

# Chapter 1

## Advances for Treatment of Knee OC Defects



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**Abstract** Osteochondral (OC) defects are prevalent among young adults and are notorious for being unable to heal. Although they are traumatic in nature, they often develop silently. Detection of many OC defects is challenging, despite the criticality of early care. Current repair approaches face limitations and cannot provide regenerative or long-standing solution. Clinicians and researchers are working together in order to develop approaches that can regenerate the damaged tissues and protect the joint from developing osteoarthritis. The current concepts of tissue engineering and regenerative medicine, which have brought many promising applications to OC management, are overviewed herein. We will also review the types of stem cells that aim to provide sustainable cell sources overcoming the limitation of autologous chondrocyte-based applications. The various scaffolding materials that can be used as extracellular matrix mimetic and having functional properties similar to the OC unit are also discussed.

**Keywords** Osteochondral defects · Osteochondral tissue engineering · Regenerative medicine · Chondrocytes · Stem cell therapy · iPS cells · Scaffold design · and Bilayered scaffolds

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J. M. Oliveira et al. (eds.), *Osteochondral Tissue Engineering*,

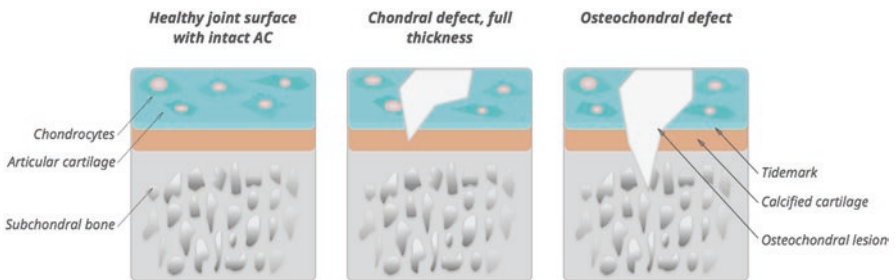
Advances in Experimental Medicine and Biology 1059,

[https://doi.org/10.1007/978-3-319-76735-2\\_1](https://doi.org/10.1007/978-3-319-76735-2_1)

## 1.1 Introduction

OC lesions are notorious for being unable to heal. They mostly affect the knee and the ankle. Usually the defects appear when the joint is used extensively, as caused by repetitive strain or direct trauma to the articulation, typical for sports activity. Genetic predisposition could be another factor responsible for OC damage [1]. Comorbidities of OC defects include joint malalignment, meniscal tear and ligamentous laxity [2]. In many cases these concomitant pathologies occur before the cartilage lesion, and known as contributors of the lesion development. Although the exact aetiology of OC defects has not yet been fully elucidated, researchers agree on two distinct phenotypes, manifested in degenerative lesions or traumatic focal defects [3, 4]. In either case, OC damages put the joint in a great risk of developing osteoarthritis; therefore, early recognition of the presence of any lesion or damage to any joint is of paramount importance [5, 6], especially because OC damages are more prevalent among adolescents and the physically more active younger population [7, 8]. Articular cartilage defects can be of many sizes and shapes and vary in depths. In most severe cases, the defects reach down to the subchondral bone. Their diagnosis is challenging, as many lesions can exist without any symptoms, and their presence is often missed in the early stages [9, 10]. Unfortunately, even the silent lesions usually progress from partial to focal defects if they are left untreated (Fig. 1.1). Having OC lesions can often cause symptoms such as discomfort, tenderness or, in more severe cases, pain, swelling of the knee and limitation in motion of the patient [11].

To establish consistency and aid communication among physicians, there were several grading scales developed, which describe the degree, severity and sometimes the location of the cartilage lesions. The most frequently used classification system was invented by Outerbridge, which was named after him, called the Outerbridge scaling system (Table 1.1) [12]. This system divides the lesions into four categories, but it does not characterize the depth of the lesion. Another widely used scaling system was developed by the International Cartilage Repair Society (ICRS), which also has four grades, complemented with a zero state marking the normal state of the cartilage. The advantage of the ICRS scaling over the Outerbridge



