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EDITORIAL

Induced pluripotent stem cells in cartilage repair

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Abstract

Articular cartilage repair techniques are challenging. Human embryonic stem cells and induced pluripotent stem cells (iPSCs) theoretically provide an unlimited number of specialized cells which could be used in articular cartilage repair. However thus far chondrocytes from iPSCs have been created primarily by viral transfection and with the use of cocultured feeder cells. In addition chondrocytes derived from iPSCs have usually been formed in condensed cell bodies (resembling embryoid bodies) that then require dissolution with consequent substantial loss of cell viability and phenotype. All of these current techniques used to derive chondrocytes from iPSCs are problematic but solutions to these problems are on the horizon. These solutions will make iPSCs a viable alternative for articular cartilage repair in the near future.

Key words: Induced pluripotent stem cells; Articular cartilage; Cartilage repair; Stem cells

Core tip: Herein we review the challenges in articular cartilage repair. Further we explain that induced pluripotent stem cells (iPSCs) represent an exciting theoretically limitless source of autologous cells for articular cartilage repair. We also discuss a novel systematic approach to optimally derive articular chondrocytes from iPSCs.

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INTRODUCTION

Nearly 1 in 2 people develop symptomatic knee osteoarthritis (OA) by age 85 years, two in three people who are obese develop symptomatic knee OA in their lifetime[1], and 1 in 4 people develop painful hip arthritis in their lifetime[2]. Over 30 million Americans suffer from arthritis and other rheumatic conditions that affect joint and connective tissue; and by 2030 nearly 25% of the American

population is expected to be affected by such conditions[3]. Joint Replacement.

Perhaps as a result or as a testament to the inability of articular cartilage to heal, knee replacement is now the most common elective surgery in the United States (Figure 1). Knee replacement though is not appropriate for young patients as it only lasts for an average of 15 years[4] so alternative cellular treatments for osteoarthritis have been sought.

Articular cartilage is made up of cells (5%) with extracellular matrix and water (95%)[5]. Articular chondrocytes express high levels of COL2A1, SOX9 and AGGRECAN[6]. Endogenous attempts at cartilage repair are ineffective in composition (primarily creating fibrocartilage with type Ⅰ rather than type Ⅱ collagen) and the reparative tissue does not provide durable healing to the adjacent normal cartilage Figure 1[6]. During embryonic cartilage formation, mesenchymal condensation is the prerequisite for the induction of chondrogenesis. Initiation of limb development starts with the lateral plate mesodermal cells, which proliferate, aggregate and form mesenchymal condensations[7]. These primordial cells differentiate into chondrocytes and form cartilage anlagen[7-10].

One major limitation when studying primary chondrocytes in culture is their loss of phenotype[11]. Research in cell-based cartilage tissue engineering has focused on identifying a cell source suitable for regenerating cartilage. Mesenchymal stem cells (MSCs) would seem to be well suited for tissue engineering and are multipotent cells able to differentiate into chondrocytes, osteoblasts, adipocytes and myocytes[12-15]. However, even though MSCs can be easily obtained from bone marrow, fat and skin, these primary cells have limited proliferation capacity when cultured *in vitro* and relatively low numbers of MSCs are capable of chondrocyte differentiation[16-21]. Autologous chondrocytes and MSCs have still been used in regeneration of articular cartilage[22-24]. However there are limitations in terms of the ability of adult differentiated chondrocytes to heal a cartilage defect, the numbers of cells that can be obtained using these autologous cells due to their obscurity, and due to the limited maintenance of their phenotype with cell division[16]. The only exception to the inability of a cartilage defect to heal effectively and seamlessly appears to be in a fetal lamb model in which partial thickness articular cartilage defects did heal to subsequently normal appearing cartilage[25].

As a result our group and others have become interested in the use of induced pluripotent stem cells (iPSCs) that can be derived from a patient skin biopsy, transformed into iPSCs and then into articular chondrocytes with theoretically large numbers of cells without the concerns of disease transmission from allogeneic cell transfer. In this review we will discuss the current status and recent progress in the development of articular chondrocytes from iPSCs.

