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REVIEW ARTICLE

Emerging opportunities for induced pluripotent stem cells in orthopaedics

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Abstract The discovery of induced pluripotent stem cells (iPSCs) has revolutionized biomedicine. Although the potential of iPSCs for tissue regeneration, disease modeling and drug screening has been largely recognized, findings of iPSC research to date are mostly focused on neurology, cardiology and haematology. For orthopaedics, growing interest in the unique cell type has prompted more researchers to get involved in iPSC research. In this article, we introduce the brief history of cellular reprogramming and different reprogramming methods that have been developed, discuss the biology of iPSCs and review previously reported findings of iPSC studies in orthopaedics.

The Translational potential of this article: Stem cell therapies hold great promise for treating orthopaedic diseases, manifested in recent study findings and results of clinical trials. iPSCs are a unique stem cell type derived from a patient's own cells while still possessing the embryonic stem cell-featured pluripotency for generation of all tissues in the body. The distinctive properties make iPSCs much desirable to fulfill the promise of regenerative medicine for clinical orthopaedics.

Introduction

Musculoskeletal disorders afflict millions of people around the world and cost billions of dollars for healthcare annually, according to the data published by the World Health Organization [1]. Unlike diseases of other vital organs that cause immediate life-threatening conditions such as heart failure, most musculoskeletal disorders result in pain and immobility in patients, which mainly affects their quality of life and often leads to additional medical conditions. To

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date, musculoskeletal disorders have caused health and economic burden, which is expected to be even greater in the coming decades as the world population ages [1]. Current treatment options for musculoskeletal disorders, such as drug administration and surgical intervention focussing on alleviating pain associated with the disease and restoring functions of affected tissues, provide temporal solutions with therapeutic outcomes that remain to be further improved. On the other hand, biological resolutions using stem cells to facilitate tissue regeneration as alternative approaches to replace current medical procedures for treatment of musculoskeletal disorders have been developed and extensively investigated in recent years [2]. The motivation driving the development of biological solutions is that stem cells with the capacity of unlimited propagation and multilineage differentiation are able to function as a therapeutic agent to repair degenerative tissues and restore their functions as a long-term solution. There are different types of stem cells derived either from the embryo or from postnatal tissues, and each of the cell types has unique characteristics and properties [3]. Whether a particular type of stem cells is more suitable than others for treatment of musculoskeletal disorders is not a topic of this review. Readers who are interested in this topic can find several comprehensive review articles in which pros and cons with the use of each stem cell type for orthopaedic applications are discussed [4–7].

In this review, we focus our discussion and review on the knowledge and research using direct cellular reprogramming as a tool to generate induced pluripotent stem cells (iPSCs) to study pathogenesis of ageing-associated musculoskeletal disorders and explore their potential for tissue repair. Specifically, we will introduce the background of direct cellular reprogramming, review the current knowledge of ageing-associated musculoskeletal disorders and discuss recent research findings on cell rejuvenation through direct cellular reprogramming and on generation of iPSCs for orthopaedic applications.

Cellular reprogramming

Cellular reprogramming is a laboratory procedure to convert a mature differentiated cell into a less-committed precursor. It was first demonstrated through somatic cell nuclear transfer (SCNT) more than 60 years ago and the technique of SCNT has been applied to resetting cell fate (Table 1). Although SCNT is a powerful method to reprogramme cells, the procedure is labour-intensive and technically challenging. It is also known that the success rate of SCNT is incredibly low, making it less attractive to be used for regenerative medicine. The approach using defined factors to reprogramme cells, termed "direct cellular reprogramming", was first reported about 3 decades ago, and in 2006, Takahashi and Yamanaka showed that by introducing a small set of transcription factors, Oct4, Sox2, Klf4 and c-Myc (termed OSKM), into a somatic cell, the cell can be induced to dedifferentiate into a pluripotent stem cell, and other transcription factors, such as LIN28 and NANOG, have also been identified and used together with a part or all of OSKM to increase the efficiency of cellular reprogramming (Table 1).