**Fig. 1.1** Schematic representation of the structure of the osteochondral unit and the osteochondral defect

**Table 1.1** Classification of osteochondral injuries according to the ICRS grading

ICRS grading system	
Grade 0	<b>Normal</b> Having a healthy appearance, no signs of cracks
Grade 1	<b>Almost normal</b> 1.a Soft indentation 1.b Superficial cracks and fissures
Grade 2	<b>Abnormal</b> Lesions extending down to <50% of cartilage depth
Grade 3	<b>Severe lesion</b> 3.a Cartilage defects >50% of cartilage depth 3.b Cartilage defects reach down to the calcified cartilage 3.c Cartilage defects reach down to the surface of subchondral bone but do not penetrate 3.d cartilage is bulging around the lesion site
Grade 4	<b>Very severe lesion</b> 4.a Cartilage lesion reaches down to the subchondral bone but not in the entire diameter of the lesion 4.b Cartilage lesion penetrates the subchondral bone fully and across the entire diameter of the lesion

**Table 1.2** Classification of osteochondral injuries according to the Outerbridge grading system

Outerbridge system	
Grade I	Softening and swelling of the cartilage
Grade II	Fragmentation and fissuring in an area half an inch or less in diameter
Grade III	Fragmentation and fissuring, greater than 0.5 in diameter
Grade IV	Erosion of cartilage down to exposed subchondral bone

scaling is that it describes the lesions based on their extent as well as depth (Table 1.2) [4, 13, 14].

For the management of OC lesions, there are several conservative and operative techniques, which are applied according to the extent of damage, age of the patient, doctor's opinion and some other factors. These approaches have a common goal of providing pain relief, repairing the damaged tissues and improving joint functionality [15]. The conservative management of cartilage lesions includes medications to fight pain and inflammation, drilling techniques, abrasion, microfracture as well as the transplantation of OC allografts and autologous chondrocyte implantation with or without matrix [16]. Despite their continuing use in the clinics, none of the mentioned approaches brought long-term solutions so far and could not fully regenerate the OC unit. In fact, according to prospective and retrospective studies, around 60% of knee arthroplasties performed were a result of previous cartilage lesions in the joint [17]. Knee arthroplasty is the most common clinical approach used to restore the joint function. Annually there are 700,000 arthroplasties performed in the USA, and it is estimated that this number will further increase, as much as reaching 3.48 million by the year 2030 [17]. It was indicated that 5% of all knee illnesses in the

general population originated from previous cartilage injuries, while 61% of the cases had OC damages, among which 19% could be specified as focal OC injury [18]. It is therefore leaving no doubt that the burden of OC injuries on today's healthcare and society is huge and its management requires the development of more effective techniques.

In this chapter, we will be briefly discussing the basics of current approaches to diagnose and treat OC defects, but our main focus will be on the future therapeutic regimens of OC lesion management with special focus on the knee joint. We will describe how the advancement of tissue engineering and regenerative medicine (TERM) brought new aspects to articular cartilage healing and will demonstrate the main components of these approaches, namely, the cell sources and matrices used in TERM.

## 1.2 Knee Joint

The largest and most complex joint of the body is the knee. It is a modified hinge joint, which actually consists of two articulations, namely, the patellofemoral and tibiofemoral [19, 20]. The knee is subjected to a great load while walking, jumping, running or performing other activities [21]. It provides stability via a combination of ligaments, tendons, synovial capsule and muscular components [22]. The osseous part is composed of the tibia, fibula, femur and patella. The surfaces of the joints are covered by articular cartilage, which allows frictionless movement [23, 24]. For extra cushioning, there is a pair of moon-shaped fibrocartilage tissues, called the menisci, between the femoral condyles and tibial plateau. Both the AC and the menisci have important shock-absorbing function in the joint [25].

### 1.2.1 Articular Cartilage

Articular cartilage is a type of hyaline cartilage which covers the joint surface providing gliding to it. It is a thin layer of tissue ranging from 3 to 7 mm of thickness at the various anatomical sites [25, 26]. The AC has a white, glassy appearance and lacks both vascularization and innervation in its healthy state. Despite its simple appearance, articular cartilage is a complex and highly specialized tissue. It consists of four layers, each of which bears with a unique structure. The main macromolecular components of the cartilage are the collagen and proteoglycan molecules, which create distinctive patterns at the different zones of the tissue and thus also define functional differences among the layers [27, 28]. Aggrecan is the most abundantly found proteoglycan molecule of the articular cartilage, while among collagen molecules, type II collagen is the most typical [29, 30]. The only cell type found in the cartilage, are the chondrocytes, which possess different properties at the different

