non-physiological, high levels of oxygen (20%) compared with in vivo level in tissues that are around 5%. It is increasingly recognised that some stem cells that are considered quiescent can be more easily "activated" by exposure to physiologically low oxygen levels, as demonstrated for muscle stem cells extracted from old muscles and "senescent" adipogenic precursor cells.[34,35] Hypoxia conditioning has been suggested to improve the stem cell survival and enhance myocardial regeneration (reviewed in Dall et al[36]), with cardiomyocyte activation and cycling in vivo strongly associated with hypoxia signalling.[37] Furthermore, after experimental myocardial infarction, exposure to severe systemic hypoxaemia induces metabolic reprogramming of adult cardiomyocytes, resulting in cell cycle re-entry, reactivation of cardiomyocyte mitosis and improved heart regeneration.[38] Such relatively simple adjustments in

culture conditions, for oxygen and other parameters such as growth factors and extracellular matrix, to more closely represent the situation in living tissues, can significantly alter interpretations of cell behaviour and improve cellular responses for tissue engineering.

New opportunities are also presented by increasing recognition of major roles for non-coding RNAs (ncRNAs) in the complex molecular regulation of cell biology and tissue formation,[39] although there are relatively few in vivo studies to date for microRNAs (that can circulate in the blood as exosomes) and long ncRNAs.[40,41] These ncRNAs have major implications as targets for new drug interventions, and for in vitro and in vivo studies to enhance tissue formation.

2.3 | Scaffolds: new materials, revascularisation, biomechanics and 3D bioprinting

The complex 3 dimensional (3D) extracellular matrix (ECM), secreted by the resident tissue cells acts like an architectural scaffold as well as providing many molecular signals to enhance tissue formation during development or regeneration. Such natural 3D scaffolds have advantages in terms of their molecular composition, architecture, physiological activities, mechanical properties and biodegradability. For many tissues, native ECM can be obtained by removing all living cells from the specific tissue, with careful digestion resulting in an acellular scaffold while maximizing retention of ECM components, including proteoglycans and their glycosaminoglycan chains, as shown for acellular myomatrix derived from skeletal muscles.[42] Successful clinical transplantation of acellular human scaffolds has been achieved for various tissues (see Part B). While native ECM can be sourced from non-human mammalian tissues, concerns about issues of immunogenicity and safety (including possible transmission of zoonotic diseases and introduction of new pathogens into the human population) have led to major expansion of interest in synthetic biomaterials to conveniently construct 3D scaffolds. Scaffolds for tissue engineering must be biocompatible (not evoke an immune response), have the correct biomechanics, and they frequently contain structural ECM proteins like collagen, fibronectin, elastin, fibrin, and laminin.[43] Such natural or artificial scaffolds are often combined with diverse source of stem cells, including hiPSCs.

Silk is a natural material (produced by insects) that is biocompatible and biodegradable and has been used clinically in surgical silk sutures for many years. Silk fibroin protein can be spun to various thicknesses and cells readily attach and grow on this surface and thus it is attractive as a substrate for bioengineering purposes. The composition varies between different types of silk moths and a comparison of 4 sources of silk in tissue culture showed an excellent capacity to support myogenesis.[44] Acellular silk sheets have been used in vivo to repair tympanic membranes perforations in the ear of rodent models: they are biocompatible,[45] with excellent acoustic and mechanical properties,[46] and hold promise for clinical applications. Other natural materials include alginate (a polysaccharide derived from seaweed) and even apple derived cellulose 3D scaffolds have been used for mammalian cell culture[47]: many creative possibilities can be explored in vitro.

Synthetic polymers such as hydrogels and polyacrylamide are widely used to construct sheets of cells and 3D scaffolds. These can be engineered for specific composition, rigidity and pore size to try and mimic the architecture and properties for the target tissue, and combined and cross-linked with growth factors, ECM components, nanoparticles and many other molecules to enhance cellular responses: such complexes represent a major area of research for bioengineering.[48] One use is for cell sheet engineering, where temperature-responsive culture dishes allows harvesting of cells (without trypsin) on intact polymer sheets and progressive layering of sheets with different types of cells can build up various tissues, including the use of endothelial vascular cells to construct vascularised tissues. There is much interest in strategies to improve the blood supply in order to form thick implantable viable tissue constructs, with much research focussed on strategies to enhance vascularisation of muscle tissues.

2.4 | Vascularisation of muscle constructs

The combination of sheets of endothelial cells and muscle precursors can build vascularised heart muscle, or can be directly transplanted into host tissues without the use of scaffolding, as reviewed in Yang et al[49] and discussed for skeletal muscles.[43] The use of spun nanofibre mesh as a support for alternating layers of sheets of muscle precursors, and human vascular endothelial cells plus fibroblasts, is another strategy to address revascularisation and build thick 3D structures in vitro.[50] Revascularisation of skeletal muscle constructs has attracted much attention with in vivo studies of muscle implants containing C2C12 myogenic cells and human umbilical vein endothelial cells (HUVEC) into immunocompromised rodents emphasising that coculture of these cells promotes in vivo vascularization of engineered tissues.[51] The subsequent use of adult human endothelial cells (instead of HUVECs) produced more potent pre-vascularisation of the muscle construct and formation of chimaeric vessel with host vasculature, emphasising the important role of angiogenic factors produced by the graft tissue[52]: see also discussion under Part B. The ECM composition can also influence the efficacy of revascularisation as demonstrated when scaffolds combined with tropoelastin (that is responsible for elasticity of blood vessels) were implanted into mouse abdominal muscle, resulting in enhanced perfusion of the penetrating vasculature and improved integration.[53] The importance of tensile forces in angiogenesis and improved vessel structure for tissue repair is also demonstrated.[54] Real time monitoring of revascularisation in vivo of (small) tissue engineered skeletal muscle implants (donor neonatal rat muscles into nude immunocompromised host mice) using a mouse dorsal window implantation model, showed a steady ingrowth of blood-perfused (host) microvasculature with increasing force contraction over 2 weeks.[55] The importance of a robust vascular supply for successful translation is also emphasised for scaled-up cardiac tissue engineering for clinical treatment of heart failure.[56] These approaches generally await further in vivo validation especially for larger constructs using animal models (also see Part B), before possible progression to clinical applications.