To force ectopic expression of the transcription factors in cells, approaches based on viral transduction or nonviral transfection have been used to deliver reprogramming vectors. Virus-based approaches using retroviral or lentiviral vectors to deliver reprogramming factors are generally more efficient than nonvirus-based ones; however, retroviral and lentiviral transduction randomly integrating exogenous transgenes into the host genome likely causes insertional DNA mutations. Considering the feasibility for clinical applications, alternative reprogramming methods not resulting in modification of the host genome are more feasible than those using viral vectors. Strategies allowing removal of exogenous genetic constructs from the host genome or those that avoid integration of transgenes with the host genome, including the Cre-loxP system [15], episomal vectors [16], PiggyBac transposons [17], the Sendai virus vector [18] or recombinant proteins [19], have been adopted for cellular reprogramming.

Biology of iPSCs

Forcing the ectopic expression of pluripotent markers in a somatic cell can transform the cell into a pluripotent stem cell, which is termed iPSCs. It has been just over a decade since iPSCs were first created in 2006. Although we have gained a significant amount of knowledge regarding iPSC generation and characterisation over the years, our

Table 1 Milestones of cellular reprogramming.

Approach	Significance	Reference
Somatic cell nuclear transfer (SCNT)	Concept of SCNT first introduced	[8]
	Landmark SCNT study using frog cells	[9]
	Reprogramming of mammalian cells to create Dolly, the sheep	[10]
	Cloning of nonhuman primates	[11]
Direct cellular reprogramming	Conversion of fibroblasts into myoblasts through ectopic expression of a key transcription	[12]
	Creation of induced pluripotent stem cells using a set of four defined transcription factors	[13]
	Identification of transcription factors other than Yamanaka factors for reprogramming	[14]

understanding of biology and physiology of the cell is still limited. Nonetheless, with the help of new analytical methods, such as RNA sequencing (RNA-seq) and DNA methylation analysis, we have begun to understand in more detail what changes occur to a reprogrammed cell at the molecular level and how these molecular changes take place in the cell. This has helped our capability to create iPSCs with consistent phenotypic characteristics in a controlled way, allowing for a better understanding of tissue development and regeneration and health concerns, such as ageing and cancer.

It has been shown that the efficiency of reprogramming somatic cells into iPSCs is low because success of the process requires cells to complete several molecular phases to overcome epigenetic hurdles and activate the endogenous pluripotency network. During the multiphase process for cell conversion, fewer cells in culture after each phase are capable of undergoing the subsequent phase of further transformation. In fact, the efficiency of iPSC generation is low, roughly about 0.0006–1.4%, depending on the cell type and reprogramming methods [20]. For a successful transition from the differentiated state to the undefined pluripotent state, a somatic cell has to undergo complete phenotypic and transcriptional changes. For example, after receiving reprogramming factors, fibroblasts begin to rapidly proliferate and downregulate fibroblast-specific transcription. After completing the early phase, the cell enters into the middle phase by undergoing a mesenchymal-to-epithelial transition to acquire epithelial characteristics and activating some of the embryonic stem cell (ESC) genes [21]. During this phase, epithelial-like cells begin to form colonies that express ESC markers, such as alkaline phosphatase and stage-specific embryonic antigen 1 (SSEA1) for mice [22] or SSEA3 and SSEA4 for human [23]. It requires the coordination of tightly regulated molecular events in a pre-iPSC to complete a phase of the reprogramming event before the cell enters into the next phase. In the late phase, endogenous ESC markers, such as OCT4, SOX2 and NANOG, are expressed to establish the pluripotency network in the cell to complete the process of cell fate conversion [22].

Somatic cells able to conquer all required phases of cell dedifferentiation likely turn into iPSCs with pluripotent characteristics and properties that function and behave similarly to ESCs. In culture, iPSCs form colonies and can be identified by their round cell morphology with large and obvious nucleoli in the scant cytoplasm. Molecularly, the cell expresses endogenous ESC genes, including OCT3/4, SOX2 and NANOG, and surface markers, such as TRA-1-60 and TRA-1-81, in addition to SSEA3 and SSEA4 [24]. As ESCs, iPSCs are mitotically active and can proliferate indefinitely. Studies have shown that the growth of iPSCs in culture can achieve a great number of population doublings and is not restricted by the Hayflick limit of cell division. This is mainly because of high telomerase activity through which human telomerase reverse transcriptase is extensively involved in maintaining the length of telomere in iPSCs. In terms of pluripotency, iPSCs in culture spontaneously form three-dimensional, spherical embryoid bodies consisting of cell types of all three germ layers. When subcutaneously injected into an immunodeficient mouse, iPSCs form a teratoma composed of endodermal, mesodermal and ectodermal tissue structures with morphologically defined tissue-specific cells. If culture with defined chemical and physical signals is used to differentiate iPSCs, the cell can be induced to become tissue lineage–specific cells, such as osteoblasts, neurons and cardiomyocytes.