layers of the tissue. Accordingly, the (i) uppermost layer called the superficial or tangential zone has rather flattened cells localized densely. Both the cells and collagen fibrils lie parallel to the surface herein. This layer has lesser amounts of proteoglycan molecules, among which biglycan and decorin are the most representative [31, 32]. The superficial layer makes up 10–20% of the whole cartilage. The chondrocytes found in this layer also produce a protein called lubricin, which is a mucous glycoprotein. Lubricin serves as a lubricant on the cartilage surface and together with the superficial zone protein creates a film which equips the cartilage with the mentioned low-friction properties [24, 33]. This layer is also rich in fibronectin and water. The (ii) intermediate zone builds up 40–60% of the total volume of the cartilage. The cells are localized less dense, have a spheroid morphology and are larger as compared to the upper layer [34]. The extracellular matrix is abundant in proteoglycan and thick collagen fibrils. The organization of the fibrils show less structure [35, 36]. The (iii) deep or radial zone of the articular cartilage makes up 30% of the total tissue volume. This layer has the least amount of cells and water, but the most of the proteoglycan molecules and the largest collagen fibrils [37, 38]. The chondrocytes are stack in columns and have spherical shapes. Both the collagen molecules and chondrocytes lie perpendicular to the cartilage surface. The (iv) calcified cartilage is located beneath the radial zone, also called the basal layer [25, 39, 40]. It is separated from the radial zone by the tidemark, which is narrow layer, metabolically active for the calcification. The tidemark is an important interface between the soft and calcified cartilage. It also has a crucial role in damping the loads. The calcified cartilage creates a connection between the hyaline cartilage and subchondral bone [41, 42]. This layer has a sparse amount of chondrocytes, which are smaller in size and have a hypertrophic phenotype. These chondrocytes are metabolically less active and are completely embedded in the extracellular matrix. Most typical collagen in this zone is the collagen type X [43, 44]. Collagen type X is believed to have an important role in mechanical load transmission [31, 45]. Any structural change to the articular cartilage as a result of either biomechanical or biochemical changes will ultimately result in poor mechanical properties and cartilage degeneration.

### ***1.2.2 Subchondral Bone***

The subchondral bone is located underneath the calcified cartilage. Together they compose the OC unit. The AC acts in concert with the subchondral bone to absorb the mechanical load in the joint [46]. The subchondral bone consists of two anatomical entities, the subchondral plateau and the subchondral spongiosa [41]. The subchondral bone is richly perforated by veins and arteries as well as by nerves. The subchondral bone is an important exchange interface for the AC. It has been demonstrated that the AC and the subchondral bone have an intense crosstalk, especially during the onset of joint degeneration [40, 47, 48].

### 1.3 Limitations of Current Clinical Approaches

There are several major limitations to effective treatment of OCDs, and their diagnosis is also extremely challenging in many cases. Often subtle injuries show little or no dysfunction, which results in their delayed detection. But even if diagnosis is performed accurately, due to the low intrinsic capacity of the cartilage, conservative care is often unable to provide a long-term solution. Many of the current techniques focus on symptom management and functional improvement of the joint [7, 49]. Common treatment strategies are the use of non-steroidal anti-inflammatory drug (NSAID) agents, which are often used together with cast immobilization, or in cases where surgical intervention is required microfracture, drilling techniques and the use of allografts or autologous chondrocyte transplants are the routined applications [16].

#### 1.3.1 *Diagnosis of OC Lesions*

Patients with acute joint injury usually suffer from pain, swelling and joint effusion, which appear as the consequence of the influx of inflammatory mediators. They usually are also limited in motion and have the joint inflexible in many cases or report mechanical symptoms such as clicking and locking of the knee. OC injury often goes together with the damage of the menisci and the ligaments or results in loose bodies in the joint cavity. In many cases on the other hand, OC lesions can go unnoticed, having no symptoms, and thus being challenging to diagnose [28].