2.5 | Recent focus on mechanobiology of cell interactions

During the 1970-1980s, Elizabeth Hay was one of the first scientists to reveal the role of ECM in regulating cell behaviour, and the importance of ECM in epithelial to mesenchymal cell phenotype transitions (reviewed in Hugo et al[57]). These concepts were expanded by Mina Bissell in the 1990s through her studies on mammary cancer cells, to show that the ECM exerts physical and biochemical influences which are transduced by cell surface receptors through the cytoskeleton to the nucleus to effect changes in gene expression that can alter cell phenotype: this led to a greater appreciation of the microenvironment of a cell (often called a niche) and the influence of 3D cultures.[58] The effect of matrix on phenotype is further demonstrated by an altered fibrotic environment (with increasing glycation and cross-linking as occurs in ageing and some muscular dystrophies) that can convert myogenic precursor cells into a fibrogenic fate and prevent myogenesis; thus the extrinsic ECM can trump the intrinsic initial cell programming.[59] The ECM may exert these effects on many cell types through the molecular and protein composition and signalling interactions (that have been widely studied), biomechanical properties (eg, stiffness or elasticity) nanotypography and also electric fields,[60] and it can be difficult to separate out these potentially different roles of the ECM in vivo. There is huge interest in the effects of new biomaterials on the biology of cells and tissues with an emphasis on the need to develop ECM-mimicking biomaterials.[61] Computational modelling also provides new strategies for tissue regeneration.[62]

Mechanobiology is focussed on biomechanics, biosensing and mechanotransduction of cells and is attracting increasing attention. Cells in vivo are exposed to a variety of mechanical loads, including shear fluid forces (endothelium), compression (bone) and tensile forces (epithelium) and the complex cellular response depends on both force magnitude and rate[63]; the in vivo significance of ECM influences on mechanotransduction for many tissues is well reviewed.[64] Biomechanical studies in vitro often use single cells in a substrate such as hydrogel of varying "stiffness" that exert degrees of force corresponding to physical properties of specific tissues: the properties of cell-substrate interactions and cellular and nuclear deformation can be analysed using special platforms to stretch cells combined with advanced optical and atomic force microscopy.[63] Stiffness gradients of hydrogel or polyacrylamide and stretch bioreactors are also used to study the effects of mechanotransduction, as shown for MSC migration and fate[65]; and adipogenesis was significantly upregulated by gels that mimicked the native stiffness of adipose tissue (2 kPa).[66] For contractile tissues such as skeletal muscles, mechanical forces and structural strength are of particular importance (as well as electrical stimulation) and there is much interest in such bioengineered muscle constructs.[67]

2.6 | 3D printing of scaffolds with cells to form tissues

The development of 3D printers has revolutionised many forms of fabrication and early interest for tissue engineering of 3D tissues, was