iPSCs for orthopaedic research

The emergence of iPSCs is considered a major scientific breakthrough in biology and medicine. Owing to the ESC-like capacity of differentiating into all types of tissue cells in the body, iPSCs hold great potential for cell therapies, by which the cell can regenerate damaged tissues/organs and restore their functions affected by diseases, such as Alzheimer's disease and heart failure. Furthermore, research evidence has shown that many aspects of cellular ageing can be reset by reprogramming, which has opened the door to new research endeavours to study how cellular reprogramming rejuvenates ageing cells, identify regulatory factors involved in the process of ageing and develop therapeutic interventions for age-related diseases. Finally, studies have demonstrated findings of iPSCs derived from donors with genetic diseases. This suggests the potential of using the patient's iPSCs to model a human disease in culture, a powerful new tool to screen therapeutic drugs for effective personalised treatments. In the following paragraphs, we will review and discuss recent findings of how the iPSC technology has impacted musculoskeletal research and changed orthopaedic medicine.

Mitigation of age-related musculoskeletal disorders

The ageing process is closely linked with diseases and subsequent mortality. While affecting nearly every tissue in the body, ageing greatly impacts the musculoskeletal system, causing age-associated degenerative diseases. For example, osteoarthritis (OA), also known as a degenerative joint disease, most commonly affects synovial joints. Although the aetiology of OA is multifactorial, age is closely associated with the disease. Ageing appears to be associated with a reduction in the effectiveness in turnover of matrix proteins, contributing to the progression of joint degeneration [25]. In addition to regulation of matrix production, age-related changes in the extracellular matrix (ECM), such as decrease in hydration, aggrecan size and growth factor level and increase in advanced glycation end product formation, collagen cleavage and matrix calcification, have been reported [26]. All of these changes, either individually or together, result in decrease in the strength and fatigue resistance of cartilage, making the tissue more vulnerable to mechanical damage. Ageing also leads to changes in the structure and property of bone. For instance, a study has shown that ageing is associated with a reduction in the formation of new periosteal bone and an increase in the resorption of endosteal bone, resulting in cortical and trabecular bone thinning and increased cortical porosity, which in turn reduces bone quality and strength [27]. The net result of subperiosteal bone formation and irreversible bone lost along the endosteal surface is a

process independent of hormonal control, suggesting that age-related mechanisms are involved [28]. Sarcopenia is age-related muscle loss, which could happen to both physically active and inactive people after a certain age. It is known that skeletal muscle mass declines approximately 4% per decade from the ages of 25—50 years and increases to 10% per decade thereafter [29]. Given that skeletal muscle is injured by its own contractions, the tissue undergoes repetitive recovery for maintenance; however, the muscle recovery becomes less effective with an age-associated reduction in hormone production, such as growth hormone and insulin-like growth factor-1.

Recent findings have suggested that cellular reprogramming is a powerful approach for studying ageing and age-related diseases because it provides researchers a way to reset the ageing clock of a cell. It has been shown that through cellular reprogramming, an ageing cell can become young again, exhibiting rejuvenated cell phenotypes and improved biological activities both *in vitro* and *in vivo*. Lapasset et al. demonstrated that cellular reprogramming can reset telomere length, gene expression profiles, oxidative stress and mitochondrial metabolism of senescent and centenarian cells for their rejuvenation. Ageing cell-derived iPSCs were induced to differentiate back to fibroblasts with enhanced capabilities to proliferate at a similar rate as young cells and undergo reduced cellular senescence, resulting in increased population doublings in culture compared with nonreprogrammed parental cells [30]. Similarly, Ohmine et al. has shown that iPSCs generated from keratinocytes of elderly patients with type 2 diabetes acquired a rejuvenated state, characterised by elongated telomeres, suppressed senescence-related $p15^{INK4b}$/$p16^{INK4a}$ gene expression and decreased oxidative stress. When induced for pancreatic cell differentiation, reprogrammed ageing cells became functional insulin-producing islet-like progenies, indicating that cellular ageing of keratinocytes had been at least partially reversed [31]. In addition to these *in vitro* studies, a recent study has demonstrated that *in vivo* cellular reprogramming can reverse ageing and prolong lifespan of mice. Specifically, Ocampo et al. used the so-called "partial cellular reprogramming" approach to induce the short-term cyclic expression of OSKM to ameliorate cellular and physiological hallmarks of ageing and increase lifespan in mice with Hutchinson—Gilford Progeria Syndrome and improve the recovery from metabolic abnormality and muscle injury induced by beta cell— and muscle cell—specific toxins in old wild-type mice [32]. This seminal finding has suggested that the strategy of partial cellular reprogramming is promising for reversing effects of ageing and mitigating age-related diseases. To date, there are no reports of *in vivo* cellular reprogramming in the field of orthopaedic research. However, considering that degenerative musculoskeletal diseases are associated with ageing, it seems reasonable to infer that cellular reprogramming, capable of reversing the ageing progress, can alter the pathogenesis and development of the diseases for the development of effective treatments. Studies to confirm the inference may soon become a focused area of orthopaedic research.