Currently, the diagnosis of OC lesions relies on patient medical history, patient self-assessment, symptomatic findings, furthermore radiographic and magnetic resonance imaging, as well as arthroscopy [50]. Plain radiography is usually considered to be the gold standard as it is largely available and cost-sensitive [51, 52]. However, radiographic images may not depict the presence of small superficial cracks but can only identify more prominent lesions and loose bodies in the joint cavity. Magnetic resonance imaging (MRI) is a more precise method allowing higher resolution and thus providing more information but is also more costly [9, 53]. MRI often goes together with the application of X-ray computed tomography (CT) [54]. Both MRI and CT can use contrast agents to enhance tissue differentiation and enable better evaluation. Contrast agents are especially important for the visualization of cartilage and other soft tissue components in the joint [54, 55]. There is a large variety of contrast agents available. The use of metal chelates, i.e. gadolinium-based agents, is common for MRI application, while for CT iodine-based agents such as sodium iodide and sodium diatrizoate hydrate are the most established [56–60]. Arthroscopy is an intra-articular method used to visualize and examine the cartilage pathology. Arthroscopy is performed by orthopaedic surgeons, and during the procedure a small camera is led to the joint cavity via a small incision, which can accurately locate and classify the damage [61, 62]. Given its in

situ nature, arthroscopy is a very precise technique, but consequently it is also more invasive compared to other imaging approaches. However, arthroscopy is not only used for diagnosis but also for the therapy of the OC tissue which we will introduce in the next section [61, 62].

Taken together all possibilities for diagnosis, unfortunately it is still a hurdle to detect less pronounced cartilage damages. Early diagnosis and accurate evaluation of articular lesions should be immediate, as all defects can eventually progress to focal lesions.

### ***1.3.2 Conservative and Surgical Care***

OC injuries are traumatic in nature. When patients present with OC injury without any severe symptoms, typically ICRS grades I and II (Table 1.2), they are usually advised to rest, and often cast is used to immobilize the joint. The treatment regimen also includes the use of NSAIDs. The goal is to help the potential oedema resolve and avoid the development of any necrosis [49, 63]. In grades III and IV, the injury is accompanied by pain and larger damage to the joint; therefore, usually a surgery is imperative. Current surgical concepts for the restoration of articular cartilage structure and function mostly include approaches such as arthroscopic lavage and debridement, drilling, microfracture and the implantation of OC allografts and autologous chondrocytes [64–67].

Arthroscopic lavage and debridement involves the incision of the joint and insertion of a small camera, called the arthroscope, for the evaluation of damage as mentioned before. When the location and degree of defect are assessed, it is washed thoroughly to remove all loose bodies and damaged OC tissue from the cavity [68, 69]. Microfracture and drilling are marrow stimulation techniques and are used in an attempt to initiate cartilage regeneration [70, 71]. After perforating the subchondral bone, the blood is drained into the joint covering the cartilage surface and lesions with stem cells and bioactive molecules, such as growth factors which are believed to facilitate the cartilage repair capacity. The drilling holes are in close proximity to each other, approximately 3–4 mm apart [72, 73]. The ultimate goal is to reproduce biomechanically functioning cartilage. However, this procedure mostly leads to the formation of fibrocartilage only, which has knowingly reduced capacity to bear loading. OC graft implantation is used when there are larger defects present with size around 2 cm<sup>2</sup> [66, 74]. Initially, autografts were harvested from the same patient's intact joint region, usually from the patellofemoral site. However, due to anatomical misalignment, this procedure often failed. Advancement was brought by the multiple use of cylindrical shape osseous grafts, a procedure called mosaicplasty, developed by Hangody and Bobić [75, 76]. Further popularity of the usage of graphs came when standardization of tissue and cell storage was established by the American Association of Tissue Bank and US Food and Drug Administration in 1998. Clear guidance on how long cells and tissues stay viable in refrigerated condition allowed commercialization and wider use of OC allografts [66].

While immunological intolerance is relatively rare, the lack of tissue incorporation and a possibility to disease transmission remain a relative complication to this procedure.

## 1.4 The Future of OC Repair: Advanced Therapies

The future of OC lesion repair has been taken over by the emerging approaches of TERM strategies, where the focus is on replacing and regenerating the damaged tissues, instead of restoring the function only [77, 78]. The number of TERM techniques has been substantially increasing over the past years, resulting in a wide range of procedures to tackle tissue regeneration. Given the prevalence and low chances of healing on its own, OC lesions are prime candidates for stem cell therapies and tissue engineering approaches.

### 1.4.1 Cell Sources

The only cell type which has the perfect capacity to regrow the native cartilage is the chondrocytes. However, continuous supply of fresh chondrocytes is hard, if not impossible, to maintain. Researchers and clinical experts are constantly looking for other more optimal cell sources and feasible strategies to repair OC lesions. Here we will list and describe the most promising cell candidates for cartilage regeneration.