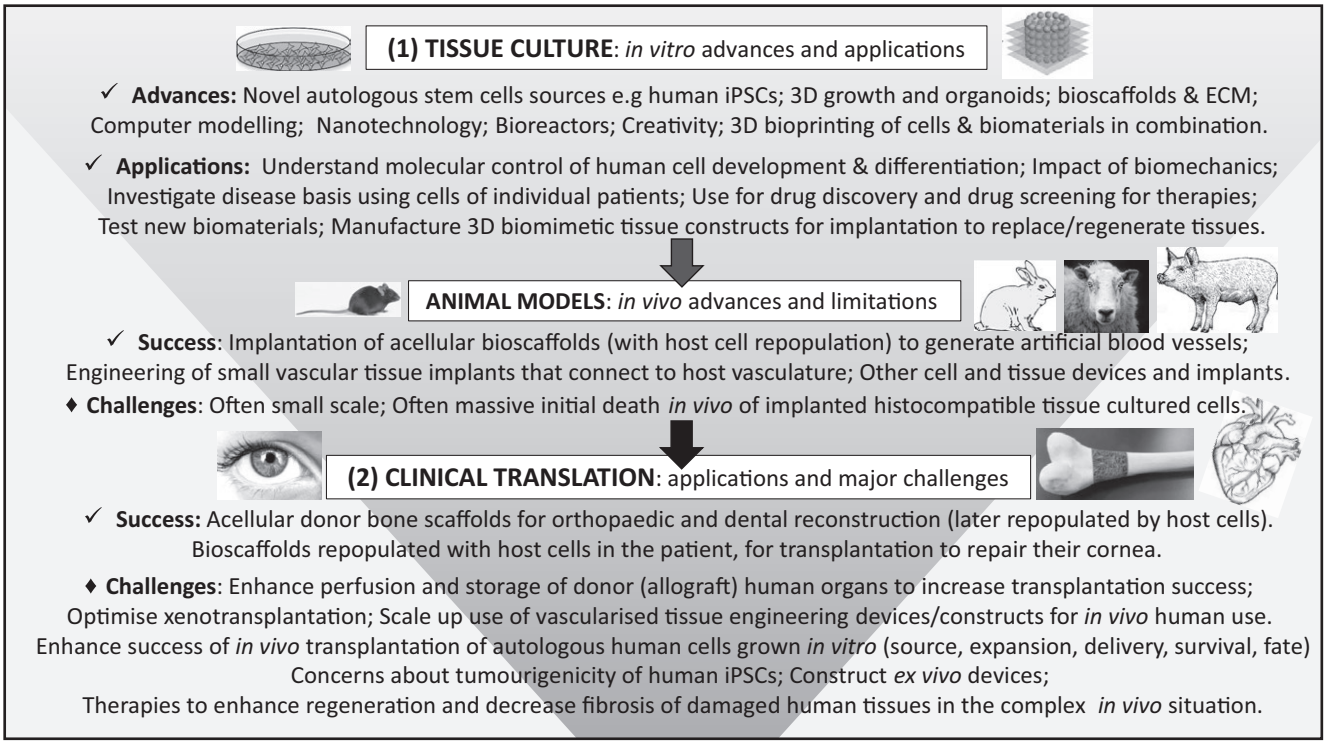

FIGURE 1 Simple summary of recent activities in Tissue Engineering and Regenerative Medicine. This emphasises (1) the expanding activities in tissue culture combined with progress for in vivo applications using animal models (eg, rodents and larger species) and (2) major problems and challenges to overcome to expand success of clinical translation to humans

illustrated by a simple 3D construction of beating cardiomyocytes in 2003.[68] This technology that uses bioprinting to precisely dispense different cell types and biomaterials layer by layer, has significantly advanced construction of biomimetic living tissues,[69] and future vascularized 3D soft organs might help solve the organ transplantation crisis.[70] This approach may also be used to construct devices outside (ex vivo) but attached to the human body to maintain some vital function, for examples a substitute living kidney as an alternative to dialysis: such devices are being developed using extracorporeal, bioartificial kidney membranes with fully differentiated and functional human tubular epithelial cells.[71] Such 3D biomimetic constructs (greatly assisted by availability of hiPSCs), are of particular interest to the pharmaceutical industry for drug discovery research,[72] as discussed in detail for skin,[73] and may be able to partially substitute for testing of drugs in animals. While there are major obstacles to overcome, 3D bioprinting presents creative possibilities for future uses in tissue transplantation and regenerative medicine. These exciting achievements and scope for tissue culture studies are indicated in Figure 1, along with the progress using animal models and challenges to be overcome for human applications.

3 | PART B: MAJOR CHALLENGES FOR TRANSLATION TO CLINICAL APPLICATIONS

3.1 | Complexity of in vivo situation compared with tissue culture conditions

Clinical applications of bioengineering are complex with many technological challenges to be solved. The important issue of tissue volume for organs is a big challenge, especially when scaling up from pre-clinical studies using rodent models to the relatively huge size of humans, with implications also for essential angiogenesis and vascular architecture, plus reconnection of nerves during innervation: these are not major issues for construction of tissues in vitro. The implications of growth need to be considered in this context,[74] as do the dynamics and composition of the ECM, including the basement membrane that is in intimate contact with the surface of many cells, especially as this is poorly represented in vitro. The ECM is critical for the transfer of mechanical forces for contractile tissues like cardiac and skeletal muscles[75] and scale also has major implications on forces exerted on the ECM and tissues, especially on muscles and bones: these forces need to be considered when extrapolating results from experiments in small laboratory animal models to larger humans.

The roles of many interacting cell types in vivo are essential for optimal regenerative responses, yet only a single pure cell type is usually studied in tissue culture. This is illustrated for fibroblasts (a major component of the interstitial ECM), since it has been shown that interactions between fibroblasts and skeletal muscle cells have beneficial effects in tissue engineered constructs of skeletal muscle[76] and during skeletal muscle regeneration in vivo in mouse models,[77] with strong positive regulatory influences confirmed for human fibroblasts and muscle cells[78]: yet fibroblasts are specifically excluded in vitro from most studies of cultured muscle cells. Fibroblasts also have beneficial effects during heart development, in addition to well-documented adverse effects related to fibrosis.[79] In adult tissues the matrix may play a far greater role than is generally appreciated, especially where it is disturbed after disease and injury, plus there are striking changes during ageing: an increasing centre stage for the ECM is envisaged in regenerative medicine.[80,81]

Also critically important in vivo is the interaction of host inflammatory and immune cells with the transplanted cells and tissues (especially initially during the early stages): this interaction is also not present in vitro. In addition, fluxes in systemic factors play important roles in cell and tissue function in vivo with hormones and other circulating molecules (eg, nutrients, growth factors and miRNAs in exosomes) contributing to signalling. Therefore, given the in vivo complexity (relative to tissue culture conditions) and confounding effects of scale difference between rodent models and humans, it is really not surprising that translational to clinical success has been so slow.

It is emphasised that there are increasing ethical and other concerns regarding the commercial marketing of unproven stem cell therapies for clinical use, as discussed by a recent paper[23] that involved 20 international institutions including 5 from Australia.

3.2 | Evaluating autologous human iPSCs for transplantation

The extraordinary success with generating new sources of human stem cells, especially hiPSCs, provides many opportunities for uses in tissue culture (described above) and also the generation of large number of human cells of a specific lineage ex vivo, thus solving a major problem of cell supply for transplantation in vivo. These remarkable autologous human cells can be transplantated in vivo as cells alone or combined with bioscaffolds, or integrated into external devices ex vivo, to substitute or repair a diversity of tissues. With such revolutionary potential for hiPSCs, what are the impediments to clinical success?