DEVELOPMENT OF IPSCS

Many attempts have been made in the last decade to obtain various MSCs, derived from iPSCs, in ample quantity and high purity after differentiation *in vitro*[26-33]; and the International Society for Cellular Therapy has defined three primary criteria for cells to meet the definition of MSCs. First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14, CD11b, CD79alpha or CD19 and HLA-DR surface molecules. Third, MSCs must be able to differentiate into osteoblasts, adipocytes and chondrogenic cells *in vitro*[34]. In the past, undifferentiated iPSCs have contaminated the differentiated population of MSCs, and they can contribute to teratoma tumor formation; and a uniformly differentiated cell population is necessary for clinical use[35]. iPSCs were developed by Yamanaka by taking differentiated cells and reprogramming them to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with cells. Specifically he demonstrated induction of pluripotent stem cells from mouse adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions[36,37]. These cells, which his group designated iPSCs, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of these iPSCs into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Their work demonstrated that pluripotent stem cells could be directly generated from fibroblast cultures by the addition of only a few defined factors[38].

The fibroblasts used to derive iPSCs can be obtained from a skin punch biopsy done in clinic at the time of patient presentation. iPSCs have the potential to self-renew and differentiate into many adult cell types[39] and represent a theoretically nearly unlimited supply of cells for studying normal cell function and modeling of disease[16,17,27,31,40]. More recent publications have proven the beneficial effect of cells derived from stem cells[41,42]. Stem cell derived cardiomyocytes improve myocardial performance in animal models[42]; and stem cells derived from neuroprogenitor cells lead to regeneration of functional neurons in *in vivo* models[4,43]. Stem cells derived from retinal epithelial cells improve vision in rodents and humans[33,44]. iPSCs, also potentially provide cell sources for the development of regenerative therapy in articular cartilage repair[45-48]. The chondrogenic cells derived from iPSCs are similar to the fetal lamb chondrocytes, (effectively able to repair cartilage) based on their rapid proliferation and ability to make healthy appearing tissue[38,47-51]. iPSCs can also be manipulated to correct genetic defects, a very important consideration for genetically inherited diseases, including RA. Genetic manipulations could indeed allow *de novo* produced articular cells to be resistant to inflammatory stimuli and to produce tissues insensitive to degrading enzymes. Based on these considerations and evidence that human iPSCs can be directed to undergo differentiation into various cell types, iPSCs are currently the best option to develop strategies for tissue repair in articular cartilage.