#### 1.4.1.1 Chondrocytes

Regeneration of OCDs requires the application of chondrocytes and osteoblastic cells. Autologous chondrocytes were for long the commonest choice for conservative and advanced approaches of cartilage therapy [61]. Autologous chondrocytes are the patient's own cells harvested from a nondamaged area of the joint. The derivation of biopsies from a healthy area of the cartilage can potentially initiate secondary osteoarthritis, which is a disadvantage of this technique [79]. Also, not all patients have enough healthy areas of the joint. Owing a restricted size of biopsies, the acquired cell number is limited. Therefore, to achieve sufficient amount of cells, the chondrocytes must first be expanded *in vitro*. This is a risk factor as the chondrocytes can lose their phenotype easily when not in their native environment [80, 81]. Once they bear with reduced chondrogenic capacity, their clinical use and therapeutic outcome are impeded [82]. Therefore, there had been various approaches developed to prevent dedifferentiation during *ex vivo* expansion, including the use of growth factors in the culture media, providing dynamic hydrostatic pressure to the cells while in culture and limiting the levels of oxygen in the environment [83, 84]. Another crucial factor is the amount of time of expansion; the shorter time the



chondrocytes spend in culture, the better the chances are to preserve their phenotype. However, these cells have a very low proliferation rate, and consequently the shorter the time, the smaller cell number is being achieved. Ergo, donor site morbidity, cell dedifferentiation and limitations in cell number are the most critical detriment of the use of autologous chondrocytes [82].

To overcome obstacles given by the limited availability of autologous chondrocytes, non-autologous chondrocytes had also been considered and explored as a potential cell source to manage OC defects [85]. The use of allogeneic or xenogeneic chondrocytes has the advantage of acquiring larger cell populations while avoiding damaging the healthy sites of the joint and thus the development of secondary osteoarthritis. One could even obtain cells from younger populations with better chondrogenic capacity [86]. However, this technique also has drawbacks, namely, immunological intolerance and the possibility of disease transmission.

### 1.4.1.2 Stem Cells

Stem cells had been widely explored to overcome the limited supply of primary cells. Continuous cell sources could be provided by using off-the-shelf stem cell techniques. Most research involves the application of mesenchymal stem cells (MSCs), but embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) had also been considered as ideal candidates for OC regeneration (Table 1.3).

**Table 1.3** Advantages and disadvantages of the various cell candidates for the regeneration of the osteochondral unit

Cell types	Benefits	Challenges
Autologous chondrocytes	Native phenotype Immunocompatibility	Insufficient cell number Prone to dedifferentiation
Allogeneic chondrocytes	Larger cell number Off-the-shelf solution	Risk of disease transmission Lack of donor availability Immune rejection
Adult stem cells (MSCs, ADSCs, SMSCs, BMSCs)	Reliable potential for differentiation Large availability Various tissue sources Easy extractability No ethical complications	Large variety of proliferative capacity, phenotype Heterogeneous cell population Differentiation problems
Embryonic stem cells (ESCs)	Multiple cell types can be produced Immortal cell source Immune-privileged cells	Risk of teratoma formation Ethical complications
Induced pluripotent stem cells (iPSCs)	Unlimited cell supply Non-invasive extraction of donor cells Large variety of cell types can be differentiated	Difficulty to achieve uniform differentiation Risk of teratoma formation

Mesenchymal stem cells (MSCs) are multipotent cells which can differentiate into osteoblast or chondroblast. They can be originated from various sources, such as the bone marrow, adipose tissue, synovium, infrapatellar fat pad, muscle, dermis, blood or the umbilical cord [87]. MSCs are defined by a minimal prerequisite established by the International Society for Cellular Therapy. This includes the expression of surface markers CD105, CD73 and CD90 and the exclusion of surface molecules CD45, CD34, CD14 or CD11b, CD79 $\alpha$  or CD19 and HLA-DR in standard culture conditions. They also have to be plastic adherent and capable of differentiating into chondroblasts, osteoblasts and adipocytes *in vitro* [88].

Chondrogenic differentiation of MSCs is induced via the application of growth factors and by the manipulation of their culture environment. It is usually divided into three stages starting with (i) cell condensation and the expression of adhesion molecules, such as N-cadherin and tenascin-C, which facilitate the cell-cell interaction. This is followed by (ii) the activation of transcription mediators including bone morphogenetic proteins (BMPs), Sox-9 and FGF signalling pathways. The last step is (iii) ECM deposition and pre-chordial cell formation which is continued by full chondrocyte formation [89]. MSCs isolated from different sources may differ in their differentiation potentials [90].