Regeneration of musculoskeletal tissues

The discovery of iPSCs has catalysed the development of non—ESC-based regenerative therapies, as manifested through recent clinical trials (Table 2). Similarly, there is an emerging interest in the potential of iPSCs for the treatment of musculoskeletal diseases. In the past 2 decades, mesenchymal stem cells (MSCs) isolated from adult tissues have been widely studied as therapeutic agents in preclinical and clinical trials. However, accumulated evidence has shown several limitations associated with the use of adult tissue—derived MSCs for orthopaedic regenerative medicine. For example, procedures to harvest MSCs are considered invasive, and only few MSCs can be obtained from a single donor. In addition, harvested MSCs are a heterogeneous population that exhibits donor-to-donor variation in phenotypes [38], and their properties and functions are also dependent on age and health condition of a donor [39]. In culture, adult tissue—derived MSCs quickly lose their proliferation and differentiation capability and become senescent with cell passages [40]. On the other hand, iPSCs have been shown to be able to overcome these limitations, and MSCs

Table 2 Approved clinical trials of iPSC therapies.

Target disease	Cell type	Status	Reference
Age-related macular degeneration	Autologous iPSC-derived retinal pigment epithelial cells	Suspended	[33]
Age-related macular degeneration	Allogeneic iPSC-derived retinal pigment epithelial cells from human leucocyte antigen—matched donors	Ongoing	[34]
Heart diseases with damaged myocardium	iPSC-derived cardiomyocytes	Ongoing	[35]
Parkinson's disease	iPSC-derived dopaminergic progenitors	Ongoing	[36]
Spinal cord injury	iPSC-derived neural stem/progenitor cells	Ongoing	[37]

iPSC, induced pluripotent stem cell.

derived from iPSCs (iPSC-MSCs) become an alternative type of multipotent cells for orthopaedics. It seems that iPSC-MSCs feature advantageous characteristics of both iPSCs and MSCs without limitations associated with MSC isolation and culture [41]. This has been supported by recent findings demonstrating that iPSC-MSCs are capable of extensive proliferation, similar to iPSCs, and as MSCs, they possess the immunomodulatory capacity [42], without causing tumorigenesis [43].

Osteogenesis of iPSCs

Protocols used to induce ESCs for osteogenic differentiation have been applied to iPSCs, by which pluripotent stem cells were grown in an embryoid body or a microcarrier and treated with retinoic acid and differentiation inducers that include ascorbic acid, beta-glycerophosphate, dexamethasone, bone morphogenetic proteins (BMPs) and vitamin D3, commonly used in osteogenic culture of tissue-derived MSCs. Several groups have implemented different approaches to enhance osteogenesis of iPSCs. For example, Kao et al. [44] have demonstrated that resveratrol, a natural polyphenol antioxidant, facilitates osteogenic differentiation in both iPSCs and ESCs. By overexpressing the key transcription factor Runt-related transcription factor 2, Tashiro et al. [45] were able to enhance osteogenic differentiation of mouse iPSCs. Osteogenesis of iPSCs can also be enhanced through coculturing with primary bone cells [46]. A number of engineering approaches have been used to increase osteogenesis of iPSCs, including using an electromagnetic field to stimulate the cell for osteogenic differentiation [47] and optimising the composition of three-dimensional scaffolds to provide an osteoconductive environment to encourage the differentiation. Levi et al. [48] have reported that biomaterial scaffolds containing hydroxyapatite, poly-L-lactic acid and BMP2 improve the survival and osteogenesis of seeded iPSCs, and Ji et al. have demonstrated that the amount of nanohydroxyapatite in nanohydroxyapatite/chitosan/gelatine scaffolds controls osteogenic differentiation of human iPSCs [49].