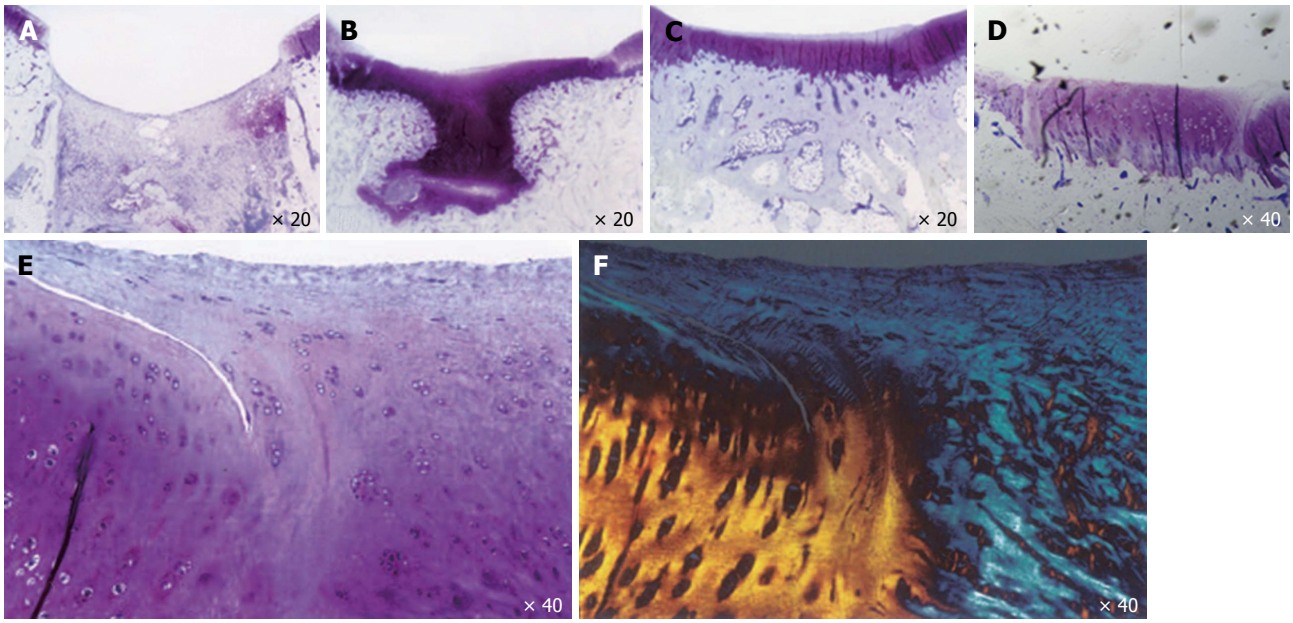

Figure 1 Articular cartilage healing in a microfracture model in adult rabbits. Articular cartilage healing at day 7 (A), 21 (B), 42 (C), and day 84 (D-F). E and F: Lack of healing of reparative cartilage to "normal cartilage" is shown by toluidine blue and polarized light micrographs at day 84.

DEVELOPMENT OF ARTICULAR CHONDROCYTES FROM IPSCS

iPSCs can be derived from a small skin biopsy done with minimal intervention before orthopaedic surgery and can be amplified into virtually limitless amounts of homogeneous cell populations. iPSCs could thus be better than other cell sources to create highly reproducible orthopaedic biologic implants such as for articular cartilage (requiring large amounts of cells). Interestingly, iPSCs apparently produce differentiated cells that exhibit young rather than adult properties, including faster proliferation and creation of healthier, longer-lasting reparative tissues such as the cartilage repair observed in the fetal lamb[25,36,47-50,52-55].

Recent reports have demonstrated the ability to induce differentiation of iPSCs into different lineages (similar to embryogenesis) by using small molecules, cytokines and overexpression of transgenes[40,45,56-62]. There are several existing protocols for generating mesenchymal progenitors or MSCs from ESCs and iPSCs that utilize embryoid bodies and/or co-culture with primary cells[26,29,30,40,46]. These protocols are important steps in developing the use of iPSCs for articular cartilage repair but they have limitations in terms of using either an embryoid body stage or feeder cells which lead to cell heterogeneity or the sue of serum which decreases reproducibility.

Two large groups have had a specific interest in chondrogenic differentiation from iPSCs. Tim Hardingham's group has developed techniques using a number of growth factors to differentiate iPSCs to impressive chondrogenic cells with feeder cells and use fibrin as a control group which we believe actually inhibits *in vivo* cartilage repair[63,64]. Craft *et al*[65] developed a protocol with an embryoid body stage with healing in an *in vivo* model with impressive cartilage formation without an adequate control group. Recently, a third group made chondrogenic cells without the use of feeder cells and do not use an embryoid body stage but at the end of their protocol it is not clear why the cells are in suspension, moreover their toluidine blue staining is not similar to that of the adjacent articular cartilage indicating a difference in the sulfated glycosaminoglycans[30,51,66-69].

CURRENT CHALLENGES IN THE USE OF IPSCS IN ARTICULAR CARTILAGE REPAIR

Chondrogenic differentiation from iPSCs has been demonstrated by monolayer cell culture and in coculture experiments with primary chondrocytes in 3D culture systems such as condensed cell bodies and pellet cultures, but the necessity of coculture conditions increases the chance of contamination of differentiated cells with feeders or other undesired cells[6,28,70].

A strategy for large-scale production of chondrogenic cells from human ESCs and iPSCs *in vitro* without the use of serum or feeder cells and without the necessity of a condensed cell body step. To aid in the development of an optimal protocol and to avoid the use of feeder cells, serum and the formation of embryoid bodies we plan to use a Quality-by-Design (QbD)-based method similar to that used in the pharmaceutical industry. Specifically the FDA recommends using QbD-based methods to develop new drugs and cell-based treatments for patients[71]. QbD is a systematic approach that utilizes experimental design and statistical methods in order to gain an in-depth understanding of the effects of input parameters