Synovium-derived MSCs (SMSCs) were demonstrated to have excellent chondrogenic potential, greater than the MSCs isolated from the donor-matched bone marrow, periosteum, muscle and adipose tissue [91]. Furthermore, SMSCs are closely related and share many characteristics with the chondrocytes. AC and the synovium originate from the same pool of precursor cells and have a similar gene expression profiles, i.e. superficial zone protein, collagen type II and aggrecan are all expressed by both the chondrocytes and SMSCs [92, 93]. SMSCs also have a relatively high rate of proliferation as compared to other MSCs. Furthermore, they were shown to deposit greater amount of extracellular matrix, as compared to BMSCs. This is especially important since ECM deposition is believed to delay cell senescence and dedifferentiation [94, 95]. Additionally, SMSCs are able to form hyaline cartilage *in vitro* which holds extreme value for OC regeneration [96]. SMSCs are harvested via arthroscopy or from the synovial fluid. Unfortunately, the yield of cells is relatively low, having only around 14 cells per mL of synovial fluid, which have the capacity to form CFU-F colonies [97].

MSCs of other origin, such as bone marrow-derived stem cells (BMSCs) or adipose-derived stem cells (ADSCs), can also differentiate into chondrocytes or osteoblasts. Despite these cells having less chondrogenic potential, they bear with other advantages making them desirable for the OC tissue repair. In particular, ADSCs are routinely available from excised fat or lipoaspiration, which yields on average around 400,000 cells per mL, and thus provide the sufficient amount of cells easily [90]. Thorough molecular analysis had been performed to characterize these cells, and their trilineage mesodermal potential has been confirmed by several studies [98]. Different induction conditions had been identified where adipogenic, osteogenic and chondrogenic differentiation can be stimulated [99–101]. These include the presence of growth factors, hormones and mechanical stimuli, as well as 3D scaffold cultivation using scaffolding materials with different composition.

Studies using alginate scaffolds demonstrated a superficial chondrogenic capacity of chondrocytes differentiated from ADSCs as compared to normal chondrocytes after 21 days of in vitro culture in alginate scaffolds [102]. On the other hand, when using hyaluronic acid scaffolds, collagen type II expression was similar in both ADSC and chondrocyte cultures and was lower in BMSC cultures. Numerous papers had been discussing whether BMSCs or ADSCs have stronger ability to differentiate into the chondrogenic lineage. Most of them agree that BMSCs possess more chondrogenic potential. Interestingly, it was also shown that although ADSCs and BMSCs have similar surface receptor profile, they may require different induction conditions [103]. For instance, stimulating BMSCs and ADSCs by transforming growth factor- $\beta$  results in different chondrogenic profile as measured by chondrogenic marker expression, such as aggrecan and collagen type II. It was pointed out that while bone morphogenetic protein-6 (BMP-6) had successfully resulted in increased expression of aggrecan by ADSCs, BMSCs required stimuli provided by TGF- $\beta$  to achieve the same [104]. Unfortunately, BMSCs yield significantly less cells upon harvest as compared to ADSCs, and they are harder to obtain involving a more invasive procedure, called bone marrow aspiration. It was shown that 1 mL marrow yields between 100 and 1000 cells.

ESCs and iPSCs have also tremendous potential as a cell source for the manufacture of OC therapy. The advantage of ESCs and iPSCs over adult stem cells is that they can be expanded indefinitely without undergoing senescence. Blastocysts which are unsuitable for in vitro fertilization are used to acquire ESCs. ESCs are the inner cell mass of blastocyst. Consequently, scientific use of ESCs is ethically unaccepted in many countries despite the immense interest and potential of their application. There are four main categories of methodologies to generate chondrogenic cells from ESCs, namely, (i) targeted differentiation, (ii) differentiation of ESCs via their coculture with mature chondrocytes, (iii) embryoid body formation with stimuli of growth factors and (iv) spontaneous differentiation into MSCs and subsequent chondrogenic differentiation [105]. Besides ethical concerns, teratoma formation is another problem encountered in the application of ESCs in regenerative therapy.

