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Mini Review

iPS cell technologies and their prospect for bone regeneration and disease modeling: A mini review

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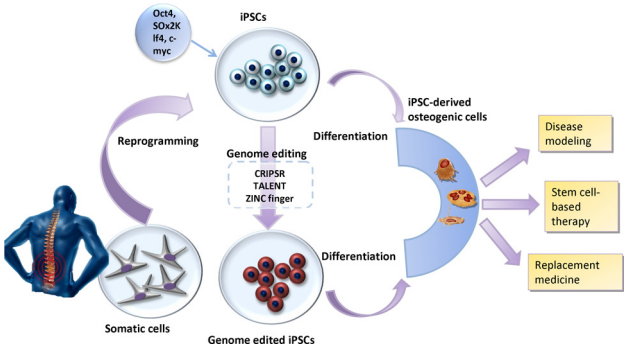
GRAPHICAL ABSTRACT



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ABSTRACT

Bone disorders are a group of varied acute and chronic traumatic, degenerative, malignant or congenital conditions affecting the musculoskeletal system. They are prevalent in society and, with an ageing population, the incidence and impact on the population's health is growing. Severe persisting pain and limited mobility are the major symptoms of the disorder that impair the quality of life in affected patients. Current therapies only partially treat the disorders, offering management of symptoms, or temporary replacement with inert materials. However, during the last few years, the options for the treatment of bone disorders have greatly expanded, thanks to the advent of regenerative medicine. Skeletal cell-based regeneration medicine offers promising reparative therapies for patients. Mesenchymal stem (stromal) cells from different tissues have been gradually translated into clinical practice; however, there are a number of limitations. The introduction of reprogramming methods and the subsequent production of induced pluripotent stem cells provides a possibility to create human-specific models of bone disorders. Furthermore, human-induced pluripotent stem cell-based autologous transplantation is considered to be future breakthrough in the field of regenerative medicine. The main goal of the present paper is to review recent applications of induced pluripotent stem cells in bone disease modeling and to discuss possible future therapy options. The present article contributes to the dissemination of scientific and pre-clinical results between physicians, mainly orthopedist and thus supports the translation to clinical practice.

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Introduction

Currently, stem cell-based therapies and research represent a significant advance in bone regeneration. Recent therapeutic options for bone disorders have included restricted or modified activity, immobilization of injured or diseased structures using splints and casts, non-steroidal anti-inflammatory drugs, corticosteroid administration, physical therapy, acupuncture, extracorporeal shock wave therapy, and surgical manipulation. However, attention is increasingly turning to the application of stem or progenitor cells as the basis for bone tissue regeneration. Several recently published animal studies show promising results for bone, tendon and cartilage regeneration. Bone marrow-derived mesenchymal stem cells (MSCs) were the first stem cell type investigated and remain the gold standard for many researchers [1]. However, MSCs must be isolated from various donors and are usually quite heterogeneous. Furthermore, therapy for skeletal disorders has various limitations, such as the age of pathologically related impairments regarding cell survival, proliferation activity and the potential of multilineage differentiation [2].

A major scientific breakthrough in biomedical research is related to the formation of induced pluripotent stem cells (iPSCs) by Takahashi and Yamanaka in 2006 [3]. By transferring a mixture of nuclear transcriptional factors (Oct4, Sox2, Klf4, and c-myc), terminally differentiated adult cells were successfully reprogrammed into iPSCs and closely resembled human embryonic stem cells [4,5]. So far, different human somatic cells have been reprogrammed into iPSCs. As the field grows, improved combinations of scaffolding biomaterials and bioreactors are creating a more suitable stem cell microenvironment for new tissue formation. Nevertheless, safety remains an important issue, especially with the potential of tumour formation [6].

The main purpose of the present review was to summarise the current state of IPSC technology and to discuss its prospects for regeneration and modeling bone disorders.

