Chapter 14
Challenges for Cartilage Regeneration

Fariba Dehghani and Ali Fathi

Abstract Articular cartilage is a resilient connective tissue, which covers the surface of bones to facilitate their movements against each other. Due to unique mechanical properties, cartilage has a prominent role in locomotion and mobility of the human body. This tissue however has limited capability of regeneration and repair due to its low metabolism and avascular structure. Trauma, degenerative conditions and inflammatory arthritis lead to lifetime disability states and pain. The scope of this chapter is to first provide an overview of mechanical, biological and micro-architectural properties of articular cartilage and the effect of aging on these characteristics. Then the cartilage treatment techniques that have been proposed for different types of cartilage defects are discussed. Cell-based therapies, such as autologous chondrocyte implantation (ACI) technique, have been developed to achieve reproducible results regardless of patients' age, gender and physical conditions. The second generation of ACI is a tissue engineering-based technique, which includes the use of appropriate cell type, bioactive molecules such as growth factors and proper scaffold to regenerate cartilage. The favourable types of cells, biological compounds and properties of biomaterials for cartilage regeneration have also been discussed in this chapter. Finally, the biomaterial products that have been examined in clinical trial for cartilage repair are outlined, and their properties and clinical results are discussed.

Keywords Articular cartilage • Tissue engineering • Scaffold • Tissue regeneration • Biopolymer

14.1 Articular Cartilage Properties and Aging

Hyaline articular cartilage covers the ends of articulating bones [1]. It is a resilient connective tissue with low friction and high load-bearing capacity. Cartilage serves a critical role in mobility of one bone against another. Chondrocyte is the main cell

F. Dehghani (✉) • A. Fathi
School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, NSW 2006, Australia
e-mail: Fariba.Dehghani@Sydney.edu.au

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type scattered within an extracellular matrix (ECM) of cartilage [2]. Breakdown or any degree of damage in this tissue leads to substantial reduction in mobility of the human body and pain and subsequently has negative impact on patients' lifestyle. Cartilage has limited capability of regeneration and repair due to its low metabolism and the absence of vascularisation in its structure [3, 4]. To design and engineer a cartilage, it is essential to have better understanding over the mechanical and biological properties of this tissue. Mechanical, physiochemical and biological properties of articular cartilage are mainly affected by its biochemical composition as well as its structural properties that are reviewed in this section.

14.1.1 Biochemical Composition

The composition of articular cartilage varies during the tissue development. In a mature cartilage, 70–80 % of its content is water. The solid fraction of articular cartilage is mainly collagen (50–75 %), proteoglycan (15–30 %) and other protein molecules [5, 6]. The collagen network provides shear and tensile stiffness, whereas proteoglycan molecules bring about compressive stiffness to cartilage ECM. The collagen network also suppresses the high swelling tendency of the proteoglycan molecule and preserves the functional integrity of cartilage [7]. The mixture of collagen and proteoglycan, therefore, forms an integrated mesh-like network with superior mechanical properties. Concentration of collagenous proteins and proteoglycan along with non-collagenous proteins in the structure of articular cartilage substantially affects the mechanical properties and functionality of articular cartilage.

Collagen has a primary role in the function and structure of all connective tissues throughout the human body. Collagen is composed of repeating amino acid sequences (mainly glycine, proline and hydroxyproline) and possesses a triple helix structure. In articular cartilage, collagen type II is predominant [8], which provides shear and tensile stiffness to cartilage ECM. In addition to collagen type II, cartilage ECM is composed of other types of collagen.

Other fibrillar and globular collagen types, such as types V, VI and IX, also exist in cartilage ECM [9]. The roles of these types of collagen are still unknown, but it is believed that they support the integration of cartilage structure by affecting the intermolecular interaction in collagen type II [5, 6].

Proteoglycans are large macromolecules consisted of a protein segment in core, covered with polysaccharide chain, known as glycosaminoglycan. The molecular weight of proteoglycans varies in the range of 50,000 kDa to 100 kDa [10, 11]. Proteoglycan networks function as a mesh, which covers the organised collagen network. Majority of this biopolymer is one of the factors that provide structural integrity to cartilage. The primary proteoglycan in articular cartilage network is aggrecan. This proteoglycan consists of a hyaluronan core with chondroitin and keratin sulphate in side chains. The presence of these two carboxyl and sulphate groups gives articular cartilage network a negative charge (known as a fixed charge

density) [12]. This overall negative charge leads to hydrophilic and high swelling properties to cartilage network [7, 13]. The mechanical functionality of cartilage on the other hand is highly dependent on the fluid pressurisation within the tissue. Loss of proteoglycan can, therefore, decrease water intake capacity and thus fluid pressure within the cartilage network. Lack of fluid pressure in cartilage substantially decreases the mechanical strength of this tissue [13].

Cartilage ECM also contains a small fraction of non-collagenous proteins and other matrix constituents. Non-collagenous proteins include cartilage oligomeric proteins, fibronectin, thrombospondin, matrix glycine–leucine–alanine, chondrocalcin, superficial proteins and elastin [14]. Other matrix constituents of cartilage ECM are lipids, phospholipids and inorganic crystal compounds [15, 16]. The role of each of these molecules in the function of articular cartilage is still not clear. Collagen and proteoglycan are the two predominant compounds that affect the cartilage functionality. In this chapter, therefore, the main focus is on collagen type II and proteoglycan rather than other minor compounds [7]. In addition to biochemical composition, the microstructures and micro-layouts of articular cartilage affect the biomechanical and biological properties of cartilage.

14.1.2 Cartilage Microstructure

The structure of articular cartilage substantially varies from the surface of articular cartilage to bone [17, 18]. The biochemistry, cell morphology and cell arrangement vary in different regions within articular cartilage. The schematic overview of articular cartilage is shown in Fig. 14.1. The tissue can be divided into superficial, middle, deep and calcified regions.

The top surface of articular cartilage is covered with very thin proteinaceous layer, termed as lamina splendens [18]. The zonal region right beside the lamina splendens is a superficial zone, which has a thickness within the range of 4 to 8 mm for the healthy cartilage of a man [17]. The cells in this region are densely packed with discoidal shapes that are oriented along with the collagen fibres [19]. The middle or transitional layer zone occupies 40–60 % of the total articular cartilage thickness (equivalent to 16–22 mm in healthy male cartilage) [17]. The deep zone is the last region of purely hyaline tissue. The thin layer, which separates the deep zone from calcified region of articular cartilage, is called tidemark. The calcified region is the transitional zone between elastic cartilage and rigid bone tissues [20].

14.1.3 Cartilage Mechanical Function

The main role of articular cartilage is to act as low-friction, load-bearing, wear-resistant surface for mobility of bones over decades of continuous use. The force applied on cartilage depends on the location of the joint. The forces exerted on the

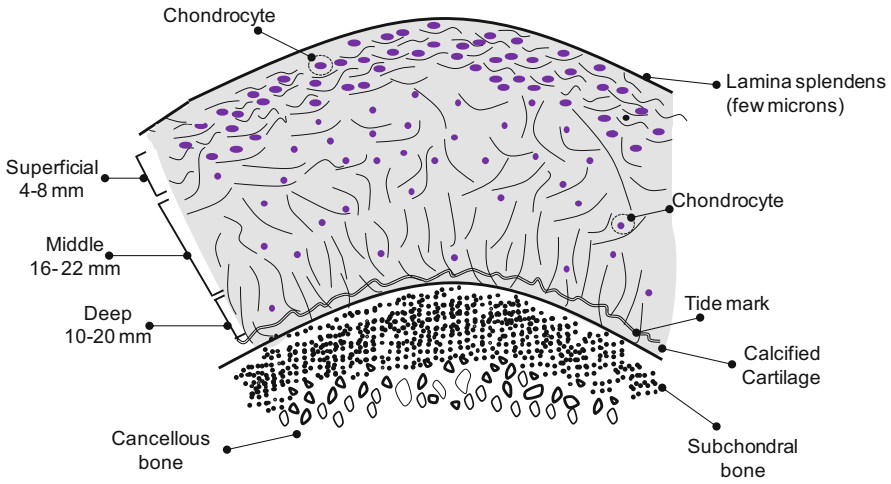

Fig. 14.1 Schematic layout of articular cartilage and thickness of different zones of articular cartilage in a healthy knee joint (The image is generated by accumulating data from different sources [18, 21–23])

shoulder, ankle, heap and knee are 1.5, 2.5, 3.3 and 3.5 times of the human body weight, respectively [24]. The biochemical characteristics of articular cartilage directly affect the performance of the tissue in the joint and its mechanical functionality. Any change in mechanical and biochemical properties of articular cartilage might dramatically alter the loading profile exerted on the joint, which may degrade tissue and eventually lead to loss of articular cartilage [3, 4].

The deformation of cartilage plays an important role in its mechanical functionality, which is governed by the rate of absorbance or release of fluid, mainly water, through its solid structure [25, 26]. For example, sudden loading on cartilage releases water from its structure; this subsequently absorbs the impact of stress and covers the tissue. In addition, release of water leads to expansion of cartilage which in turn increases the surface area of contact (between cartilage and the bone) and thus decreases the impact of stress on skeleton [25, 26].