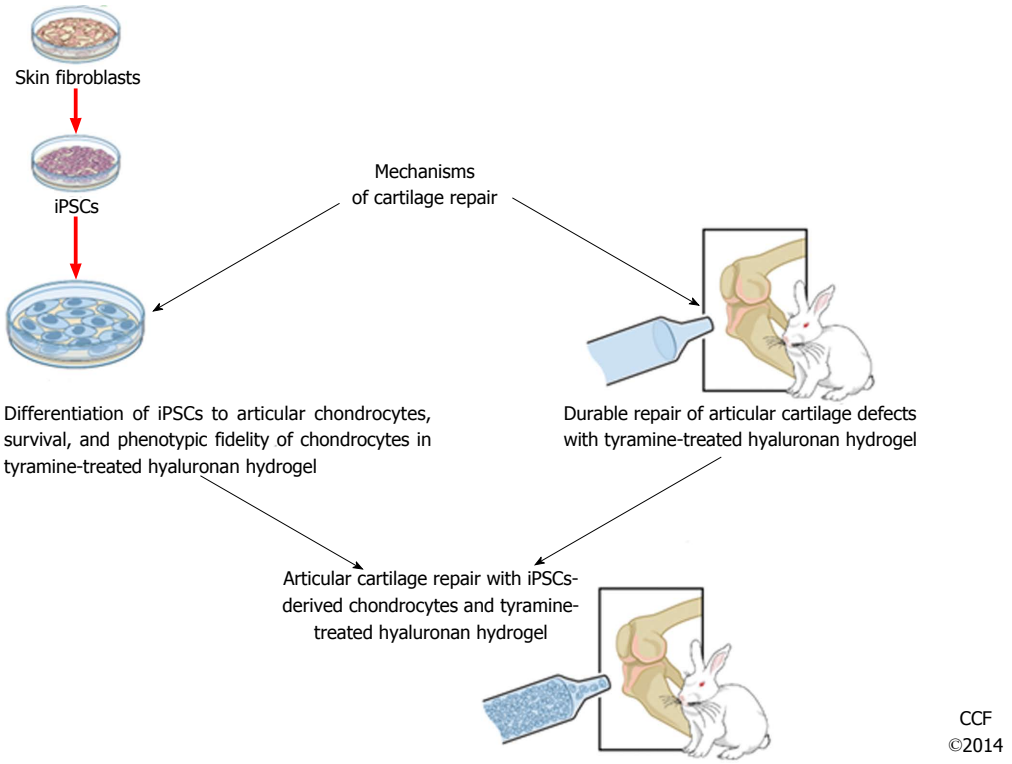

Figure 2 A broad outline for the use of induced pluripotent stem cells in articular cartilage repair. iPSCs: Induced pluripotent stem cells.

and obtain optimal results and quality[72]. We have begun to apply QbD by implementing the Design-of-Experiment theory and by combining it with Multivariate Data Analysis will more thoroughly and systematically optimize protocols for chondrocyte differentiation from iPSCs.

DISCUSSION

One of the main challenges in using iPSCs for either therapeutic applications or *in vitro* modeling is the difficulty in achieving uniform differentiation of the desired cell type. One cause for a lack of uniform differentiation is the use of serum in the differentiation process of cells, which is imprecise due to batch variability and the presence of undefined extracellular factors within serum. The other primary cause for heterogeneity is the use of feeder cells or an embryoid body stage.

Coculture of MSCs with primary chondrocytes to get chondrogenic differentiation has been used to avoid the inconsistent differentiation of primary MSCs in a cartilage regeneration model[73-75]. However coculture is problematic as there are contamination issues when the desired cells need to be separated from the feeder cells as mentioned above[30].

Thus current issues which need to be addressed to further the use of iPSCs in articular cartilage repair and are critically important in cartilage regeneration in an articular cartilage repair model are: (1) Chondrogenic potential and fidelity of the cells; (2) Long term survival of the cells in the repair tissue; (3) Healing to the adjacent endogenous "normal" cartilage in comparison to an adequate untreated control group; and (4) Contamination with (a) undifferentiated cells that form teratomas with embryoid body formation or (b) with feeder cells used in coculture (Figure 2). Despite these hurdles our group and others have preliminary solutions to these issues. Our group believes that a more systematic approach similar to that used in the pharmaceutical industry could add important information to optimize chondrocyte generation from iPSCs with QbD techniques. We predict that the use of iPSCs clinically for cartilage repair holds the most promise to provide a biologic solution for cartilage damage in the near future and that we and others will be able to optimize protocols applicable for clinical use in cartilage repair in the near future.

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