Methods for iPSC generation

The most used method for establishing iPSC lines had been insertion of a mixture of reprogramming factors (Sox2, Oct4, c-myc, Klf4 and Lin28) into the genome of somatic cells by using delivery vectors [7]. Substantial advances have been made in searching for new strategies to increase the effectiveness of reprogramming techniques, as well as new approaches for improving biosafety by reducing the number of genomic modifications required to complete the process [8]. Recently, methods used to transfer genes into target cells can be divided into: (a) integrative viral vectors (viral delivery system, transfection of linear DNA), (b) integrative free vectors (piggyBac transposon, plasmid/episomal plasmid vectors, minicircle vectors), and (c) non-integrating methods (direct protein/microRNA delivery, small molecules) (Fig. 1, Table 1) [4,5].

Integration methods apply viral vectors (e.g. retroviral and lentiviral) to transfer selected genes into the host genome. Their advantage is the undeniably high efficiency; however, these methods possess considerable risk of tumour formation. Because of this, different approaches have been also employed [9].

The most promising reprogramming approaches seem to be non-integrating techniques. For instance, the method of protein transduction can replace the use of transcription factors. The conjugation of proteins with short peptides responsible for cell penetration can be used for protein delivery into the cells. The majority of murine and human iPSCs were produced according to this method using purified polyarginine-tagged Oct4, Sox2, Klf4, and c-myc [10]. MicroRNAs (miRNAs) and small molecules have been also examined for their potential to enhance the reprogramming efficiency or replace reprogramming genes. miRNAs are an essential component of the gene network and are regulated by genes of pluripotency. Therefore, the expression of pluripotent stem cell-specific miRNAs, reprogramming gene-related miRNAs and the inhibition of tissue-specific miRNAs may support cell reprogramming in iPSCs [11].

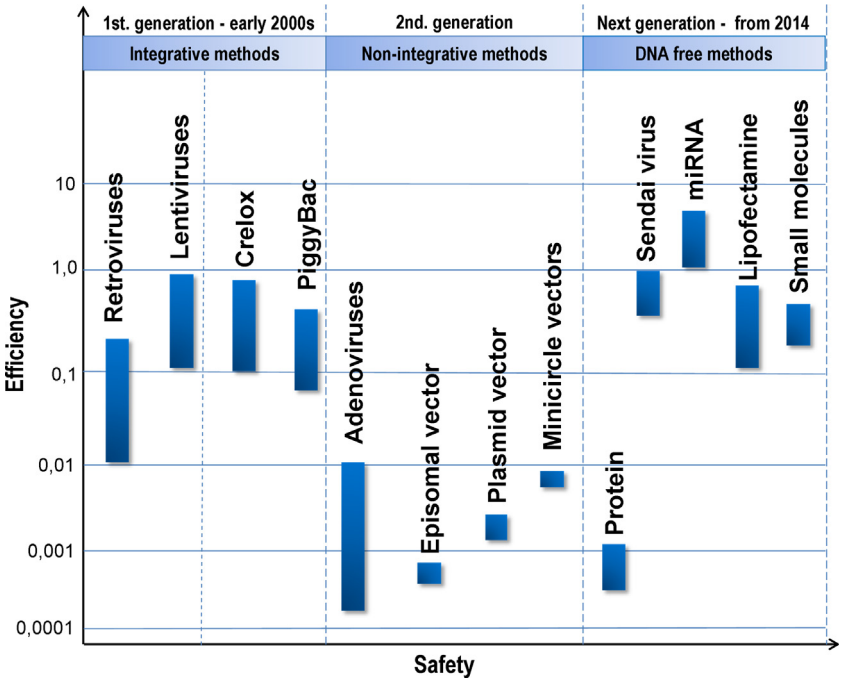

Fig. 1. Methods involved in the transfer of genes into the target cells.

Table 1
The overview of the reprogramming methods.