Chondrogenesis of iPSCs

Similarly, protocols for induction of chondrogenic differentiation of iPSCs are adopted from those used to induce chondrogenesis of ESCs, which have been developed to recapitulate the process of chondrogenesis *in vivo* that includes phases of condensation of mesenchymal cells, proliferation of chondroprogenitors and differentiation of chondroblasts. The complete transition from pluripotent stem cells to mature chondrocytes requires well-coordinated signalling from molecules including BMPs, fibroblast growth factors (FGFs), transforming growth factors (TGFs), Wnt and cell adhesion molecules, such as N-CAM, N-cadherin and β-catenin. In addition, ECM macromolecules, such as collagen type 2, hyaluronan, aggrecan or fibronectin, can also act as signalling molecules to regulate chondrogenic differentiation. A number of protocols have been developed to induce differentiation of human ESCs and iPSCs into chondrocytes, which can be classified into three types according to the strategy used: coculture with primary chondrocytes [50], derivation through embryoid body formation [51] and induction of a combination of growth factors [52]. On the other hand, several groups have taken a different approach by treating iPSCs temporally with a series of defined media to induce the sequential formation of intermediate cell populations, including mesendodermal and mesodermal cells and chondrocyte progenitors, during chondrogenesis [53]. Considering the critical role of three-dimensional culture in induction of chondrogenesis, biomaterial scaffolds have been included to enhance chondrogenic differentiation of iPSCs. Representative examples shown in studies that involve polycaprolactone/gelatine [54], polycaprolactone [55] or alginate matrix [56] demonstrate that not only the chondrogenesis of iPSCs is enhanced by use of a scaffold but also the regulation of the differentiation is dependent on the composition of a scaffold.

Myogenesis and tenogenesis of iPSCs

Other than osteoblasts and chondrocytes, iPSCs can also be differentiated into myoblasts and tenocytes, which offers hope for patients afflicted with diseases or sports injuries of skeletal muscles or tendons/ligaments. Several groups have recently reported successful derivation of skeletal muscle cells from iPSCs by overexpressing myogenic transcription factors, MyoD and Pax3, or through stepwise induction using small molecules and cytokines to modulate myogenic-associated signalling [57,58]. Notably, a research group has demonstrated that by taking advantage of the epigenetic memory of parental cells, muscle cell—derived iPSCs with the intrinsic propensity for myogenesis can be effectively induced to turn into myoblasts [59].

Although many efforts have been made to explore the potential of iPSCs for osteogenesis, chondrogenesis and myogenesis, studies investigating tenogenic differentiation of the cell are just beginning to emerge. Among the few studies, one reported by Xu et al. [60] has demonstrated that iPSCs were induced to differentiate into neural crest stem cells for tenogenesis and that iPSC-derived neural crest stem cells helped repair tendon in a rat model. Another study by Zhang et al. [61] used a different approach by which they directed iPSCs into MSCs as a cell resource for tenogenesis and repaired Achilles tendon with iPSC-MSCs. Although these results are encouraging, there remain many challenges in effectively driving iPSCs into functional tenocytes. It is of importance that future studies aim to fill the knowledge gap.

Modelling of orthopaedic diseases and drug screening

Although it may take a few years before iPSC-based therapies become clinical treatments, generation of the cell from patients with diseases for *in vitro* disease modelling and drug screening has been put into practice, and a number of groups have demonstrated that iPSCs generated from patients with neurological, cardiac, haematologic or hepatic disorders were used to study disease formation and screen pharmacological compounds for treatment of the