iPS cells are not burdened by ethical issues and were proven to be safe and stable when used for cartilage formation [106]. Since their first introduction in 2006, iPSCs were widely explored for various applications [107–109]. Originally, they were generated in a mouse model using fibroblasts. Four factors were manipulated, namely, two transcriptional factors, the octamer-binding transcription factors 3 and 4 (Oct3/4) and the Kruppel-like factor 4 (Klf4), and two tumour-related genes, the v-myc avian myelocytomatosis viral oncogene homolog (c-Myc) and Sox-2 [107]. Successful chondrogenic differentiation of iPSCs derived from various sources, such as fibroblast-like cells, neural stem cells and human osteoarthritic chondrocytes, was also demonstrated [110, 111]. Generation of iPSCs and subsequent differentiation into chondrogenic lineage from murine neural cells were achieved by the simultaneous overexpression of Oct4 and Klf4. Another approach, using somatic cells, had successfully generated iPSCs via a reprogramming approach using c-Myc, Klf4 and Sox-9 which were later able to differentiate into the chondrogenic lineage [112, 113]. Interestingly, even the reprogrammed cells show differences in their

chondrogenic potential. Namely, iPSCs originating from chondrocytes show superior properties than iPSCs generated from neural or fibroblast-like cells [97]. iPSCs have tremendous potential for regenerative approaches. It is possible to establish allogeneic iPSC libraries, based on HLA phenotypes. These cells could be induced to differentiate into chondrogenic or osteogenic phenotype and could serve as off-the-shelf solution to heal OC lesions providing a truly advanced solution [89].

## **1.4.2 Biomaterials**

Apart from the cell and molecular component, another pillar of the TERM approaches is the carrier. Although cells might be administered without scaffolding material, they often require support provided by a 3D structure to survive and keep their phenotype. Scaffolds need to be compatible with the cells as well as mechanically matching the environment of implantation. To design appropriate scaffolds for the OC unit, one must understand the biology of both the cartilage and the subchondral bone; therefore, it is particularly challenging. The scaffolds should integrate to their environment by being biocompatible and slowly degrade as the cells rebuild the tissue and thus also have to be biodegradable. There have been a myriad of materials proposed already originating from both natural and synthetic polymers [114]. The usage of inorganic materials, such as glasses and ceramics and metallic materials, had also been introduced. In this section we will review the advantages and disadvantages of these different materials.

### **1.4.2.1 Natural Polymer-Based Materials**

Natural polymers are derived from the nature; therefore, they bear with high biocompatibility properties which is an advantage over the synthetic polymers. They are of animal or vegetal origin, or obtained from algae, and can be synthesized as well [115–118]. They can also aid cells in their tissue interaction, synthesis and development due to molecular domains present on their structures. Collagen polymers, for instance, contain ligands, which promote cell adhesion and matrix deposition [119, 120]. Collagen-based scaffolds showed improvement when regenerating cartilage; however, they also bear with relatively weak mechanical properties. Since both the cartilage and bone contain large amounts of collagen molecules, collagen-based scaffold seems to be an ideal candidate for OC regeneration. Current commercially available applications of collagen include Zyderm, CaReS (ArthroKinetics, Essingen, Germany) and Carticel (Genzyme Inc., Cambridge, UK) [121, 122]. Another favoured natural polymer is the alginate. Alginate is a linear, unbranched copolymer of L-glucuronic and D-mannuronic acid [123]. Ca-alginate porous scaffolds combined with gelatine were able to differentiate MSCs into both

chondrogenic and osteogenic lineages [124]. Alginate-based 3D scaffolds were shown to have the capacity to redifferentiate chondrocytes [125]. Among the cationic polymers, chitosan is the most popular. Based on the source and method of preparation, various chitosan materials can be attained, which also bear with different properties. Successful subchondral bone and cartilage generation had been achieved by chitosan matrices [126]. Chitosan polymers have structural similarities with two major cartilage molecules, namely, hyaluronic acid and glycosaminoglycan molecules; thus, it is also prepared as a blend [127]. Silk polymers obtained from silkworm cocoon or spider silk are also widely explored for the use of TERM approaches [128]. They can be combined with synthetic polymers, which are used to improve their mechanical stability and tailorability/processability. For instance, when silk was prepared in a combination as silk fibroin/nano-CaP to obtain bilayered scaffolds, it showed superior mechanical properties compared to silk-only scaffolds [129].

Natural matrices have the advantage of being biocompatible and biodegradable and having more similarity to biological macromolecules; therefore, the native environment can recognize it and process metabolically. However, natural matrices have the danger of transition hazardous agents and have purification issues; therefore, their use on the clinics is hampered. They also bear with weaker mechanical properties as compared to synthetic polymers.