Method	Transgene expression	Advantages	Disadvantages	References
Retrovirus/ Lentivirus	Yes	Relatively easy to use; medium to high efficacy (0.1%)	Integration of foreign DNA into genome; residual expression of reprogramming factors; increased tumour formation	[3]
Adenovirus	No	Non-integrative; infects dividing and non-dividing cells	Low efficiency (0.0001%)	[52,53]
Episomal plasmid vector	No	Non-integrative; simple to implement to laboratory set-up; less time consuming	Very low efficiency ($3–6 \times 10^{-6}$); the use of potent viral oncoprotein SV40LT antigen	[54,55]
Minicircle plasmid vector	No	More persistent transgene expression; lack bacterial origin	Very efficiency (0.005%)	[56]
PiggyBac transposons	Excision of transgene by transposase	Elimination of insertional mutagenesis; no footprint upon excision; higher genome integration efficiency	Excision may be inefficient, potential for genomic toxicity	[57]
Sendai virus	No	Medium to high efficiency; Non-integrating; robust protein-expressing property; wide host range	Involve viral transduction	[58,59]
Protein	No	Free of gene materials; direct delivery of reprogramming factor proteins	Extremely slow kinetics, low efficiency (0.001%); difficulties in generation and purification of reprogramming protein	[60]
miRNA	No	Higher efficiency (1,4–2%)	Requires high gene dosages of reprogramming factors and multiple transfection	[61]
Small molecules	No	Easy of handling; no need for reprogramming factors	2×10^{-3}; more than one target, toxicity	[62]

Transfection of mature miRNA from the miR-200c, miR302s, and miR369s families or infection with a lentiviral construct over-expressing miR-302/367 clusters were reported to reprogram mouse and human adipose stromal cells or fibroblasts, respectively, into iPSCs [12]. However, for the therapeutic applications of iPSCs, the genome of reprogrammed cells cannot contain any genomic insertion of transgene sequences. Small molecule compounds (inhibitors of histone deacetylases; histone demethylases; DNA methyltransferases, etc.) could be a potential alternative to resolve this problem because of their ability to target various cellular pathways that control cell features. An inhibitor of transforming growth factor beta (TGF-β) can replace Sox2 and induce Nanog expression, while a mixture of different small molecules can replace both Sox2 and c-myc. Moreover, several Oct4-activated molecules have been studied in this context [13]. Their biological actions are rapid, reversible, and dose-dependent, allowing strict control over specific outcomes by affecting their concentrations and combinations. A recent report showed that iPSCs could be generated from mouse somatic cells using a cocktail of seven small molecule compounds [14]. All this evidence suggests that chemical reprogramming approaches have a potential use in generating functionally suitable cells for safe applications in human medicine.

Bone disorders treatments

Bone is a highly specialized tissue that undergoes constant renewal through coordinated destruction and concomitant reconstruction mediated by osteoclasts and osteoblasts. Recently, the damage or loss of bone tissue and dysosteogenesis still represents a serious problem in orthopaedics [15]. A similar situation occurs in dental medicine because of the increased need for dental implants and for massive bone substitution in the atrophic alveolar ridge and the maxillary sinus [16].

Recently, the gold standard to enhance bone regeneration, is bone grafting. However, bone grafts available from bone tissue are very limited, and harvesting can be often associated with donor morbidity and several complications, such as pain, infection, fractures, and host immune reactions [15,17]. The alternative method is cell replacement therapy. Stem cells with osteogenic potential are important in the bone tissue engineering approach, thus the key to make a success is to obtain the ideal cell source [18]. The present approach involves the use of autologous cells to replace damaged tissue. Recent stem cell research has provided new possibilities for bone regeneration.

iPSCs represent a novel cell type exhibiting advantages of MSCs and ESCs. Recent discoveries have demonstrated the ability of iPSCs to differentiate into osteoblasts or osteoclasts, suggesting that iPSCs could have a substantial role to enhance the bone regeneration (Fig. 2). Moreover, they should be considered as patient-specific, thus overcoming ethical and immunological issues. They are mainly produced by using different genetic manipulations from various somatic cells. Recently, more attractive cell types over iPSCs are iPSC-derived mesenchymal stem cells (iPSCs-MSCs). The possibility to use iPSCs/iPSCs-MSCs for autologous cell replacement in impaired bone tissue makes them a promising candidate for cell-based therapies of bone defects and injuries [19–21]. Recently, de Peppo et al. [18] demonstrated the successful generation of mature, phenotypically stable bone substitutes engineered from human iPSCs.