diseases. In the current paradigm of new drug development, pharmacological compounds are screened and tested in culture of immortalised human cell lines and laboratory animals; however, both screening platforms pose limitations in selecting "right hits" of chemical compounds. Ideally, cells harvested from human disease tissues are a preferred choice for drug screening, but difficulties exist in accessing such tissues and expanding the cell in culture. With the discovery of iPSCs, researchers now can generate unlimited human disease cells from the patient's iPSCs for drug screening and toxicity testing as a new paradigm for drug development. For example, a study has shown that neural crest precursors generated from iPSCs derived from patients with familial dysautonomia, a fatal genetic disorder, were used to screen thousands of small-molecule compounds, and one of the identified hits was able to effectively rescue the expression of a key molecule associated with the disease [62]. Given that the average cost for the development of a new drug exceeds $2.5 billion dollars, the new paradigm of iPSCs for drug screening holds promise for pharmaceutical companies to reduce the cost and time needed for the process. Furthermore, with the idea of precision medicine recently emerging, using iPSCs to identify patient-specific drugs may revolutionise how diseases are treated.

The idea of iPSCs for disease modelling is also appealing because it opens up the possibility of "diseases in a dish" as a new avenue to study pathogenesis of diseases. Given that cellular reprogramming affects only the epigenetics of a cell through DNA methylation and histone modification without altering the genomic information, disease-specific DNA mutations can be retained in the patient's iPSCs; therefore, when the patient's iPSCs are induced to differentiate into disease-relevant cell types, the cell can recapitulate, at least in part, cellular and molecular changes caused by diseases. This unique feature makes iPSCs suitable for disease modelling, particularly for monogenic diseases, such as Huntington's disease and Timothy syndrome. Huntington's disease is an autosomal dominant neurodegenerative disease that is caused by a mutation in Exon 1 of the Huntington gene, which results in neuronal dysfunction and death. Researchers of the Huntington's Disease iPSC Consortium have demonstrated that differentiated neural cells derived from Huntington's disease iPSCs showed disease-associated changes in electrophysiology, metabolism, cell adhesion and cell death compared with control cells [63]. Several other groups at around the same time reported their independent findings that all together demonstrate the feasibility of iPSC-based Huntington's disease modelling [64,65]. Before the discovery of iPSCs, the progress in the study of Huntington's disease was limited by difficulties in obtaining a patient's neural tissues and creating appropriate disease models. Using iPSCs for disease modelling may one day become a standard procedure to determine pathophysiological mechanisms and identify cures, as commonly used transgenic mice in biomedical research.

Compared with the number of reported studies focussing on neurological and cardiac disease modelling and drug screening, there have been fewer studies carried out to explore the potential of iPSCs for modelling of orthopaedic disorders to date. Nevertheless, with increased awareness of the iPSC potential for disease modelling and maturation of the cellular reprogramming technology, it is anticipated that more research effort on iPSC-based modelling of orthopaedic diseases will take place. Among previously published studies, several groups have focused on modelling of fibrodysplasia ossificans progressiva (FOP) using iPSCs from patients with the disease. FOP is an inherited disorder that is caused by mutations in the *activin A receptor type 1* (*ACVR1*) gene, specifically the base encoding the amino acid R206H. The mutation in the gene alters the sensitivity of ACVR1 to BMPs in cells of muscle, tendon, ligament, bone and cartilage, resulting in constitutive activation of the receptor and downstream signalling pathways, in turn forming bone outside the skeleton and causing overgrowth of bone and cartilage. In the result reported by Matsumoto et al. [66], iPSCs derived from patients with FOP showed an increase in chondrogenesis and mineralisation in culture compared with control cells, and the increased mineralisation resulted from abnormal regulation of ACVR1 in FOP-iPSCs was attenuated by an inhibitor of BMP signalling. The findings of this study have demonstrated the feasibility of patient-specific iPSCs for modelling of a monogenic disease. Similarly, other studies have shown that iPSCs derived from patients with Marfan syndrome (MFS) or skeletal dysplasia, such as thanatophoric dysplasia (TD) and achondroplasia (ACH), were generated to model pathophysiological characteristics of disease cells during skeletal generation in culture. MFS is a genetic disorder caused by mutations in the gene encoding for FIBRILLIN1, an ECM protein, which leads to an increased production of TGF-beta (TGFB) and upregulation of TGFB signalling. Using fibroblasts harvested from a patient with MFS, Quarto et al. [67] generated MFS-iPSCs and characterised the cell and control human MFS-ESCs. They found that MFS-iPSCs were able to faithfully phenocopy the skeletogenic phenotype exhibited in osteogenic induction culture of MFS-ESCs to model the disease in a dish. Their findings suggest that abundant human MFS-ESC—like cells can now be obtained through cellular reprogramming without limitations for gaining valuable information to improve our understanding of the disease that we have known little about. Recently, Yamashita et al. [68] have performed an elegant study, in which they not only demonstrated the success of patient-specific iPSCs for modelling of human TD1 and ACH, two subtypes of skeletal dysplasia, but also identified statin as an effective therapeutic molecule for treatment using the iPSC disease models they created.