Loading and deformation of articular cartilage generate a combination of compressive, tensile and shear stresses along with friction throughout the tissue [27]. Some of the important mechanical behaviour of articular cartilage is summarised in Table 14.1 and are discussed in this section. It is important to note that all mechanical properties of cartilage are continuously changed during human growth and affected due to health condition [28–32].

14.1.3.1 Compression Behaviour

Compressive loading is one of the primary types of mechanical stress exerted on articular cartilage. The confined compressive modulus experienced by articular

Table 14.1 Important mechanical properties of native cartilage

Mechanical properties		Refs
Compression modulus (MPa)	0.08–2.1	[31, 33, 34]
Tensile modulus (MPa)	4.8–25	[25]
Strain at failure (mm/mm)	30 %	[35]
Practical strain rate	0–1 mm/s	[33]
Shear modulus (MPa)	0.07–0.65	[36, 37]
Coefficient of friction	0.001	[38]

cartilage varies in the range of 0.08 to 2.1 MPa from superficial to deep layers of adult bovine cartilage, respectively [31, 32, 39]. The mechanism of cartilage to cope with high compressive stresses is predominantly described by permeability of cartilage structure.

Cartilage structure has a low liquid permeability, leading to high resistance of tissue against fluid flow within its structure. Under compression, therefore interstitial fluid is subjected to very high pressure. After unloading, the fluid is redistributed (from high pressure to low pressure spots) within the tissue and imparts viscoelastic properties to the cartilage [26, 40, 41]. Volumetric changes and viscoelastic properties of articular cartilage are, therefore, the two predominant factors affecting the compressive behaviours of articular cartilage.

Volumetric changes occur as fluid moves out from the structure of articular cartilage under compression. Upon the unloading of the tissue from compression stress, cartilage recovers its initial dimension and absorbs the lost fluid. In this cycle hence, the solid structure exhibits both high viscoelasticity and water uptake properties to be able to cope with compression stresses and also keep its original shape [38]. Over a course of a day, however, the bulk of cartilage is compressed in the range of 15–20 % of its initial volume, which can be fully recovered within a period of inactivity (e.g. sleeping period) [42].

14.1.3.2 Tensile Properties

Tension in cartilage occurs either when two cartilages slide against each other and pull towards a single direction or when a cartilage is compressed, pulling the surrounding tissue towards the loading region. As cartilage is subjected to tension, collagen fibres within the tissue structure align and stretch along the loading axis. As the strain is in the range of 0–15 %, the crosslinked collagen network starts stretching, and thus the cartilage exhibits linear response [43]. As the strain increases, the crosslinked collagen fibres themselves start stretching. In higher range of strain (above 15 %) therefore, cartilage exhibits higher stiffness [44, 45]. The structural and physical properties of articular cartilage therefore affect the behaviour of this tissue under tension condition. These include collagen fibre density, their length and degree of crosslinking and strength of ionic bonds between collagen and proteoglycan networks [46, 47].

The tensile modulus of mature articular cartilage is significantly higher than its compression modulus, and it varies in the range of 4.8–25 MPa from deep to the superficial layers, respectively [25]. The tensile modulus of articular cartilage increases by maturation [29, 48].

14.1.3.3 Shear Stress

Articular cartilage is subjected to shear stress in its deep tissue when the joint undergoes rotational or translational movement. In theory, pure shear just causes stretching of tissue (it causes no compressive stress on the tissue). The shear stress on cartilage is independent on the fluid pressure within its structure, and thus it can be used to characterise the solid fraction of cartilage regardless of fluid dynamics in it [49]. The equilibrium shear stress in cartilage varies in the range of 0.05–0.7 MPa [36, 37].

14.1.3.4 Friction Behaviour

Friction quantifies the resistive force between two subjects as they are in contact with each other and move laterally relative to each other. Native cartilage gives mobility to the bone by its lubrication properties. Under high dynamic and static load conditions, the coefficient of friction for human cartilage is very low, and it is in the order of 0.001 [38]. Several theories have been proposed to explain low friction force between cartilage surfaces. These include but not necessarily limited to elasto-hydrodynamic lubrication [50, 51] and fluid pressurisation theories [52, 53]. It shows that the synovial fluid becomes thicker due to the deformation of applied compression on cartilage, which gives superior lubrication properties to articular cartilage. Compression and lubrication properties of cartilage therefore are closely correlated.

14.1.4 Articular Cartilage Aging and Pathology

Articular cartilage is a highly resilient connective tissue with very important biomechanical role in the human body. Cartilage has an essential function in the movement of the human body and mobility of bones against each other. Any breakdown in this tissue results in substantial reduction in standards of living and substantial level of pain. Cartilage from skeletally immature, mature and older patients exhibits very different biomechanical and biological properties. As cartilage ages, it becomes more prone to injuries [54, 55]. The damage to this tissue might result from torsional loading, joint misalignment, foreign bodies in the joint and osteoarthritis. It is critical to first understand the aging process of cartilage and

then the factors that lead to cartilage damage to be able to develop an effective treatment strategy for articular cartilage defects.

Similar to many organs, aging has significant impact on the characteristics of cartilage. Skeletally mature, immature and old cartilages are different in respect to their thickness [54, 55], vascularisation, chondrocyte population [56] and chondrocyte regeneration quality [57, 58]. Immature cartilage contains blood vessels as it is still undergoing tissue formation (endochondral ossification). It is also thicker than mature cartilage and its thickness decreases by aging [54, 55]. The population of chondrocyte cells also decreases overtime by aging [56]. In addition to chondrocytes' cell reduction in mature cartilage, aging also brings lower metabolic activity, increases apoptosis (a process of programmed cell death) and elevates passive responses of cells to growth factors [57, 58].

Biomechanical properties of cartilage change by aging. For example, the degree of crosslinking in collagen increases [59] as protein and lipids are covalently bonded with sugars (i.e. glycation). The length and molecular weight of proteoglycan also decrease as its protein content decreases [60]. These variations reduce the stiffness/strength of cartilage and induce the risk of tissue failure [61]. Studies showed that generally a cartilage approaches its peak in elasticity in 40 years of age, while the viscoelastic energy reaches its maximum value earlier within 16–29 years old. Both elasticity and viscoelasticity decline at a steady rate [62]. Collagen alignment also decreases in the middle and deep zone of cartilage by age which in turn decreases the tensile properties of cartilage [63].

Both biological and biomechanical properties of articular cartilage decrease by time. The problematic issues are, therefore, the lack of healing responses and also the decrease in the initial mechanical properties of cartilage. The overall changes in the biochemical properties of articular cartilage are schematically shown in Fig. 14.2.

Repeated non-physiological loading on tissue over the years and biological, biomechanical and other systematic changes on articular cartilage are the factors that make the tissue vulnerable to damage. Cartilage damages are either due to injuries or osteoarthritis. In addition to the impact of aging on the nature of articular cartilage, other factors such as hormonal therapy and diseases play a role in properties of cartilage. For instance, oestrogen replacement in ovariectomised sheep leads to substantial changes in the structure and mechanical properties of articular cartilage, which is relevant to menopausal women [64]. In addition, anti-inflammatory treatments such as intra-articular administration of methylprednisolone diversely affect the properties of articular cartilage [65]. Some diseases such as diabetes also have negative impact on cartilage properties [66].

14.1.4.1 Cartilage Injuries

Sudden impact, repeating loads, damage in other connective tissue and also foreign bodies affect the loading profile on cartilage and, therefore, result in cartilage damage as shown schematically in Fig. 14.3. The effect of load and stress on

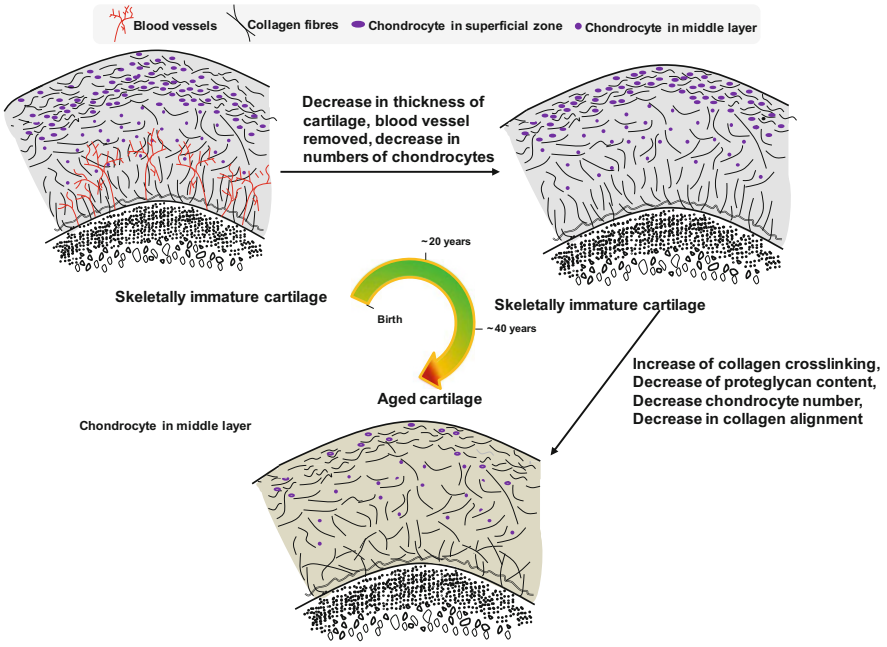

Fig. 14.2 Biochemical changes in the structure of cartilage from skeletally immature cartilage to mature and aged cartilage (The image is regenerated by accumulating data from different references [54, 55, 60–63])