Osteogenic differentiation of iPSCs

MSCs have been gradually translated into the regeneration of bones. They possess the potential for intense regeneration and plasticity. However, there are still some important issues related to their applications, such as limited availability of autologous MSCs, low proliferation rate that rapidly decreases with donor age, immunogenic concerns, an invasive harvesting procedure in case of bone marrow MSCs, etc. Thus, iPSCs may represent a new and more suitable alternative to MSCs (Table 2).

Recently, it was shown that iPSCs can be differentiated into osteoblasts and are therefore expected to be useful for bone regeneration. According to several studies, the differentiation protocols for osteogenic lineages developed for ESCs are similar to those for iPSCs [22–24]. Foetal bovine serum, ascorbic acid, b-glycerophosphate, and dexamethasone are basic components, which are commonly used in osteogenic medium formulas [25]. Bone morphogenetic proteins (BMPs) and calcium regulating hormone vitamin D3 are used to enhance osteogenic differentiation [23]. Other enhancers of osteogenic differentiation contain members of the TGF-β family [26]. Bilousova et al. [27] used retinoic acid to differentiate murine iPSCs into cells to form calcified structures, both *in vitro* and *in vivo*. Tashiro et al. [28] reported an enhanced osteogenic differentiation of mouse iPSCs by exogenous overexpression of the key osteogenic transcription factor *Runx2*.