#### 1.4.2.2 Synthetic Polymer-Based Materials

As mentioned before, the main advantage of using synthetic polymers is owing complete control over their structure and having better mechanical properties. Synthetic polymers also do not have issues with disease transmission or limitation in polymer supply. However, biocompatibility can become a major issue when using matrices fabricated from synthetic polymers. The most commonly used polymers for cartilage and bone engineering are poly(ethylene glycol) (PEG) and its derivatives poly(L-lactic acid) (PLLA) and poly(lactide-co-glycolide) (PLGA). These synthetic biodegradable polymers are often used as a blend of each other or combined with natural polymers to promote biological integration [78, 130, 131]. Despite their easy manufacture and large availability, synthetic polymers must be explored via the combination of natural polymers to enable better biocompatibility and orchestrate cellular events *in vivo*, e.g. cell adhesion, proliferation and matrix deposition. Accordingly, several studies demonstrated chondrogenic differentiation of PLLA in combination with polymers of natural origin, such as chitosan and silk, proving their potential for OC regeneration [132, 133]. Besides the lack of cell recognition sites, synthetic materials also tend to degrade easily once implanted promoting the development of inflammation. Thus, despite their advantages, synthetic polymer-based scaffolds are only ideal to be used as blend with natural matrices.

### 1.4.2.3 Other Materials (Ceramics, Glasses, Metallic Materials)

Ceramics, such as calcium phosphate ceramics and hydroxyapatite, are favoured because of their biocompatibility and osteoconductive and osteoinductive nature [85]. They were shown to promote the production of bone-like apatite when implanted, therefore being able to integrate better and induce bone regeneration [134, 135]. As it was recently summarized and demonstrated, 3D-printed Ca ceramics have tremendous potential to establish patient-specific bone grafting application; however, poor mechanical stability must be first addressed and solved [136]. Metallic materials include the application of stainless steel, titanium, titanium alloys and cobalt-based alloys. The main advantage of these materials is the structural architecture and mechanical property similar to the native grafts. Porous titanium bases were recently fabricated in combination with hydrogels for OC regeneration [137]. These biphasic scaffolds had the advantage of bearing with mechanical stability at the bone phase and having viable cell-seeded hydrogel integrated on top to support cartilage regeneration [137]. The major disadvantage of metallic materials is the lack of degeneration after implantation.

## 1.5 Final Remarks

There is no doubt that regeneration of OC damages is of major importance. Considering the poor healing capacity of the OC unit, so far only repair techniques had been applied, which fail to provide long-term solutions and full regeneration of the joints. Due to lack of regeneration, the danger of developing osteoarthritis is high in joints affected by OC injury. Orthopaedic surgeons and researchers are working on solutions, which can heal the damaged area by introducing cells, bioactive molecules and support matrices to the defect site. Whereas, in case of larger injuries, the application of in vitro engineered allografts could bring solutions, TERM approaches have the potential to overcome several limitations of the existing applications, such as insufficient cell number or cell differentiation, as well as can tackle the issue of lack of donors for OC grafts. TERM approaches hold great promise but also face many challenges. Finding the appropriate cell source, and establishing the environment to these cells, in which they are able to keep their phenotype or conversely differentiate into the desired phenotype, is extremely difficult, especially if we consider off-the-shelf solutions. Moreover, these cells usually need 3D support in vitro or when implanted. This is provided by scaffolding materials, which come in many shapes and forms. Finding the appropriate combination which then will facilitate tissue formation is hard. Furthermore, these materials have to be able to integrate into the native tissue and withstand the mechanical forces therein. This is especially difficult in the knee articulation, which is the largest joint in our body experiencing mechanical load over 20 MPa. Considering that the OC unit is composed of both the articular cartilage and subchondral bone, biphasic scaffolds obtained by the combination of materials and cells show promise for OC

regeneration. Many routes had been proposed for material fabrication. Novel strategies using the combination of bioceramics and polymeric materials or the blend of natural and synthetic materials are considered as optimal candidates. Among the cells, MSCs including SMSCs, BMSCs and ADSCs as well iPSCs are the favoured ones due to their large availability and chondrogenic potential. The design of strategies for OC regeneration is open for further development and requires the combination of multidisciplinary approaches to reach its goal. Nevertheless, if cell and molecular biology, material science and orthopaedics are effectively brought together, chances are that we will be able to reach clinics with excellent and sustainable regenerative approaches for the OC unit.

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