3.3 | Genetic fidelity and tumours

There remain serious concerns regarding whether epigenetic modulations and other alterations to DNA of the mature original nucleus used to generate pluripotent iPSCs (and also stem cells derived by SCNT), result in true fidelity and equivalence of these stem cells to an ESC, or whether aberrant development of hiPSCs might result in tumours forming in the human in vivo environment? Pluripotent stem cells can give rise to many lineages dependent on the extrinsic conditions, including aberrant tumourigenic cell lines and teratomas. Indeed, early studies showed that the capacity to form teratomas was greater for hiPSCs than for hESCs,[82] plus other studies also indicate a propensity for iPSCs to form tumours in vivo. The cell environment is easily controlled in tissue culture, whereas the complex in vivo environment means that conditions are almost undefined in many mammalian tissues. Therefore, since the mature in vivo environment may lack constraints to limit cell differentiation to only the required specific lineage and cell types, tumours might also arise. One approach to minimise

this risk of cancerous cells inadvertently arising, is to first convert the pluripotent iPSCs to the specific lineage ex vivo, and then these lineage restricted precursor cells are transplanted into the host.

Questions concerning the immunogenicity of iPSCs and other stem cells have also been raised and this remains a little controversial. These concerns about safety of hiPSCs transplanted in vivo for tissue engineering and regenerative medicine are the subject of ongoing investigations, as discussed in reviews.[83]

3.4 | Survival in vivo and other obstacles to successful transplantation of mammalian cells

The potential use of hiPSCs and organoids for clinical transplantation has increased exponentially, although concerns regarding safety and feasibility persist.[32] Some obstacles related to general feasibility of "stem cell therapies" are illustrated for skeletal muscle precursor cells (myoblasts or myogenic stem cells) classically considered to be derived from satellite cells that are quiescent reserve cells lying between the sarcolemma and basement membrane of adult myofibres,[84] with a focus on myoblast transfer therapy (MTT) as a gene replacement strategy for dystrophic skeletal muscles of Duchenne Muscular Dystrophy (DMD) boys. This attractive strategy aims to replace the missing dystrophin gene by exploiting the fact that multinucleated myofibres are formed by myoblasts fusing together, so that the gene products of normal donor muscle nuclei (with the dystrophin gene) can replace the missing dystrophin (of the host nuclei) to restore dystrophin protein in hybrid myofibres. Since dystrophic myofibres (for DMD and the mdx mouse model) exhibit high levels of myofibre necrosis with resultant myogenesis during regeneration, there is plenty of opportunity for myoblast fusion and integration of the donor muscle nuclei into the regenerating host myofibres. When donor normal (histocompatible) male mouse myoblasts were injected into dystrophic muscles of adult female mdx mice and were tracked using labels (such as the Y-chromosome probe that identifies donor male nuclei), this revealed rapid and massive death of donor myoblasts, with 90% lost within 1 week.[85] This may well account for the failure of most MTT clinical trials for DMD. It seems that exposure to tissue culture conditions such as serum and proteases contribute to the rapid demise of (even histocompatible) donor cells in the host environment.[86,87] It should be possible to overcome these major problems and yet, despite alternative source of donor myoblasts from stem cells and about 30 years of research, there has been very limited success with MTT.[88] Indeed, recent studies with MTT in non-human primates, report a similar high death of donor myoblasts.[89] This frustrating situation emphasises (i) the need for robust and appropriate pre-clinical data to justify possible progression to clinical trials and (ii) the need for suitable labels to track the fate of donor nuclei/cells—including initial cell survival, and subsequent proliferation and integration. Where initial survival of tissue cultured donor cells is measured after injection into host tissues, it seems that a similar rapid loss of donor cells may occur generally, eg, as discussed for the heart[81] where rapid loss (~89%-98%) of peripheral blood mononuclear cells was observed at 6 days after intramyocardial or intra vascular delivery in an ischemic swine model. Although myogenic stem cell therapy has been promoted for age-related loss of muscle function (sarcopenia), the rationale for this is questioned,[90] especially considering the serious issues outlined above.

Clinically, while several hundred patients with heart failure have received intracardiac injections of autologous myoblasts (from skeletal muscle), with benefits reported for functional recovery, this appears to be due to paracrine effects without integration of the donor myoblasts or generation of new cardiomyocytes[91]; these results remain controversial as benefits were not observed by all groups and there is a well-documented increased risk of arrhythmia.[19] The recent demonstration that induced hypoxia in vivo after induction of myocardial infarction can activate resident cardiomycte proliferation in situ and improve cardiac muscle regeneration in mice, has promising implications and presents an attractive alternative approach[38]; the challenge remains to translate this into a clinical application.

The poor survival in vivo after injection of isolated cultured skeletal muscle precursor cells, is in stark contrast with the outcome using grafted fresh skeletal muscle tissue that (after necrosis) readily forms large amounts of new muscles in vivo, even in very old animals, and persists.[90] One limitation for regeneration of muscle grafts is the speed of revascularisation by host vasculature (that precedes inflammation and myogenesis in the graft) and this becomes an increasing problem with larger tissue volumes that are used for clinical muscle reconstruction. Enhanced host angiogenesis with more rapid speed of revascularisation of the muscle grafts in mice resulted from pre-treatment of the donor muscle grafts in vivo with viral delivery of the potent angiogenic factor VEGF (vascular endothelial growth factor), and this was associated with more rapid new muscle formation.[92] The strategy of pre-treatment of donor muscles to accelerate host revascularisation of the implanted muscle graft (or construct—as discussed in Part A) is of clinical interest.