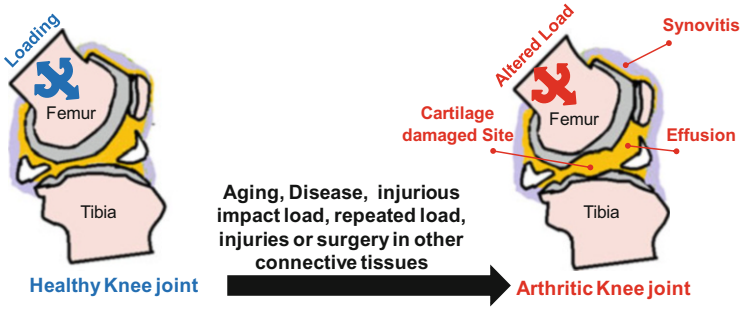

Fig. 14.3 Cartilage injuries, causes and their effect on loading profile in connective tissue

cartilage is a function of frequency of loading and their period of exposure. Due to the viscoelastic nature of articular cartilage and based on the theory of confined compression moduli, both load and strain rates significantly affect cartilage stiffness which should be taken into account for determining the load of injury. For instance, during the normal physical activity, the knee cartilage is subjected to 3.5 times the body weight [24]. Therefore, for a male with an average weight of 70 Kg, the force on his joint is subject to around 1.5 MPa stress by assuming the surface

area of tibial plateau of 1670 mm^2 [67]. During running, this load approaches to 50 $MPa.s^{-1}$ and 100 $MPa.s^{-1}$ that depends on the stress rate. However, the impact of loading from injury on cartilage is any load with time between two peak loads in the order of milliseconds (e.g. <30 ms) along with at least one of the following criteria: (i) stress rate greater than 1000 $MPa.s^{-1}$, (ii) loading rate above 100 $kN.s^{-1}$ or (iii) strain rate in excess of 500 $mm.mm^{-1}.s^{1}$[68]. It is important to note that these criteria are only applied to healthy cartilage; aging and other systematic changes in cartilage make cartilage more vulnerable to damage.

14.1.4.2 Osteoarthritis

Osteoarthritis (OA) is one of the major health issues worldwide and it is a burden for elderly population. Symptomatic OA manifests itself with severe pain in the joint and lack or even loss of mobility in the defected joint [69, 70]. The cost of treatment and healthcare for promoting the lifestyle of patients who suffered from OA is enormous. The Australian Institute of Health and Welfare reported that OA affects more than 1.3 million Australians in 2007 [71]. The existing trend suggests that in 2050, more than seven million Australians will be affected by OA [72].

The OA mostly caused by biochemical and biomechanical changes that systematically occur in articular cartilage. It might also be attributed to traumatic joint injuries such as accident and sport. It is estimated that approximately 11 % of Australian working force (young and middle-aged population) suffers from arthritis (mainly OA) [72].

14.1.5 Natural Articular Cartilage Repairing Process

There are two types of cartilage damages: partial- and full-thickness defects [73]. Partial-thickness damage is limited to the cartilage, and the subchondral bone is not violated in this type of damages. The site of defect has, therefore, no access to bone marrow-derived stem cells [3]; the cartilage thus lacks in intrinsic capability to heal the defected site [3, 74]. The defected site in this case might significantly expand by time since there is no healing process naturally commenced by the tissue [74]. This damage eventually leads to OA by aging. On the other hand, in full-thickness cartilage defect, the lesion has access to bone marrow-derived stem cells which allows the cartilage to undergo some spontaneous healing process [75].

In the full-thickness damage, also called osteochondral defect, the healing process extends greatly after 2 weeks. The subchondral blood vessels bring progenitor cells (mainly mesenchymal stem cells) into the defected site. These cells are more bioactive in generating cartilage ECM compared to natural chondrocytes. The migration, differentiation and proliferation of mesenchymal stem cells (MSC) at the defected site take place 2 weeks post injury. The blood from subchondral bone forms a fibrin clot, which contains platelets. These can secrete biofactors to recruit

mesenchymal stem cells to form cartilage. MSCs produce collagen type II and collagen type I to fill the defected site. After approximately 6–8 weeks, the defected site is filled with collagen type I and type II [76–78]. From this point onwards, the production of collagen type II is completely switched to collagen type I. After 1 year the repaired cartilage consists of hyaline and fibrocartilage tissue [75, 79]. Fibrocartilage tissue suffers from lack of mechanical strength and physical stability [80]. These lead to cartilage matrix degeneration through fibrillation [81], chondrocyte loss and dedifferentiation and GAG loss [82]. Subsequently, deep cracks appear within the structure of cartilage after the first year, and subsequently complete failure of cartilage occurs [76–78].

Lack of chondrogenesis cell in partial- and also formation of fibrocartilage in full-thickness articular cartilage damages might lead to the OA occurrence in a patient. Therefore, articular cartilage damage must be treated with therapeutic strategies at very early stages.

14.2 Therapies for Articular Cartilage Damages

Several cartilage repair techniques have been proposed to treat different types of cartilage defects. Conventional therapeutic strategies are classified in two groups, therapeutic intervention without biological compounds or with active biologics. In this section an overview of these two groups of therapies is provided.

14.2.1 Therapeutic Intervention without Active Compounds

No biological compound is used in this group of therapeutic interventions. The efficiency of these treatment techniques relies on their stimulation impact on chondral or subchondral tissue for cartilage regeneration. The main techniques in this category are lavage, chondral shaving, debridement, Pridie drilling and microfracture that are described briefly in this section.

14.2.1.1 Lavage and Arthroscopy

Lavage or irrigation of the defected site with solution of sodium chloride, Ringer or Ringer and lactate has been practised for treatment of OA of the knee. The defected site in this technique is rinsed, using closed-needle hole or arthroscopic techniques [83]. It is claimed that thorough rinsing of tissue removes intra-articularly active pain signaling molecules from the defected site, thus leading to the relief of pain in patients. The irrigation of cartilage surface might also lead to extraction of proteoglycan and aggrecans from the superficial surface of articular cartilage, which might temporarily improve the adhesion of repaired cells at the defected site

[84, 85]. In clinical studies, however, lavage (irrespective of whether it is performed with closed-needle hole or arthroscopic techniques) appears to resolve the issue for a short period of time [86, 87]. This approach is more effective for the patients with history of trauma (such as sports injury, tearing of the ligament or meniscus or traumatic structural lesions) compared to the cases with other types of OA [84, 88].

14.2.1.2 Chondral Shaving

Chondral shaving is conducted with arthroscopic techniques to mechanically remove the defected site, using appropriate surgical instrument. In recent years, it is only performed for treatment of chondromalacia patellae pain. The results of in vivo studies suggest that this procedure does not have any impact on cartilage regeneration in mature rabbits after 12 weeks. The remaining cartilage also underwent degeneration due to apoptotic cell loss [89–91].

14.2.1.3 Debridement

This approach involves meniscectomy process in which the chondral defected site is mechanically isolated, the lesion site underwent lavage process and all free bodies are removed from the joint [92]. The removal of chondral defect tissues leads to cell apoptosis and thus cartilage degeneration at the surrounding tissue. In addition, the meniscectomy process leads to skeletal malalignment and significant changes in the loading profile at the joint. The results of in vitro, in vivo and clinical studies demonstrated that debridement alone exacerbates the osteoarthritic conditions [93, 94].

14.2.1.4 Pridie Drilling

In this technique, therapeutic holes at the defected site are drilled from the articular cartilage surface into the subchondral bone marrow. The drilling is performed close to the defected articular cartilage sites. This stimulates the spontaneous repair reaction at the defected site [95–97]. The intervention to drill the holes (2 mm–2.5 mm in diameter) includes such methods as osteochondritis dissecans, which is a painful process. It is claimed that this technique is beneficial for OA patients by promoting the migration of chondrogenesis cells to the surface of the defected site [95, 98, 99]. In vitro and in vivo studies showed that Pridie drilling on rabbit resulted in formation of fibrocartilage tissue. This method may be accompanied with inflammation at the defected joint [100]. Clinical studies, however, showed that Pridie drilling is a safe treatment for cartilage repair for a short period only [101].