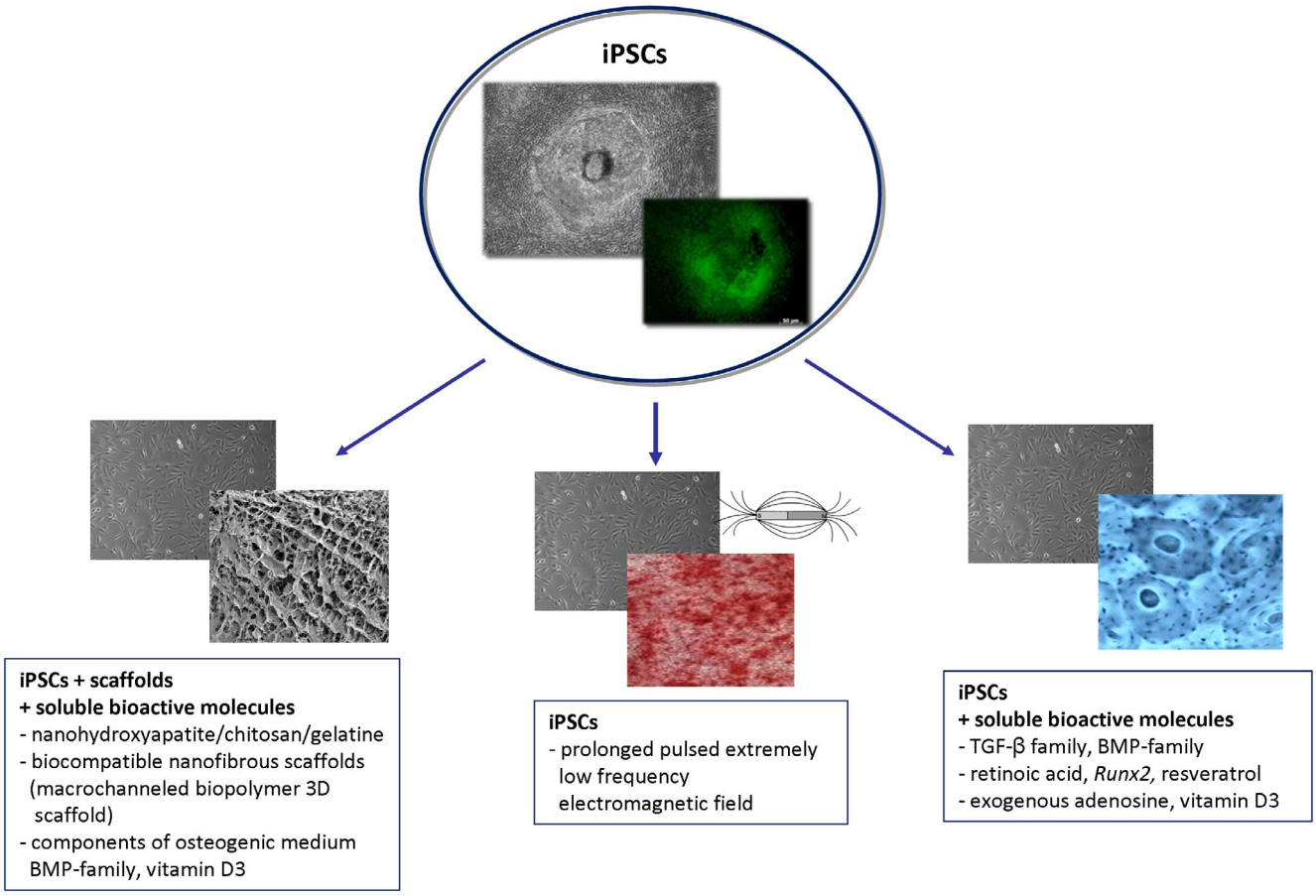

Fig. 2. Overview of iPSCs-based therapeutic approaches for the treatment of bone disease.

Runx2-transduced iPSCs displayed more than 50% higher alkaline phosphatase activity in comparison with non-transduced cells. Another approach based on intercellular interactions and the secretion of different soluble bioactive molecules is the differentiation in co-cultures with primary bone cells [29,30].

On the basis of differentiation protocols for ESCs, the production of bone matrix-forming osteoblasts has been reported from mouse to human iPSCs [31]. The first study describing the osteogenic potential of iPSCs was published by Kao et al. [32]. Researchers obtained osteocyte-like cells after culturing iPSCs in osteogenic medium and found that resveratrol, a natural polyphenol antioxidant, has a supporting effect on the osteogenic differentiation of iPSCs. Apoptosis induced by dexamethasone in osteocyte-like cells was effectively suppressed by pre-treatment with resveratrol. Recently, Ji et al. [33] investigated the osteogenic differentiation of human iPSCs from gingival fibroblasts regulated by nanohydrox yapatite/chitosan/gelatine 3D scaffolds with nanohydroxyapatite (nHA) in different ratios. Osteogenic differentiation was notably increased when composite HCG-311 (3 wt/vol% nHA) scaffolds were used both *in vivo* and *in vitro*. This finding suggests the significant role of different nHA ratios in the osteogenic differentiation of human iPSCs.

Kang et al. [34] reprogrammed iPSCs to functional osteoblasts by simply using only the small molecule exogenous adenosine. The iPSCs treated with adenosine expressed the molecular signature of osteoblasts. Subsequently, the osteoblasts were used to repair large cranial defects through the formation of new bone tissue. This approach offers a simple and cost-effective strategy to differentiate iPSCs into cells of osteogenic lineages.