3.5 | Implantation of scaffolds in humans

Clinically, implanted acellular natural biomatrices are now used as bone scaffolds for orthopaedic and jaw reconstructions, with bone tissue banks established for this purpose.[93] However, there are reports that it can be difficult to eliminate persisting allogeneic cells from the donor bones.[94] Such acellular native bioscaffolds (with subsequent population by autologous host cells) are attracting much attention, although this is daunting for organs.[95] Clinical success with autologous cells grown on an artificial scaffold ex vivo and implanted into humans, is seen with replacement of corneal epithelium using limbal stem cells cultured from the healthy eye of the patient (the eye is an immunoprivileged site and this may assist survival of the cultured cells).[96] Another strategy aims to grow constructs within the patient's own body for subsequent transplantation to the required location. Clinically, the success of using teeth to restore vision after corneal blindness is particularly creative (autologous osteo-odonto keratoprosthesis); in this situation, the patient's own tooth dentine supports growth of autologous cells on artificial corneas implanted into the check of the patient, for subsequent transplantation into the blind eye.[97] A similar principle

has been used in animal models to construct artificial blood vessels, required for vascular bypass grafting. Artificial blood vessel grafts were manufactured within the future recipient, when conduits of sialic tubing implanted into the peritoneum were populated by autologous (host) cells and ECM, and subsequent grafting into the same animal showed that the tissue was almost indistinguishable from native vessels.[98] Biomechanical properties are very important in the cardiovascular system and recent advances using synthetic copolymers as biodegradable elastomeric scaffolds for this purpose show further promise in animal models.[99] Cardiovascular diseases are a major cause of global deaths and, in the absence of suitable healthy autologous vascular grafts, there is a crucial clinical need for tissue engineered blood vessels (TEBV) and also heart valves[100] for cardiovascular regeneration: although there have been significant advances in these fields using animal models, clinical applications are still mainly in the developmental stage.[101,102]

3.6 | Construction of living tissues for in vivo clinical transplantation

The criteria typically required to manufacture tissues for in vivo transplantation are comprehensively discussed above. The bioengineering of human skeletal muscle tissues serves to illustrate the scale of the challenges: this requires various sources of stem cells, plus the vital need for good vascularisation (to supply nutrients and oxygen and remove carbon dioxide and waste) and effective innervation (to signal muscle contraction).[43] While several groups have shown that vascularised small constructs of muscle cells and human endothelial cells, transplanted into immunocompromised host mice can successfully integrate with the host blood supply (discussed under Part A), these important proof of principle studies use only a tiny implant, eg, 6 mm diameter and 1 mm thick.[52] Clinically, the vascularization of thick tissue constructs and integration with the host vasculature is increasingly difficult for much larger volumes of donor tissue constructs and is a bottleneck for tissue engineering and regenerative medicine: this is challenging.

Animal models are essential for pre-clinical studies to address the key issues of donor cell survival and fate, the immune response and revascularization of any tissue or organ construct. Many aspects can be studied in animals that cannot be addressed in humans (using genetically modified and immunocompromised as well as normal host mice, plus rabbits and sheep as larger models). Once proof of concept is demonstrated, formal pre-clinical trials are required in animals (in most situations), using conditions that mimic as closely as possible the equivalent clinical situation. When the technology is considered efficacious for human use, there remain major regulatory hurdles that need to be overcome associated with clinical trials and commercialisation: many regulations aim to ensure patient safety, including the risk profile of the technology, the reagents and processes in manufacture, possible storage, and transport to the bedside.[10] Thus the final stages of commercial upscaling of a procedure and approvals for clinical applications can be time consuming and complex.

CONCLUSIONS

The huge advances for tissue culture studies include the generation of hiPSCs and the use of 3D printing with new biomaterials for generation of bioscaffolds with increasing appreciation of biomechanics. Combined, these present enormous opportunities in vitro for studying basic biology, drug testing and high throughput screening, and for tissue engineering of tissues, organs and devices: this is an exciting field. The major obstacles relate to clinical translation. The intent of preserving living donor organs (natural or engineered) for successful storage in organ tanks, would greatly assist the logistics and success of matching suitable donors with patients desperately awaiting such transplants. For clinical purposes acellular scaffolds, that provide a new home for host cells, are already being used in various situations and are likely to have expanded applications. The major clinical obstacles relate to problems with the transfer of living cells from tissue culture conditions into the human body; this applies to many isolated cells, tissue constructs and artificially engineered organs. Plus vascularisation of implanted tissues/organs at the human scale is very challenging. While the rapid and creative advances in tissue bioengineering hold great promise for the future of regenerative medicine, balanced and critical evaluation of these new technologies, including robust ethical discussions, is required for realistic consideration and promotion of potential clinical applications.

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REFERENCES

1. Langer R, Vacanti JP. Tissue engineering. *Science*. 1993;260:920-926.
2. Langer RS, Vacanti JP. Tissue engineering: the challenges ahead. *Sci Am*. 1999;280:86-89.
3. Guillot PV, Cui W, Fisk NM, Polak DJ. Stem cell differentiation and expansion for clinical applications of tissue engineering. *J Cell Mol Med*. 2007;11:935-944.
4. Lanza R, Langer R, Vacanti JP. *Principles of Tissue Engineering*. San Diego, CA: Elsevier Science; 2011.
5. Barker CF, Markmann JF. Historical overview of transplantation. *Cold Spring Harb Perspect Med*. 2013;3:a014977.
6. Giwa S, Lewis JK, Alvarez L, et al. The promise of organ and tissue preservation to transform medicine. *Nat Biotechnol*. 2017;35:530-542.
7. Hodgetts SI, Stagg K, Sturm M, Edel M, Blancafort P. Long live the stem cell: the use of stem cells isolated from post mortem tissues for translational strategies. *Int J Biochem Cell Biol*. 2014;56:74-81.
8. Cooper DKC. A brief history of cross-species organ transplantation. *Proc (Bayl Univ Med Cent)*. 2012;25:49-57.