14.2.1.5 Microfracture

The biological basis of this technique is the same as Pridie drilling. The only differences between Pridie drilling and microfracture techniques are the size and numbers of holes [102]. In microfracture technique, micro-sized pores (from 500 μm to 1 mm in diameter) are generated within the entire articular cartilage lesion site. This technique is efficient for lesions with average size of less than 2.3 cm^2. The depth of holes is approximately 4 mm and 3–4 holes are generated per cm^2 of the cartilage defected site. The smaller diameter of holes in this technique, compared to Pridie technique, leads to less adverse impact on biomechanical properties of the subchondral bone [103]. Animal studies confirmed the regeneration of tissue by using this method. In clinic, the microfracture technique is mainly applied for articular cartilage damages in young athletes [102]. Positive results are reported for cartilage treatments by microfracture; in 75 % of cases, pain relief and improvement in joint functionality are reported. However, application of this method for the patients with OA with lack of adequate number of bone marrow-derived mesenchymal stem cells is not promising [88, 104].

14.2.2 Allogeneic Osteochondral and Chondral Grafting (Full Osteochondral Allograft)

In this technique, allogeneic osteochondral or chondral graft is used to fill the articular cartilage defected site, which has no intention to stimulate cartilage repair responses. It relies on replacing the defected tissue with healthy cartilage that is usually derived from cadavers [105, 106]. Patients with substantial osteochondral defects such as tumour reaction, osteonecrosis, broad OA, osteochondritis dissecans and extensive trauma have benefited from this treatment technique. However, there are immunological problems associated with this technique which might lead to complete rejection of the grafted tissue. Animal studies showed that allogeneic grafts survived under immunosuppressive conditions. In addition, matching the histocompatibility between the grafted and native tissue is required to reduce cell-mediated cytotoxicity and antibody titre [107, 108]. Clinical studies with this treatment technique revealed that the immunoresponses in the human body are less than those in animals [109–111]. In addition, the osteochondral transplant grafts survive for a longer period of time even after freezing and lyophilisation [109–111]. The success rates of 65–85 % are reported for this treatment technique even after follow-up period of 10 years [112, 113]. The application of this method, however, is limited due to scarcity of fresh donor and problems associated with the handling and storage of frozen allograft tissues. It is also critical to contemplate the risk of disease transmission for allogeneic osteochondral and chondral grafting [88].

Table 14.2 Results from clinical studies of MF and FOA techniques for articular cartilage defect treatment

Treatment technique	No. of joint	Average age (yrs.)	Lesion size (cm^2)	Results, year (successful cases %)[1]	Refs.
MF	11	38.5	2	1 (100), 1.5 (0)	[114]
FOA	20	42	NR	2(90), 5 (35), 10 (15)	[115]
	14	37	NR	2(57), 5(43), 10 (29)	[116]

[1]For example 2 (90) represented: after 2 years of follow-up time, there are 90 % of successful cases

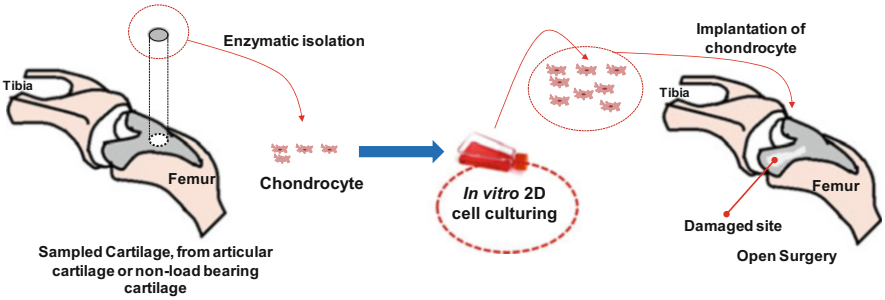

Fig. 14.4 Schematic overview of classical ACI technique for articular cartilage treatment

Among all the previously mentioned techniques for cartilage repair, microfracture (MF) and fresh osteochondral allograft (FOA) are the only methods with acceptable biological basis. Different human studies investigated the efficacy of these techniques for treatment of articular cartilage defect. The results from the clinical studies on efficacy of MF and FOA techniques are summarised in Table 14.2.

14.2.3 Autologous Implantation Technique

Different cell-based therapies have been proposed such as autologous chondrocyte implementation (ACI) technique for treatment of cartilage defects [117]. ACI is established in 1994 [117] and thereafter fully approved by the US Food and Drug Administration (FDA) in 1997 [118]. This treatment technique is schematically shown in Fig. 14.4. The first generation of ACI technique provided significant and long-term benefits for patients as per providing tissue functionality and pain relief with better lifestyle. The major problematic issues in this method are the serious damage that imposed at the donor collection site [88]; monolayer in vitro culture of chondrocyte, which leads to the formation of fibrocartilage [119]; and also hypertrophy or ossification of the patched periosteum [120].

14.2.4 Motivation for Tissue Engineering

A demand for tissue engineering of cartilage arises from two perspectives, the high risk of failure in using traditional treatment techniques and the large numbers of patients suffering from articular cartilage defects. Articular cartilage damages can occur during childhood, in young adults and also in elderly people. Osteochondrosis and osteochondritis are joint defects that occur mainly in children due to the lower capacity of skeletally immature cartilage to high range of stresses. The rate of knee cartilage injuries is more than 25 % of participants in any sport activity. In addition, articular cartilage damage in elbow and shoulder joints commonly occurs in athletes who play baseball and cricket, for example [121–123]. It is estimated that one out of three children suffers from severe cartilage damage requiring medical treatment. The cost of treatment for articular cartilage damage is estimated to be $ 1.8 billion per annum considering that more than 30 million school-aged children just in the USA participate in sport activities [124].

During the last decade, the first generation of ACI technique has been the major approach for the treatment of cartilage defects [118]. Cartilage tissue engineering is considered as a second generation of ACI and has been proposed to address the issues with current cartilage treatments to improve autologous chondrocyte implementation techniques [125–129]. Tissue engineering involves using suitable type of cells, scaffolds and bioactive molecules such as growth factors [130]. Tissue engineering approaches will be discussed comprehensively in the next section.

14.3 Tissue Engineering of Cartilage

Tissue engineering is the modern approach in treatment of articular cartilage defects using suitable types of cells, biomaterials and bioactive molecules such as growth factors [130]. The schematic overview of tissue engineering approaches for treatment of damaged cartilage is shown in Fig. 14.5. Briefly, suitable cell types such as chondrocyte and stem cell (e.g. from autologous sources) are harvested from patients, and then the cells are cultured in vitro. Chondrogenesis biofactors may be used to promote the capacity of cells for cartilage regeneration. A biomaterial matrix is required for 3D cell growth, which is comprised of scaffolds for in vitro cell growth or a cell carrier system for in vivo applications. For cartilage tissue engineering, there are two approaches, namely, in vitro and in vivo cartilage regeneration. In the former, cells are seeded on the scaffolds and the construct is cultured for a specific period of time. The resulted autologous tissue is then transplanted into the patient via arthrotomy (open surgery) or arthroscopy. In the latter, the suspension of cells in bioengineered matrix is injected into the articular cartilage damaged site. Selection of proper cells and incorporation of chondrogenesis biofactors and the bioengineered matrix have pivotal role in

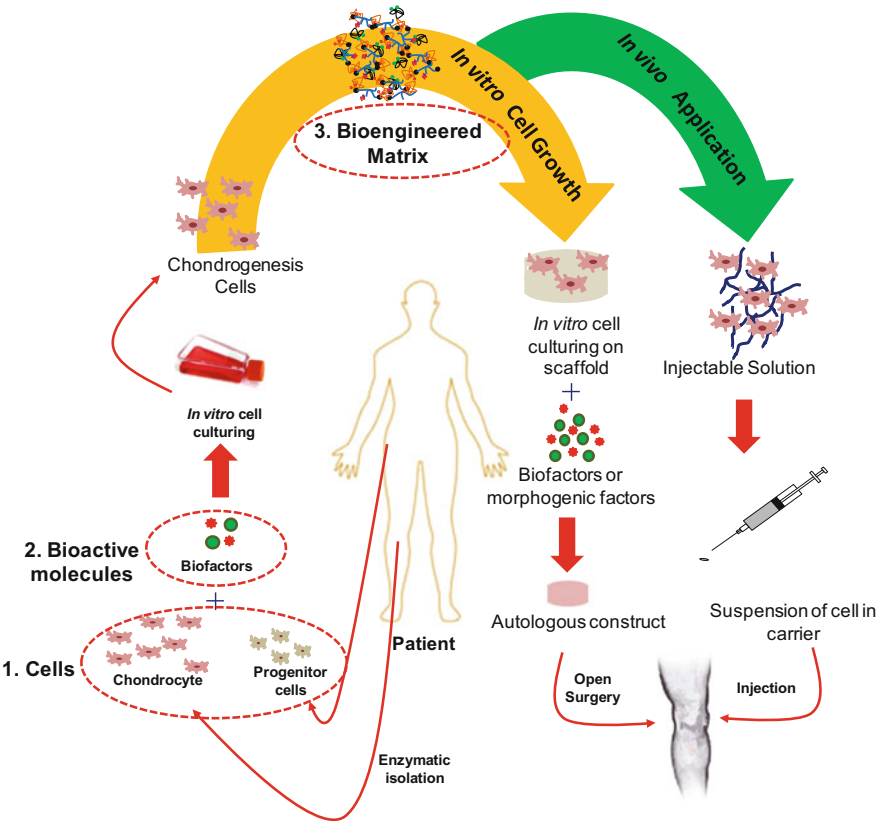

Fig. 14.5 Modern ACI treatment technique for articular cartilage repair

modern ACI technique [131]. Suitable cell types, bioactive compounds and characteristics of biomaterials for cartilage tissue engineering are discussed below.