The adjustment of protocols from tissue culture plastic dishes to 2D or 3D scaffolds also plays an important role in the differentiation of iPSCs to osteogenic cells. The extracellular matrix (ECM) affects the structure and biological properties of cells. It has been shown that the best biocompatible nanofibrous scaffolds should mimic the function of native ECM. Many scaffolds have incorporated nanostructures into their formulations in order to enhance mechanical properties. Xin et al. [35] reported that nanoscale interactions with the ECM components of bone tissue can influence the behaviour of stem cells. Jin et al. [36] cultured iPSCs in a macrochanneled poly (caprolactone) biopolymer 3D scaffold under osteogenic conditions. Subsequently, the scaffolds colonized by iPSCs were transplanted into the subcutaneous site of arrhythmic mice. These findings indicated the formation of distinct levels of ECM and their mineral deposition within the structure of scaffolds. Several studies published a positive effect of scaffolds with phosphate minerals on osteogenic differentiation of MSCs and on osteogenic differentiation *in vivo* [37,38]. With respect to this knowledge, Kang et al. [39] used biomaterials containing calcium phosphate minerals to promote the osteogenic differentiation of iPSCs. The iPSCs were cultured in both 2D and 3D cultures using mineralized gelatin methacrylate-based scaffolds without any osteoinductive factors. After 28 days of cultivation, the majority of cells expressed an osteocalcin, suggesting effective osteogenic differentiation of iPSCs in a mineralized environment.

Significant progress in the osteogenesis of iPSCs was made by Levi et al. [40], who studied the influence of a skeletal defect environment combined with an osteogenic scaffold micro-niche on survival and osteogenesis of implanted iPSCs. Scaffolds contained

Table 2
The comparison of MSCs and iPSCs characteristics.

Advantages	Disadvantages	References
MSCs		
Very little ethical issue	Limited availability of autologous MSCs	[63]
Resistant to malignant transformation	Several complications related with autologous MSCs harvesting (invasive method)	[64]
Successful differentiation into osteogenic lineages (multilineage potential)	Impaired self-renewal ability	[65]
Potent paracrine and anti-inflammatory properties	Age-related decreasing of proliferative potential	[64,66]
Effective in orthopedic application (preclinical and clinical studies)	Allogenic MSCs present a risk of host immune reactions	[67]
Anti-apoptotic properties	Donor-dependent ability of expansion and differentiation	[68]
	Need for differentiation protocols optimization	[64]
iPSCs		
No ethical and immunological issues	Necessary induction into high-quality progenitor cells after transplantation	[53,69]
Differentiation into 3 germ layers – pluripotency (similar to ESCs)	Risk of spontaneous teratoma formation	[4,9]
Generation from any cell source	Need for reprogramming protocols optimization	[24]
Patient-specificity (sufficient for *in vitro* bone repair)		[29,70]
Osteodegenerative disease modeling (*in vitro* disease recapitulation)		[15,42]
Unlimited self-renewal capacity		[4,5]
Effective autologous cell replacement in impaired bone tissue		[28,31]
Osteogenic capability equal or higher than MSCs		[66,70]
iPSC-MSCs have much higher capacity of cell proliferation than bone marrow-derived MSCs		[67]

hydroxyapatite, poly-L-lactic acid and BMP-2. A high survival rate and differentiated osteogenic cells were detected. Moreover, integrated cells displayed very low teratoma formation. These results suggest the direct effect of the surrounding environment on implanted iPSCs followed cell engraftment and bone formation.

Ardeshirylajimi and Soleimani [41] investigated the effect of prolonged pulses in an extremely low frequency electromagnetic field on iPSCs under *in vitro* conditions. Results showed increased proliferation activity and osteogenic differentiation, which was proved by presence of calcium mineral deposition, expression of alkaline phosphatase and different bone-related genes. Authors suggested that the combination of osteogenic medium and electromagnetic field can be another promising approach suitable for promoting osteogenic differentiation in stem cells.

iPSC-based therapy and modeling of skeletal diseases

There is a high demand for bone tissue in regenerative therapy. Osteodegenerative diseases, such as osteoporosis and osteoarthritis, still represent significant public health problems affecting a broad spectrum of the elderly population. A number of different factors may promote bone disorders related to loss of bone mass and decreased bone density. The primary drugs in clinical practice are anti-osteoporosis agents that inhibit bone resorption, such as bisphosphonates. However, these drugs are associated with numerous adverse effects. Thus, iPSCs-based therapy represents a promising new approach for bone repair and regeneration [42].