9. Liu Z, Hu W, He T, et al. Pig-to-primate islet xenotransplantation: past, present, and future. *Cell Transplant*. 2017;26:925-947.
10. Wood FM. Skin regeneration: the complexities of translation into clinical practise. *Int J Biochem Cell Biol*. 2014;56:133-140.
11. Jeon EY, Choi B-H, Jung D, Hwang BH, Cha HJ. Natural healing-inspired collagen-targeting surgical protein glue for accelerated scarless skin regeneration. *Biomaterials*. 2017;134:154-165.
12. Aghazadeh Y, Nostro MC. Cell therapy for type 1 diabetes: current and future strategies. *Curr DiabRep*. 2017;17:37.
13. Mihara Y, Matsuura K, Sakamoto Y, Okano T, Kokudo N, Shimizu T. Production of pancreatic progenitor cells from human induced pluripotent stem cells using a three-dimensional suspension bioreactor system. *J Tissue Eng Regen Med*. 2017;11:3193-3201.
14. Catts O, Zurr I. Growing for different ends. *Int J Biochem Cell Biol*. 2014;56:20-29.
15. Frith JE, Hudson JE, Cooper-White JJ. Conference scene. A bright future for tissue engineering and regenerative medicine in the Asia Pacific region. *Regen Med*. 2011;6:167-170.
16. Rosenthal N, Grounds MD. Regenerative medicine: the challenge of translation. Editorial. *Int J Biochem Cell Biol*. 2014;56:2-3.
17. Li C, Zhao H, Liu Z, McMahon C. Deer antler–a novel model for studying organ regeneration in mammals. *Int J Biochem Cell Biol*. 2014;56:111-122.
18. Grounds MD, White JD, Rosenthal N, Bogoyevitch MA. The role of stem cells in skeletal and cardiac muscle repair. *J Histochem Cytochem*. 2002;50:589-610.
19. Cai CL, Molkentin JD. The elusive progenitor cell in cardiac regeneration: slip slidin' Aaay. *Circ Res*. 2017;120:400-406.
20. Li F, Truong VX, Thissen H, Frith JE, Forsythe JS. Microfluidic encapsulation of human mesenchymal stem cells for articular cartilage tissue regeneration. *ACS Appl Mater Interfaces*. 2017;9:8589-8601.
21. Munsie M, Hyun I. A question of ethics: selling autologous stem cell therapies flaunts professional standards. *Stem Cell Res*. 2014;13:647-653.
22. Munsie M, Pera M. Regulatory loophole enables unproven autologous cell therapies to thrive in Australia. *Stem Cells Dev*. 2014;23(Suppl 1):34-38.
23. Sipp D, Caulfield T, Kaye J, et al. Marketing of unproven stem cell-based interventions: a call to action. *Sci Transl Med*. 2017;9:pii: eaag0426.
24. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;282:1145-1147.
25. Campbell KH, McWhir J, Ritchie WA, Wilmut I. Sheep cloned by nuclear transfer from a cultured cell line. *Nature*. 1996;380:64-66.
26. McLaren A. Cloning: pathways to a pluripotent future. *Science*. 2000;288:1775.
27. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861-872.
28. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318:1917-1920.
29. Voges HK, Mills RJ, Elliott DA, Parton RG, Porrello ER, Hudson JE. Development of a human cardiac organoid injury model reveals innate regenerative potential. *Development*. 2017;144:1118-1127.
30. Bartfeld S, Clevers H. Stem cell-derived organoids and their application for medical research and patient treatment. *J Mol Med (Berl)*. 2017;95:729-738.
31. Pera MF, de Wert G, Dondorp W, et al. What if stem cells turn into embryos in a dish? *Nat Methods*. 2015;12:917-919.
32. Munsie M, Hyun I, Sugarman J. Ethical issues in human organoid and gastruloid research. *Development*. 2017;144:942-945.
33. Zenke M. Stem cells: from biomedical research towards clinical applications. *J Mol Med (Berl)*. 2017;95:683-685.
34. Chakravarthy MV, Spangenburg EE, Booth FW. Culture in low levels of oxygen enhances in vitro proliferation potential of satellite cells from old skeletal muscles. *Cell Mol Life Sci*. 2001;58:1150-1158.
35. Ratushnyy A, Lobanova M, Buravkova LB. Expansion of adipose tissue-derived stromal cells at "physiologic" hypoxia attenuates replicative senescence. *Cell Biochem Funct*. 2017;35:232-243.
36. Dall C, Khan M, Chen CA, Angelos MG. Oxygen cycling to improve survival of stem cells for myocardial repair: a review. *Life Sci*. 2016;153:124-131.
37. Kimura W, Xiao F, Canseco DC, et al. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. *Nature*. 2015;523:226-230.
38. Nakada Y, Canseco DC, Thet S, et al. Hypoxia induces heart regeneration in adult mice. *Nature*. 2017;541:222-227.
39. Frith JE, Porrello ER, Cooper-White JJ. Concise review: new frontiers in microRNA-based tissue regeneration. *Stem Cells Transl Med*. 2014;3:969-976.
40. Yoshioka H, Yoshiko Y. The roles of long non-protein-coding RNAs in osteo-adipogenic lineage commitment. *Int J Mol Sci*. 2017;18:1236.
41. Butchart LC, Fox A, Shavlakadze T, Grounds MD. The long and short of non–coding RNAs during post-natal growth and differentiation of skeletal muscles: focus on lncRNA and miRNAs. *Differentiation*. 2016;92:237-248.
42. Chaturvedi V, Dye DE, Kinnear BF, van Kuppevelt TH, Grounds MD, Coombe DR. Interactions between skeletal muscle myoblasts and their extracellular matrix revealed by a serum free culture system. *PLoS One*. 2015;10:e0127675.
43. Koning M, Harmsen MC, van Luyn MJ, Werker PM. Current opportunities and challenges in skeletal muscle tissue engineering. *J Tissue Eng Regen Med*. 2009;3:407-415.
44. Chaturvedi V, Naskar D, Kinnear BF, et al. Silk fibroin scaffolds with muscle-like elasticity support in vitro differentiation of human skeletal muscle cells. *J Tissue Eng Regen Med*. 2017;11:3178-3192.
45. Shen Y, Redmond SL, Papadimitriou JM, et al. The biocompatibility of silk fibroin and acellular collagen scaffolds for tissue engineering in the ear. *Biomed Mater*. 2014;9:015015.
46. Allardyce BJ, Rajkhowa R, Dilley RJ, et al. Comparative acoustic performance and mechanical properties of silk membranes for the repair of chronic tympanic membrane perforations. *J Mech Behav Biomed Mater*. 2016;64:65-74.
47. Modulevsky DJ, Lefebvre C, Haase K, Al-Rekabi Z, Pelling AE. Apple derived cellulose scaffolds for 3D mammalian cell culture. *PLoS One*. 2014;9:e97835.
48. Naahidi S, Jafari M, Logan M, et al. Biocompatibility of hydrogel–based scaffolds for tissue engineering applications. *Biotechnol Adv*. 2017;35:530-544.
49. Yang J, Yamato M, Nishida K, et al. Cell delivery in regenerative medicine: the cell sheet engineering approach. *J Controlled Release*. 2006;116:193-203.
50. Kim MS, Lee B, Kim HN, et al. 3D tissue formation by stacking detachable cell sheets formed on nanofiber mesh. *Biofabrication*. 2017;9:015029.
51. Levenberg S, Rouwkema J, Macdonald M, et al. Engineering vascularized skeletal muscle tissue. *Nat Biotechnol*. 2005;23:879-884.
52. Perry L, Flugelman MY, Levenberg S. Elderly patient-derived endothelial cells for vascularization of engineered muscle. *Mol Ther*. 2017;25:935-948.
53. Landau S, Szklanny AA, Yeo GC, et al. Tropoelastin coated PLLA-PLGA scaffolds promote vascular network formation. *Biomaterials*. 2017;122:72-82.
54. Rosenfeld D, Landau S, Shandalov Y, et al. Morphogenesis of 3D vascular networks is regulated by tensile forces. *Proc Natl Acad Sci U S A*. 2016;113:3215-3220.
55. Juhas M, Engelmayr GC, Fontanella AN, Palmer GM, Bursac N. Biomimetic engineered muscle with capacity for vascular