14.3.1 Cells

Several different cell sources are proposed for cartilage tissue engineering. Autologous chondrocyte, isolated from hyaline or articular cartilage, however is regarded as the most common cell type. By extracting cells from patient's own body, the issue of any immune response can be avoided. In addition, chondrocyte is already differentiated into target cartilage phenotype and can secrete cartilage ECM, such as collagen and proteoglycan [132]. For the cases with extensive cartilage damage or disease, procurement of autologous chondrocyte is not possible. An alternative method is to use allogeneic chondrocyte from a donor tissue. This approach, however, is limited by disease transmission and also host body immune response.

In general, the major issue in the application of both allogeneic and autologous chondrocytes is the limited numbers of available cells, the instability of chondrocyte in monolayer culture and the lack of intrinsic repair capacity [131]. A promising alternative cell source for cartilage tissue engineering is autologous progenitor cells or stem cells [133].

Adult stem cells reside throughout the different parts of the body and can be differentiated along different pathways such as chondrogenesis and osteogenesis lineages. Stem cells from fat tissue (adipose) and bone marrow mesenchymal stem cells (BM-MSC) have been extensively used for different tissue engineering applications [133]. Stem cells exhibit multipotent differentiation capacity which can be used to improve clinical treatment techniques in various locomotion tissues [134], including the bone [135], fat [136], ligament [137, 138] and articular cartilage [136, 139].

Stem cells exhibit high proliferation and growth rate, and thus for cell procurement, little sampling from donor site is required. The sampling process for adipose stem cells is also straightforward and less invasive compared to chondrocyte procurement. The process therefore is advantaged by minimal donor site morbidity and pain level. For a direct comparison between the efficacy of chondrocyte and stem cells for cartilage repair, two populations of patients are treated with either chondrocyte or stem cells [140]. The results from this study showed that articular cartilage defect treatment with autologous stem cells is as successful as the one using chondrocyte. By considering the limited number and low quality of chondrocyte in the defected tissues, treatment with stem cells has more potential for ACI applications.

The use of BM-MSC also enables simultaneous repair of the bone and cartilage, which ultimately results in promoting remodelling and integration of the regenerated cartilage with the host tissue [141, 142]. However, the monolayer culture of both stem cells and chondrocytes possesses the issue of telomere shortening,[1] loss of proliferative rate and multipotency [143–145]. An alternative source for stem cell is embryonic stem cell that has unlimited capacity for proliferation in monolayer culturing and is promising for a broad range of tissue engineering applications [133]; however, commercialisation of embryonic stem cell-based discoveries is banned in many countries, such as European countries.

14.3.2 Bioactive Factors

Regeneration and growth of cartilage and its integration with the surrounding tissues rely on the biochemical signaling of cells within the regenerating tissue. Bioactive factors include small drug-like molecules, growth factors or any other

[1]Human (and other) somatic cells without telomerase gradually lose telomeric sequences as a result of incomplete replication.

molecule that has cell motif sites and can bond with cells to create biological responses. The intensity, duration and sequence of stimulation factors affect the metabolic activity of cells and their ECM exertion.

14.3.2.1 Chondrogenesis Small Drug-Like Molecules

Small drug-like molecules have developed to selectively regulate transcriptional factors of subcellular localisations and activities [146, 147]. For cartilage tissue engineering, kartogenin is identified as a small molecule which can promote chondrogenesis and chondrocyte differentiation of MSC by disrupting interaction of filamin-A with transcription factor CBFβ. This regulates the CBFβ-RUNX1 transcriptional program, which ultimately induces chondrogenesis. The positive effect of this molecule on chondrogenesis has been studied and proved by in vitro and two animal model studies that were subjected to osteoarthritis disease. Application of drug-like selective regulator molecules for transcriptional subcellular factors is limited for their intrinsic complexity. Chondrogenesis of BM-MSC is, therefore, mostly promoted by using exogenous anabolic factors, loaded within the network of scaffold.

14.3.2.2 Growth Factors

Chondrogenesis can be promoted by using exogenous anabolic factors [148]. The growth factors include but not limited to TGF-β [148, 149] and BMP family [150–152] that have been used to promote chondrogenesis. Most commonly used growth factors for articular cartilage applications and their in vitro and in vivo effects are summarised in Table 14.3.

TGF-β superfamily is involved in tissue repair and inflammation responses following a cartilage injury [153, 154]. Controversy results are obtained for the effect of TGF-β superfamily for cartilage tissue engineering. For therapeutic application, TGF-β superfamily can prolong cartilage life by minimising or even eliciting the biological and biomechanical changes in cartilage over the years. For tissue engineering applications, some studies confirmed the positive effect of TGF-β1 in chondrogenesis proliferation and growth. The positive effect of TGF-β1 in cartilage tissue engineering, however, depends on the differentiation state of cells; for freshly isolated chondrocytes, addition of TGF-β1 had no significant effect on cell growth. The incorporation of growth factor, however, significantly affects the proliferation and proteoglycan synthesis of chondrocyte, cultured in vitro after 1 week of isolation [155]. TGF-β1 had negative impact for cartilage repair. For instance, it is observed that the synthesis of proteoglycan by arthritic chondrocytes is decreased when adding TGF-β1 to the media [156]. This reduction may result in formation of articular cartilage with unfavourable properties.

Bone morphogenetic protein (BMP) superfamily also plays an important role in endochondral bone and cartilage formations. More than 20 types of BMP have been

Table 14.3 Different growth factors, used for articular cartilage tissue engineering, and results achieved in vitro and in vivo

Growth factor	Results	Refs.
TGF-β1	No significant effect on freshly isolated cells	[153–158]
	Improve proteoglycan synthesis on chondrocytes after 1 week of post-isolation	
	Proteoglycan synthesis decreased by arthritic chondrocytes	
BMP-1	Promotes the expression of proteoglycan and collagen more than TGF-β1	[154, 159, 160]
BMP-2	Regulates proteoglycan and collagen production and helps to adjust the biomechanical and biochemical properties of regenerated tissue	[159, 160]
BMP-4	Increases cell proliferation, proteoglycan formation as well as bone formation	[160, 161]
BMP-7	Decreases collagen type I expression, increases proteoglycan and ECM production and cellular proliferation	[162, 163]
BMP-12 and BMP-13	Promotes synthesis of GAG, less significant effect compared to BMP-2	[164]

identified. In tissue engineering applications, BMPs are commonly used for bone repair and regeneration. For cartilage tissue engineering, BMPs also enhance the osteochondral integration of the defected cartilage by promoting osteogenesis and chondrogenesis at the defected site. It is shown that BMP-1 has more stimulation effect on cells to express proteoglycan and collagen compared to TGF-β1 [165]. BMP-2 also regulates the formation of collagen and proteoglycan from chondrocytes [159, 160]. In addition it promotes the healing process of cartilage defects in vivo [154]. BMP-4 stimulates proteoglycan expression and osteochondral tissue formation and also enhances cellular proliferation for articular cartilage regeneration [160, 161]. Addition of such BMP growth factors as BMP-7, BMP-12 and BMP-13, particularly BMP-2, enhances ECM formation [160] and cell proliferation [162]. In addition, BMP-7 suppresses expression of collagen type I and differentiation of fibroblast in vivo [163], which inhibits the formation of fibrocartilage at the defected site [164].

14.3.3 Biomaterials for Cartilage Regeneration

In a tissue engineering approach, biomaterials are used either for fabrication of 3D scaffolds for in vitro tissue regeneration or synthesis of an injectable vehicle to deliver cells/drugs to the defected site of cartilage for in vivo cartilage regeneration. In both techniques, biomaterials play a critical role in cellular growth and tissue regeneration. The physicochemical and biological properties of biomaterials substantially affect the functionality of repaired cartilage [166].

14.3.4 Properties for Biomaterials

Biomaterials are used as physical supports for cell growth and to avoid spillover and asymmetric distribution of cells. This is important to promote synthesis of cartilage ECM and to regenerate functional tissues. In case of cartilage tissue engineering, the biomaterials support the newly formed tissues to promote the integration of repaired cartilage with host tissue. The physical and biological properties of biomaterial used for scaffold fabrication play important roles in cell responses, adhesion and infiltration to form functional 3D structure cartilage [166]. Biomaterials must be biocompatible and exhibit cell adhesive surface properties with suitable microstructure for 3D cell proliferation. They also must be mechanically strong to support the newly regenerated tissue in vivo.