Another important advantage of iPSCs is the possibility to create particular models of diseases affecting bones. Many genetic bone disorders have limited treatment possibilities due to the absence of appropriate animal models and inaccessibility of native bones. IPSCs-derived disease models from patients with genetic mutations enable us to understand the origins and pathologies of diseases. iPSCs have been used to model infrequent genetically influenced disorders (e.g. Fibrodysplasia ossificans progressiva (FOP) and metatropic dysplasia) [43,44].

FOP, an inherited disease which is manifested by progressive ossification of soft tissue (muscles, ligaments and tendons), is caused by gene mutations in the Activine A Receptor type 1 (ACVR1), which is part of bone morphogenic protein (BMP) signalling. Matsumoto et al. [45,46] developed a "disease in a dish" model of FOP to investigate differences in individual steps of endochondral bone ossification between FOP iPSCs and control iPSCs. Results showed increased mineralization and chondrogenesis of FOP iPSCs *in vitro*. Researchers found that mineralization can be suppressed by a small molecule inhibitor of BMP signalling. In a subsequent study using a model of FOP derived from iPSCs-MSCs, the authors demonstrated that MMP1 and PAI1 have a pivotal effect on enhancing the chondrogenic features of FOP cells [47]. Recently, Barruet et al. [48] used an FOP model derived from human iPSCs to investigate if mutation of ACVR1 R206H elevates the production of osteoprogenitors in the endothelial cell lineage. Results showed that endothelial cells expressing the ACVR1 receptor produced elevated levels of collagen proteins, which can contribute to the formation of fibrotic tissue.

Chen's group [21] has been studying craniometaphyseal dysplasia (CMD), an uncommon genetic bone disorder, characterized by progressive thickening of bones in the craniofacial region and a widening of metaphysis in long bones. Mutation for the autosomal dominant CMD is in the ankylosis gene and in Connexin 43 for the recessive form [49]. Researchers used patient specific iPSCs to identify osteoclast defects and found out altered osteoclasts in a laboratory mouse model with a Phe377del mutation. Additionally, they established a simple and efficient method to produce human iPSCs from the peripheral blood of donors suffering from CMD.

Quarto et al. [50] prepared human iPSCs from patients with Marfan syndrome (MF) to study the pathology of skeletogenesis *in vitro*. Another research group reported molecular and phenotypic profiles of skeletogenesis from iPSCs-differentiated tissues carrying a heritable mutation in FBN1. Human MF – iPSCs represent impaired osteogenic differentiation as a consequence of an alteration in TGF-β signalling [51].

Conclusion and future perspectives

Bone tissue exhibits regenerative capacity, however, ageing, disease or injury frequently result in progressive bone loss, which prevent the natural replacement of bone tissue. The accessibility and therapeutic effect of patient-specific iPSCs provides a unique approach for regenerative medicine, including bone reconstruction and orthopaedics. Still, engineered grafts derived from iPSCs are far from being standard in human medicine. In the near future, the major issue to be resolved is the safety of the method because of tumorgenesis and teratoma formation associated with the

incorporation of vectors into the host genome. Other challenges include time-consuming methods of osteogenic differentiation, poor reproducibility, low efficiency and low survival of transplanted cells. Strategies to increase cell survival in iPSC-patient specific cells after transplantation could include local immune modulation to decrease the inflammation and thereby reduce apoptosis. Different soluble bioactive factors, extracellular matrix components, and cells can also effectively influence the survival and optimal functions of transfected cells.

If treating a disease involves correcting a genetic mutation, then gene-editing technologies (CRISPR/Cas9, ZFN, TALENs, etc.) can be used as an additional step before differentiating the iPSCs into the desired cell type.

The present application of iPSCs involves laboratory scale production and testing assays. Despite the significant scientific and therapeutic prospects of iPSCs, further research focusing on an optimization of reprogramming methods will be essential to accelerate the process of iPSC translation into human medicine.

Conflict of interest

The authors declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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