integration and functional maturation in vivo. *Proc Natl Acad Sci*. 2014;111:5508-5513.
56. Dilley RJ, Morrison WA. Vascularisation to improve translational potential of tissue engineering systems for cardiac repair. *Int J Biochem Cell Biol*. 2014;56:38-46.
57. Hugo H, Ackland ML, Blick T, et al. Epithelial–mesenchymal and mesenchymal–epithelial transitions in carcinoma progression. *J Cell Physiol*. 2007;213:374-383.
58. Bissell MJ. Goodbye flat biology – time for the 3rd and the 4th dimensions. *J Cell Sci*. 2017;130:3.
59. Stearns-Reider KM, D'Amore A, Beezhold K, et al. Aging of the skeletal muscle extracellular matrix drives a stem cell fibrogenic conversion. *Aging Cell*. 2017;16:518-528.
60. Banks TA, Luckman PS, Frith JE, Cooper-White JJ. Effects of electric fields on human mesenchymal stem cell behaviour and morphology using a novel multichannel device. *Integr Biol (Camb)*. 2015;7:693-712.
61. Li Y, Xiao Y, Liu C. The horizon of materiobiology: a perspective on material-guided cell behaviors and tissue engineering. *Chem Rev*. 2017;117:4376-4421.
62. Martin KS, Virgilio KM, Peirce SM, Blemker SS. Computational modeling of muscle regeneration and adaptation to advance muscle tissue regeneration strategies. *Cells Tissues Organs*. 2016;202:250-266.
63. Haase K, Pelling AE. Investigating cell mechanics with atomic force microscopy. *J R Soc Interface*. 2015;12:20140970.
64. Hadden WJ, Choi YS. The extracellular microscape governs mesenchymal stem cell fate. *J Biol Eng*. 2016;10:16.
65. Hadden WJ, Young JL, Holle AW, et al. Stem cell migration and mechanotransduction on linear stiffness gradient hydrogels. *Proc Natl Acad Sci U S A*. 2017;114:5647-5652.
66. Young DA, Choi YS, Engler AJ, Christman KL. Stimulation of adipogenesis of adult adipose-derived stem cells using substrates that mimic the stiffness of adipose tissue. *Biomaterials*. 2013;34:8581-8588.
67. Khodabukus A, Baar K. Factors that affect tissue-engineered skeletal muscle function and physiology. *Cells Tissues Organs*. 2016;202:159-168.
68. Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR. Organ printing: computer-aided jet-based 3D tissue engineering. *Trends Biotechnol*. 2003;21:157-161.
69. Sears NA, Seshadri DR, Dhavalikar PS, Cosgriff-Hernandez E. A review of three-dimensional printing in tissue engineering. *Tissue Eng Part B Rev*. 2016;22:298-310.
70. Mandrycky C, Wang Z, Kim K, Kim D-H. 3D bioprinting for engineering complex tissues. *Biotechnol Adv*. 2016;34:422-434.
71. Mollet BB, Bogaerts ILJ, van Almen GC, Dankers PYW. A bioartificial environment for kidney epithelial cells based on a supramolecular polymer basement membrane mimic and an organotypical culture system. *J Tissue Eng Regen Med*. 2017;11:1820-1834.
72. Peng W, Datta P, Ayan B, Ozbolat V, Sosnoski D, Ozbolat IT. 3D bioprinting for drug discovery and development in pharmaceutics. *Acta Biomater*. 2017;57:26-46.
73. Abaci HE, Guo Z, Doucet Y, Jackow J, Christiano A. Next generation human skin constructs as advanced tools for drug development. *Exp Biol Med (Maywood)*. 2017;242:1657-1668. 1535370217712690.
74. Kuhl E. Growing matter: a review of growth in living systems. *J Mech Behav Biomed Mater*. 2014;29:529-543.
75. Grounds MD, Sorokin L, White J. Strength at the extracellular matrix–muscle interface. *Scand J Med Sci Sports*. 2005;15:381-391.
76. Li M, Dickinson CE, Finkelstein EB, Neville CM, Sundback CA. The role of fibroblasts in self-assembled skeletal muscle. *Tissue Eng Part A*. 2011;17:2641-2650.
77. Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G. Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. *Development*. 2011;138:3625-3637.
78. Mackey AL, Magnan M, Chazaud B, Kjaer M. Human skeletal muscle fibroblasts stimulate in vitro myogenesis and in vivo muscle regeneration. *J Physiol*. 2017;595:5115-5127.