14.3.4.1 Biocompatibility and Surface Properties

Particular attention has been paid on the biocompatibility of biomaterials. Biocompatibility is predominantly affected by the chemical and biological properties of biomaterials used for cartilage repair. A biomaterial is biocompatible when it elicits neither cytotoxic effects nor inflammatory responses within the surrounding tissue [167].

Surface properties of biomaterials affect in vitro cellular adhesion, phenotype maintenance, intracellular signaling and in vivo cell recruitment, healing and osteochondral integrations [168–170]. Cell responses to the ECM matrix and the new regenerated tissues are mediated through an interfacial layer formed on the surface of scaffold or biomaterial. This layer is formed by non-specific absorption (not chemical conjugation) of ECM proteins with a biomaterial when it is in a proper physiological environment. As an example, hydrophilic properties of a polymer such as gelatine and poly(vinyl alcohol) promote the formation of interfacial layer and thus enhance the adhesion of cells [171]. The incorporation of peptides in the form of long chain of ECM proteins (such as fibronectin, laminin, elastin and collagen) or short peptide sequences, derived from ECM proteins (such as arginine–glycine–aspartic acid), provides cell binding sites on scaffold to mediate cell responses, thereby enhancing tissue regeneration [172, 173].

14.3.4.2 Microstructural and Mechanical Properties

The microstructure, porosity and pore interconnectivity are critical factors for biomaterials that are used for tissue engineering. Porosity allows the cell migration into the 3D structure of scaffold, and pore interconnectivity is a key factor for nutrient, oxygen and waste transfer into and from the cells in scaffolds [174–178]. For each cell type a range of pore sizes is required to mimic the innate tissue and allow the cells' adhesion and regeneration [179]. For in vitro cartilage tissue

engineering, the average pore size in the range of 250–500 μm is recommended [180]. Small pore size resulted in occlusion and obstruction of pores, which then prevents cellular penetration within the 3D structure of scaffolds. Pores in the range of 75–100 μm can result in growth of un-mineralised osteoid tissues [181]. Additionally, only fibrous tissues penetrate within a scaffold with an average pores size of 10 μm or less. Low average pore size, therefore, leads to the formation of fibrocartilage rather than cartilage. Meanwhile, the mechanical strength of physical supports decreases by increasing the average pore size. Therefore, it is important to tune the void volume (or average pore size) in scaffolds for cartilage tissue engineering to both enable the migration of chondrogenic cells and maintain the required structural strength [178].

For in vivo cartilage tissue engineering, chondrogenic cells are suspended within the polymeric matrix, and the suspension is gelled in situ. There is no need for cells to immigrate through pores, and thus large pores from 250 to 500 μm are not necessary anymore. It is shown that small pores that are less than 100 μm in biomaterials induce osteochondral formation in vivo, while a larger pore size leads to osteogenesis before cartilage formation [182]. As an example, chondrocyte maintains its phenotype, and cartilage ECM is produced by encapsulating cells within an injectable chitosan/starch/β-glycerol hydrogel with average pore size in the range of 19.8–26.4 μm [183].

The biomaterials provide a temporary mechanical support to bearing in vivo loadings and stresses during the tissue regeneration. One of the key factors in successful tissue engineering is to develop a mechanically strong scaffold that its degradation rate corresponds to the rate of regeneration of ECM. External loads and stresses stimulate cell proliferation, remodelling and tissue regeneration [184–186]. It is essential that loads gradually transfer from the biomaterials (supporting cell growth and tissue regeneration) to the regenerated cartilage to promote tissue remodelling. It is therefore, critical to control the degradation rate and mechanical strength of scaffold to ensure sufficient structural integrity of matrix is retained during the regeneration of cartilage [187]. The rapid degradation of biomaterials is not desirable as it provokes excessive stresses and impact loads to the developed tissues prior to sufficient growth and remodelling cartilage. This effect might result in complete failure of regenerated tissue construct. On the other hand, the slow degradation of biomaterials may shield the cells against external stimulating stresses, which can decrease the proliferation and growth rate of chondrogenesis cells. It is important to design scaffolds with the mechanical properties that mimic cartilage tissues for in vitro and in vivo applications. The compressive modulus of native cartilage varies from 0.08 to 2.1 MPa [31, 32, 39] and its tensile modulus in between 4.8 and 25 MPa [25]. It might be possible to use biomaterials that may not fully meet these mechanical properties, as the newly formed cartilage might provide extra structural integrity after implantation.

14.3.5 Scaffold for In Vitro Tissue Engineering

Tissue engineering techniques have been proposed for the treatment of articular cartilage defects. In modern ACI, 3D scaffolds are used to address the issues associated with monolayer culture of chondrogenesis cells in vitro such as dedifferentiation and ossification of chondrogenesis cells [126]. In vitro cell studies showed that chondrocyte phenotype can be maintained up to 8 months of post seeding in 3D scaffold [188]. In the clinical approach for the treatment of cartilage defect, chondrogenesis cells (chondrocytes or stem cells) are seeded on a biodegradable scaffold and cultured in vitro. The construct is harvested in vitro, and cell viability and chondrogenesis of cultured cells are continually tested for a period of 6 weeks. Subsequently, the regenerated tissue, formed within the scaffold, is transplanted at the defected site (most commonly with open surgery). The efficacy of this technique for the treatment of articular cartilage defect is substantially affected by the characteristics of scaffolds used for in vitro cell growth [188]. Different biomaterials are attempted for the fabrication of scaffolds for cartilage regenerations. These include biodegradable synthetic and natural polymers that are either hydrophobic or hydrophilic.

14.3.5.1 Synthetic Polymers

Synthetic polymers exhibit reproducible and predictable physicochemical, mechanical and degradation properties, all of which can be closely tuned to fulfil the requirements. In addition, the risk of toxicity, immunogenicity and infections is low in the application of synthetic polymers for different biomedical applications since they constituted of well-known molecular structure. Poly(α-hydroxy esters) are widely used for cartilage tissue engineering, and they are often processed to form 3D porous structures with hydrophobic properties. Meanwhile, poly(vinyl alcohol) (PVA), poly(ethylene glycol) and polyacrylates (PEs) formed hydrophilic structures with high water content, called hydrogel. Hydrogels are a class of biomaterials, composed of natural or synthetic polymer chains with very high water content (above 30 wt%) [189]. Hydrogels become the material of choice as scaffold for cartilage tissue engineering applications attributable to their hydrophilic properties, high water content, superior permeability of nutrients, long chain molecules, proteins and oxygen [174, 179, 190–193]. Some of the in vitro and in vivo outcomes, from the applications of main synthetic polymers as scaffold for tissue growth, are summarised in Table 14.4 and comprehensively discussed in the following sections.

Table 14.4 The pros and cons observed from in vivo and in vitro applications of synthetic polymers for cartilage repair

Biomaterial	Positive outcomes	Negative outcomes	Refs.
PGA	Maintenance of chondrocyte phenotype in vitro and in vivo	Initial in vitro cell culturing is necessary to initiate ECM formation in vitro	[194–197]
PLGA	Can maintain the chondrocyte phenotype in 2 weeks of in vitro	Initial in vitro culturing is necessary prior to in vivo implantation	[194–197]
PLA	Minimising release of acid as the result of degradation	Biochemical properties inferior to native tissue	[198, 199]
	Maintaining structural mechanical support for a long time	Low cell adhesive surface properties	
	Cartilaginous appearance		
PCL	High structural integrity in in vitro culturing	Very low chondrocyte adhesion and proliferation	[200–202]
PVA	Similar water content as natural cartilage	Dedifferentiate chondrocyte due to very low mechanical properties	[203–207]
	Has high growth factor loading capacity		
	Osteochondral		
PEG-ma	Photo-crosslinkable, suitable for cell encapsulation	Lack of mechanical strength for load-bearing cartilage	[208–210]
	Chondrogenesis cells induced cartilage ECM formation by the addition of required growth factors		
PEG-da	Photo-crosslinkable and suitable for cartilage regeneration	Lack of cell motif sites	[211–215]
	Tuneable mechanical and physical properties to match the requisites for cartilage repair		
	Can act as a crosslinking agent for different PEG-based polymers		
OPF	Formation of cartilage-like tissue by using OPF hydrogel for cartilage regeneration both in vitro and in vivo	The lack of cell motif sites might lead to low chondrogenesis cell proliferation and growth	[216–218]
		Non-biodegradable properties of this hydrogel might lead to unpredictable problems and unreproducible results	

Poly(α-Hydroxy Esters)

Poly(α-hydroxy esters) (PHEs) is a class of synthetic biodegradable polymers that have been used for the preparation of scaffolds for cartilage tissue engineering.

They include poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactic-*co*-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL). Based on the position of methyl group in the lactic acid monomer, there are three stereoisomers of PLA (D (−), L(+) and D,L). The degree of crystallinity in PLLA is higher than those of PDLA and PDLLA. All PHE polymers have had the FDA approval for different biomedical applications [219].