Other than modelling of skeletal disorders, patient-specific iPSCs have been created to model cartilage diseases, particularly those caused by monogenetic mutations. Familial osteochondritis dissecans is a genetic skeletal disease with cartilage lesion that is predominately caused by a heterozygous mutation in the aggrecan gene. In a study by Xu et al. [69], iPSCs reprogrammed from fibroblasts of a patient with familial osteochondritis dissecans were induced for chondrogenesis to generate chondrocytes with changed phenotypes and associated dysregulated matrix production, which established an *in vitro* model of the disease for studying the pathogenesis and exploring effective treatments. In addition, chondrodysplasia is a collection of cartilage diseases with different forms, and one of them resulting from a mutation of the FGF receptor

3 (FGFR3) gene affects growth plate cartilage, in turn causing abnormal skeletal development. Currently, there is no effective treatment for the disease. Using an *in vivo* cartilage model created from iPSCs of patients with chondrodysplasia, Kimura et al. [70] have demonstrated that by targeting FGFR3 with an inhibitor, the pathology of hypertrophic chondrocytes was mitigated, which provides insight into the future development of potential drugs for the disease. Other than genetic cartilage abnormalities, the approach of iPSCs for disease modelling has also been implemented to study most common cartilage disorders, OA and rheumatoid arthritis (RA) [55]. In these studies, human synoviocyte- or chondrocyte-derived iPSCs were generated from patients with OA or RA and evaluated for their chondrogenic capacity. Their findings have demonstrated that the models recapitulated key changes in chondrocyte phenotypes and matrix production found in OA or RA cartilage, providing an alternative way to access the patient's cells for research.

In addition to bone and cartilage diseases, researchers have also applied the "disease in a dish" approach to model genetic skeletal muscle disorders in iPSC culture, such as Miyoshi myopathy (MM) and Duchenne muscular dystrophy (DMD). MM is caused by a mutation in the gene *DYSFERLIN*. In a study investigating myogenic differentiation of iPSCs from a patient with MM, Tanaka et al. [71] found that myotubes differentiated from the iPSC exhibited pathological phenotypes and that by forcing the expression of full-length *DYSFERLIN* in the cell, the phenotype was rescued. Another genetic muscle disease, DMD, results from absence of the protein dystrophin, which causes progressive muscle degeneration and weakness. Several groups have differentiated iPSCs derived from patients with DMD into muscle cells and characterised phenotypic changes in myogenic cells to determine pathogenic mechanisms and identify potential treatments. For example, Choi et al. [72] have demonstrated that the disease is associated with abnormal expression of inflammation or immune-response genes and collagens and upregulated BMP/TGFB signalling, and Abujarour et al. [73] have shown that by studying myogenic differentiation of iPSCs from a patient with DMD, insulin-like growth factor 1 and Wnt family member 7A (WNT7A) have been identified as molecules of potential treatments.

Conclusions

It has been more than a decade since iPSCs were first created. During that time, significant progress in the generation, characterisation and control differentiation of iPSCs has been made to establish a collection of information that not only helps better understand the fundamental biology of the cell but also paves the way for using the cell in clinical applications. However, a few challenges remain. The most salient challenge is how to direct lineage-specific differentiation of iPSCs in a controlled manner to generate homogeneous target cells. Effective derivation of homogenous tissue-specific cells from iPSCs is a fundamental requirement that has to be met to realise the hope in tissue regeneration, drug screening and disease modelling. Another pressing challenge is to ensure it is completely safe to introduce iPSCs or iPSC-derivatives in the body. With more research effort involved, these challenges will be addressed in the near future. In orthopaedics, interesting findings of iPSC studies have just begun to emerge, suggesting there are enormous opportunities for us to explore.

Conflicts of Interest

The authors have no conflicts of interest relevant to this article.

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