79. Furtado MB, Nim HT, Boyd SE, Rosenthal NA. View from the heart: cardiac fibroblasts in development, scarring and regeneration. *Development*. 2016;143:387-397.
80. Grounds MD. Complexity of extracellular matrix and skeletal muscle regeneration. In: Schiaffino S, Partridge TA, eds. *Skeletal Muscle Repair and Regeneration*. Dordrecht, the Netherlands: Springer Netherlands;2008:269-302.
81. Taylor DA, Chandler AM, Gobin AS, Sampaio LC. Maximizing cardiac repair: should we focus on the cells or on the matrix? *Circ Res*. 2017;120:30-32.
82. Gutierrez-Aranda I, Ramos-Mejia V, Bueno C, et al. Human induced pluripotent stem cells develop teratoma more efficiently and faster than human Embryonic Stem Cells regardless the site of injection. *Stem Cells*. 2010;28:1568-1570.
83. Okano H, Nakamura M, Yoshida K, et al. Steps toward safe cell therapy using induced Pluripotent Stem Cells. *Circ Res*. 2013;112:523.
84. Relaix F, Zammit PS. Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. *Development*. 2012;139:2845-2856.
85. Fan Y, Maley M, Beilharz M, Grounds M. Rapid death of injected myoblasts in myoblast transfer therapy. *Muscle Nerve*. 1996;19:853-860.
86. Smythe GM, Grounds MD. Exposure to tissue culture conditions can adversely affect myoblast behavior in vivo in whole muscle grafts: implications for myoblast transfer therapy. *Cell Transplant*. 2000;9:379-393.
87. Grounds MD. Myoblast transfer therapy in the new millennium. *Cell Transplant*. 2000;9:485-487.
88. Negroni E, Bigot A, Butler-Browne GS, Trollet C, Mouly V. Cellular therapies for muscular dystrophies: frustrations and clinical successes. *Hum Gene Ther*. 2016;27:117-126.
89. Skuk D, Tremblay JP. Cell therapy in myology: dynamics of muscle precursor cell death after intramuscular administration in nonhuman primates. *Mol Ther Methods Clin Dev*. 2017;5:232-240.
90. Grounds MD. Therapies for sarcopenia and regeneration of old skeletal muscles: more a case of old tissue architecture than old stem cells. *Bioarchitecture*. 2014;4:81-87.
91. Menasche P. Skeletal myoblasts as a therapeutic agent. *Prog Cardiovasc Dis*. 2007;50:7-17.
92. Smythe GM, Lai MC, Grounds MD, Rakoczy PE. Adeno-associated virus-mediated vascular endothelial growth factor gene therapy in skeletal muscle before transplantation promotes revascularization of regenerating muscle. *Tissue Eng*. 2002;8:879-891.
93. Winter JM, Cowie AI, Wood DJ, Zheng MH. Musculoskeletal tissue banking in Western Australia: review of the first ten years. *ANZ J Surg*. 2005;75:665-671.
94. Coutinho LF, Amaral JBD, Santos EBD, et al. Presence of cells in fresh-frozen allogeneic bone grafts from different tissue banks. *Braz Dent J*. 2017;28:152-157.
95. Stoltz JF, Zhang L, Ye JS, De Isla N. Organ reconstruction: dream or reality for the future. *Biomed Mater Eng*. 2017;28:S121-S127.
96. Arandjelovic P, Kaur P. The regenerative potential of epithelial stem cells in tissue repair. *Int J Biochem Cell Biol*. 2014;56:107-110.
97. Gulati M, Salaria SK, Anand V, Jain N, Pandey S. Periodontium bestows vision!!. *J Indian Soc Periodontol*. 2016;20:349-351.
98. Hoenig MR, Campbell GR, Rolfe BE, Campbell JH. Tissue-engineered blood vessels: alternative to autologous grafts? *Arterioscler Thromb Vasc Biol*. 2005;25:1128-1134.
99. Dargaville BL, Vaquette C, Peng H, et al. Cross linked poly(trimethylene carbonate-co-l-lactide) as a biodegradable, elastomeric scaffold for vascular engineering applications. *Biomacromol*. 2011;12:3856-3869.

100. Hasan A, Ragaert K, Swieszkowski W, et al. Biomechanical properties of native and tissue engineered heart valve constructs. *J Biomech*. 2014;47:1949-1963.
101. Dogan A, Elcin AE, Elcin YM. Translational applications of tissue engineering in cardiovascular medicine. *Curr Pharm Des*. 2017;23:903-914.
102. Elomaa L, Yang YP. Additive manufacturing of vascular grafts and vascularized tissue constructs. *Tissue Eng Part B Rev*. 2017;23:436-450.

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