PHEs can be easily processed, and their degradation and mechanical and physical properties are tuneable over a wide range by changing their molecular weight, crystallinity and copolymer composition. Their degradation process via a random, bulk hydrolysis of ester bonds in the polymer chain, however, might induce premature bulk failure of scaffolds. In addition, the release of acidic degradation products can cause strong inflammatory responses [220, 221]. These synthetic polymers degrade to monomeric acids and thereafter to carbon dioxide and water through de-esterification phase. PGA molecules are degraded to glycine and PLA to lactic acid. In vivo, polymeric degradation products are exerted by natural pathways via respiratory routes and renal filtration [222].

The kinetics of degradation in PHEs are affected by different factors, including (i) copolymer composition, (ii) molecular weight, (iii) degree of crystallinity, (iv) polydispersity index, (v) structural morphology (pore size and porosity) and (vi) distribution of chemically active compounds such as proteins in their structures [223, 224]. In PLGA scaffolds, for example, the degradation rate strongly depends on lactic/glycolic monomer ratio and crystallinity of lactic monomer [223]. PHE scaffolds exhibit two profiles of degradation, surface and bulk degradation. Surface degradation presents easier diffusion of soluble oligomer and neutralisation of the carboxylic end groups by surrounding buffer solution in vitro or in vivo. Conversely, the degradation rate in bulk is promoted by autocatalysis [225]. Therefore, PLA with a methyl pending group degrades slower compared to PGA without any pending group. In addition, the hydrolysis of amorphous polymers such as PDLLA is faster due to lack of crystalline regions [225].

The mechanical properties of PHEs depend on the molecular weight, microstructure and crystallinity of polymers. In general, PGA is more hydrophilic and rigid, whereas PLA is more flexible (higher elastic modulus) with low degradation rate. Copolymerisation of these two polymers in different ratios can be used to form PLGA polymer with optimised mechanical, degradation and water uptake properties. PLGA has amorphous structure because PLA and PGA are not tightly coupled and, therefore, it exhibits higher degradation rate compared to both PGA and PLA [226].

PLA, PLGA and PGA are used for cartilage tissue engineering in the forms of fibres (electrospun as discussed in Chap. 8) or foamed scaffolds. In vitro and in vivo studies showed the maintenance of chondrocyte phenotype on PLGA and PGA scaffolds [194, 197]. But functional cartilage could only be achieved by short-term in vitro chondrocyte culturing on the scaffolds and then in vivo implantation of constructs [195, 196]. Electrospun PLA scaffolds have been used for in vitro cartilage tissue engineering. Chondrocyte maintains its chondrogenic phenotype after 7 days of culturing on PLA [199]. One-year animal studies using allogeneic

perichondrial cells [227] and autogenous perichondrial cells [198] on PLA scaffold showed inconsistent subchondral bone regeneration.

In addition, regenerated tissues have been disadvantaged by inferior biochemical properties compared to natural cartilage [198]. The limited application of these types of polymers for cartilage tissue regeneration is due to the poor cell adhesion to their surface. PLA has been conjugated with monomethoxy poly(ethylene glycol) (mPEG) to increase its hydrophilicity and cellular adhesive properties [228]. The compressive modulus of PLA/mPEG polymer, however, decreased significantly compared to PLA [228].

Poly(ε-caprolactone) or PCL is aliphatic linear polyester, which undergoes autocatalysed bulk hydrolysis. The degradation process of this biomaterial is slow due to its semi-crystalline nature and hydrophobicity [229, 230]. The packed macromolecular arrays retard fluid diffusion into the bulk of hydrophobic PCL. This polymer is, therefore, used as a long-term implant (e.g. for years) in different biomedical applications. To increase the degradation rate of this polymer and also its processability, PCL is copolymerised with different PHEs [229, 230]. The copolymerisation, however, substantially decreases the mechanical strength of PCL copolymer and thus limited their applications for cartilage tissue engineering. In addition, the hydrophobic surface of this synthetic polymer might adversely affect the cell adhesion and proliferation. To achieve PCL-based scaffolds with more favourable biological properties, composite scaffolds of PCL and naturally derived polymers such as PCL with chitosan [231], hyaluronan, fibrin [232] and elastin [200, 201] have been prepared. In one study PCL porous scaffold has been fabricated using gas foaming by high pressure CO_2 and salt leaching techniques. Then elastin is impregnated into these pore structures. The in vitro study demonstrated that chondrocyte cell adhesion and proliferation within the 3D structure of this PCL/elastin composite scaffold are substantially enhanced compared to neat PCL [201].

Acidic degradation products of PHEs can cause adverse tissue reactions and might induce immunoresponsive reactions. To counteract the acidic degradation process for some of PHEs and also to stabilise the pH of the surrounding environment, these polymers are combined with basic compounds [223, 224]. Calcium phosphate compounds and bioactive glasses are the two main additives used for this purpose. The addition of bioactive glass might also modify the mechanical properties of scaffolds and promote osteoconductivity of biomaterial. Bioactive glasses are thus used for bone tissue engineering rather than cartilage regeneration.

One of the major drawbacks of PHEs is their hydrophobicity, which can adversely affect the cell phenotype, cellular growth and proliferation. Different techniques, such as surface modification [200, 201], NaOH surface treatment [233] and ammonia plasma treatment [234], have been attempted to promote chondrogenesis cell adhesion on the surface of PHE. For instance, coating the surface of PLLA with chitosan and collagen enhances the cellular adhesion. However, cell proliferation and differentiation in PLA/collagen scaffold is inhibited due to blockage of pores.

Poly(vinyl Alcohol) (PVA)

PVA is hydrophilic biocompatible polymer that can be crosslinked to form hydrogel. It is historically used for cartilage regeneration applications [235] and can be engineered to have similar water uptake properties as native cartilage [203]. PVA hydrogel can be cut into the required shapes and then transplant into the patient's body through an open surgery process. In vitro cell studies on pure PVA hydrogel showed that low mechanical strength of the construct might lead to dedifferentiation of seeded chondrocyte, and hence further processing is required to increase its mechanical strength. Poly(lactic-co-glycolic acid) microparticles have been embedded within PVA hydrogel network, and insulin-like growth factor-1 (IGF-1) has been loaded in the hydrogel, which showed that the sustained release of IGF-1 can enhance cartilage formation which might lead to effective integration of the construct with the surrounding tissue [207].

Poly(ethylene Glycol)-Based Polymers

Poly(ethylene glycol) (PEG), also known as poly(oxyethylene) or poly(ethylene oxide), is one of the most extensively investigated non-biodegradable, synthetic, hydrophilic polymers for different cartilage tissue engineering applications [236]. This is due to its hydrophilicity, acceptable cell compatibility and non-cytotoxicity. PEG can also be easily functionalised with different reactive end groups. Copolymer of hydrophilic poly(ethylene glycol) (PEG) and different biodegradable and biocompatible polyesters, such as polylactide (PLA), poly(-ε-caprolactone) and poly(glycolide) (PGA), have drawn great attention for their tuneable characteristic parameters [237].

PEG macromer is functionalised with methacrylate (ma) groups to form a photocrosslinkable hydrogel, suitable for cartilage tissue engineering [208]. Different chondrogenesis cell types, including chondrocytes, embryonic stem cells and MSCs, are encapsulated with PEG-ma hydrogels and induced to form cartilage tissue in the presence of growth factor [208–210]. Diacrylate (da)-functionalised macromers of PEG have also been developed for different cartilage tissue engineering applications [211–215]. The lack of cell motif sites within its structure is the main drawback of this polymer. Due to high activity of PEG-da macromer, it can be used as a chemical crosslinking agent for different PEG-based macromers [212].

More biomimetic derivative macromers of PEG have been synthesised by conjugating collagen-mimetic peptide –$(Pro\text{-}Hyp\text{-}Gly)_x$– with PEG macromers. In vitro cell study showed that MSC proliferates and both collagen and proteoglycan are produced within this hydrogel [238]. In addition, the incorporation of PEG to chondroitin sulphate-based hydrogels increases the production of cartilage ECM proteins from MSCs seeded within this hydrogel [239]. PEG can also be functionalised by the addition of fumarate groups to the macromer using fumaric acid. Oligo(poly(ethylene glycol) fumarate) (OPF) is the fumarate-functionalised,

photo-crosslinkable PEG macromer. In a rabbit model with osteochondral defects, acellular OPF hydrogel has been used, and it is observed that native MSCs migrate within the 3D structure of these hydrogels to form fibrocartilage at the lesion site. Encapsulation of external MSCs within the OPF hydrogel structure results in regeneration of cartilage [218]. Different in vitro and in vivo studies have been conducted to investigate the capability of OPF hydrogels to deliver different growth factors to the defected site to promote natural process of cartilage tissue regeneration. These studies showed the potential of OPF hydrogels for encapsulation of different growth factors for cartilage tissue engineering [216, 217]. Tuneable properties, easy to process and functionalised, hydrophilic and biocompatible properties of PEG-based hydrogels are the main advantages of this group of biomaterials for cartilage tissue engineering.

Polyacrylates (PEs)

Poly(2-hydroxyethyl methacrylate) (PHEMA), poly(methyl methacrylate), poly (ethyl methacrylate) and poly(tetrahydrofurfuryl methacrylate) are the main polyacrylate-based hydrogels used for cartilage tissue engineering. It has been attempted to use PHEMA/MMA hydrogel for articular cartilage repair. However, the regenerated tissue exhibited lower mechanical strength compared to the surrounding native cartilage. Compliance of the fabricated PHEMA/poly(methyl methacrylate) hydrogel might lead to the formation of fibrocartilage as a result of implantation of construct in vivo [240]. Sawtell et al. also reported the cartilage inductive properties of PEMA/poly(tetrahydrofurfuryl methacrylate) hydrogel. In vitro studies showed the expression of GAG from seeded chondrocytes on this hydrogel. This results indicated the potential of PEMA/poly(tetrahydrofurfuryl methacrylate) hydrogel for cartilage repair [241]. Animal studies on this hydrogel showed the regeneration of hyaline cartilage at the subchondral defected site in rabbits [242]. The non-biodegradation properties of this group of biomaterials might lead to some noncontrollable biological behaviour by the cartilaginous construct after in vivo implantation. This is the main drawback in the application of these polyacrylate biomaterials for cartilage repair.

14.3.5.2 Naturally Derived Hydrogels

The feasibility of using biopolymers such as protein and polysaccharides for cartilage tissue engineering has been examined [5, 6]. The presence of cell motif sites in naturally derived polymer promotes their cellular adhesive properties. Protein-based hydrogels that have been attempted for cartilage tissue engineering include collagen, fibrin, silk and elastin-like polypeptides (ELPs). Commonly used polysaccharides are hyaluronic acid, agarose, alginate and chitosan. The common methods used for crosslinking these polymers and a summary of their properties are listed in Table 14.5, while more details are provided in the following discussion.

Table 14.5 Natural hydrogels for in vitro cartilage tissue engineering

Biomaterial	Crosslinking method	Comments	Refs.
Collagen types I and II	Physical/chemical (mixed with alginate) glutaraldehyde)	Bioactive, but poor mechanical properties	[243–249]
		Chondrogenesis after 3 days when mixed with alginate	
		No cell degeneration after 24 weeks	
		For physical crosslinking, possible to incorporate cells and chondrogenesis biofactors	
		Chemical crosslinking slightly increases the mechanical properties, but there is a risk of cytotoxicity	
		In vivo cell study with chemically crosslinked collagen in rabbit model	
		Might be immunogenic	
Fibrin	Physical	Poor mechanical properties	[88, 250–252]
		Immunoresponsive effect	
		Promote natural healing process at the defected site	
		Chondrocyte and biofactors can be incorporated	
Silk	Physical	High mechanical strength, risk of immunoresponses	[253–256]
		Tedious purification required to minimise this risk	
		Stem cells and chondrocyte maintained chondrogenic phenotype	
		Might initiate adverse immunoresponses	
		Required complicated purification process	
Alginate	Chemical (ion induced)	Easy to produce, cost-effective	[176, 243, 257–259]
		Chondrocytes and biofactors can be incorporated within its structure	
		Low bioactivity, slow and inconsistent degradation rate	
		Low cell adhesive properties	
Chitosan	Physical/chemical (genipin and glutaraldehyde)	Easy to process and functionalised	[260–263]
		Chondrocyte maintains its phenotype in this hydrogel	
		Good cell adhesion and proliferation	
		Possess low mechanical properties	
Chondroitin sulphate	Physical	Inhibit GAG production	[259, 264–268]
		High cost	

(continued)

Table 14.5 (continued)

Biomaterial	Crosslinking method	Comments	Refs.
Elastin-like polypeptides	Physical/chemical (glutaraldehyde)	Maintain chondrocyte phenotype	[201, 269–272]
		Functionalised to form covalent bonding and crosslinking	
		Tuneable chemical structure to promote chondrogenesis	
		Lack of mechanical strength	
		High cost	

Collagen

Collagen type II and type I are used for cartilage repair as these are the key types of collagens that exist in this organ [243, 273]. Chondrogenesis cells bind to collagen hydrogel via integrins, which induces chondrogenesis signaling that promotes cartilage formation. Collagen type II initiates and maintains chondrogenesis phenotype of mesenchymal stem cells enhancing the effect of TGF-β1 on cartilage formation [243]. The main advantage associated with collagen type I is its potential to spontaneously form hydrogel at physiological temperature and pH. Despite the fact that collagen type I is only found in the structure of diseased or damaged cartilage, the studies showed that the articular chondrocyte maintains its phenotype in collagen type I. Chondrocytes synthesised cartilage ECM component (e.g. collagen type II and proteoglycan) when using hydrogels fabricated from collagen type I [248]. Chondrocytes embedded within a collagen type I hydrogel are used for the treatment of full-thickness articular cartilage defect in small animals. Moderate regeneration of articular cartilage surface is reported [248]. In another study, however, it is shown that after 2 weeks of in vitro culture, only 30 % of chondrocytes on collagen type I hydrogel maintained their chondrocytic phenotype (spherical shape) [249]. Nevertheless, when using hydrogels from collagen type II, more than 60 % of chondrocytes maintained their spherical shape [249]. Buma et al. developed a composite hydrogel of collagen types I and II to further mimic the biochemical properties of natural cartilage. In this approach deep layer of hydrogel is formed from collagen type I for subchondral recruitment of stem cells. The more superficial layers of hydrogel, however, are composed of collagen type II that maintains chondrogenesis phenotype of cells [274]. Results from the application of collagen type II hydrogels for treatment of osteochondral defect in rabbit model showed the formation of both hyaline cartilage and the bone at the defected site. However, the mechanical properties of regenerated tissues are significantly lower than native tissues [244].

Several strategies have been undertaken to enhance the mechanical strength of collagen-based hydrogels such as crosslinking [243], mixing collagen with a synthetic polymer such as PLGA [275], bioactive glass [275] and hydroxyapatite [276]. For chemical crosslinking of collagen, the amine side group of lysine and hydroxylysine is chemically bonded with a crosslinking agent. Glutaraldehyde

[245], hexamethylene diisocyanate [277] and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [246] are the examples of crosslinkers [247]. However, the effect of chemical crosslinking on mechanical properties and degradation of collagen hydrogel is trivial [243, 278].

Elastin and Elastin-Like Polypeptides (ELPs)

Elastin is 68 kDa protein comprised of approximately 800 amino acid residues [279]. It has elasticity and mechanical strength that mimic native cartilage and ligament tissues [280]. Elastin contains hydrophobic and hydrophilic domains, which provides crosslinking sites with neighbouring molecules [281]. Application of elastin in tissue engineering, however, is limited for many years by its extreme water insolubility [282]. This has been improved by increasing availability of animal-derived soluble elastin through acidic (α-elastin) [283, 284], alkaline (k-elastin) [284] and enzymatic hydrolysis of elastin [285, 286]. In addition, a recently developed recombinant full-length elastin precursor, known as recombinant tropoelastin (rhTE), has been developed suitable for biomedical applications [285]. It is viable to synthesise elastin-like polypeptides (ELPs) by polymerisation of pentapeptide motif VPGVG and control physical and functional properties at the generic or chemical level [279].

Early studies showed the positive effect of elastin in promoting production of chondrocyte ECM such as collagen type II and proteoglycan in long-term monolayer culture of chondrocyte without dedifferentiation [269]. After 10 days of culturing chondrocyte in thermosensitive ELP solution, its phenotype is maintained and both collagen type II and GAG are produced [269]. Primary chondrocyte [287] and adult stem cell [288] have been cultured using coacervated ELP structure for cartilage tissue engineering. It has been demonstrated that ELP promotes the differentiation of stem cell that has undergone chondrocytic pathway even in the absence of any specific chondrocyte growth factor [288]. Elimination of growth factor significantly reduces the cost of differentiation and cartilage regeneration [285]. However, the shear moduli of ELP are fourfold lower than that of articular cartilage. The addition of crosslinking agent is one option to enhance the mechanical strength of ELP [37, 287]. For instance ELP is functionalised with glutamine to fabricate an enzymatic crosslinking site and create hydrogel [270]. This ELP-based hydrogel exhibits chondrogenesis behaviour and produces cartilage ECM proteins [270].

ELP-based biomaterials are chemically crosslinked from their active lysine group to increase their mechanical properties [289–291]. Lim et al. fabricated ELP hydrogel by reacting lysine containing ELPs with an organophosphorus crosslinker, β-[tris(hydroxymethyl)phosphino]propionic acid (THPP) in less than 5 min [289]. Both in vitro and in vivo cell studies confirmed the formation of cartilage ECM with these hydrogels [289, 290]. The mechanical strength of elastin-based hydrogel can be enhanced by synthesising this class of molecules with more

crosslinking sites. It is also feasible to use naturally derived elastin and mix it with mechanically stronger tropoelastin to enhance their mechanical properties [292].

Level of crosslinking, formulation, molecular weight and concentration of ELPs are the important factors that may affect the properties of ELP hydrogel and its performance for cartilage repair [271]. It is found that the molecular weight of ELPs has negligible effect on the physiochemical and biological properties of regenerated cartilage [293]. However, the crosslinking and also mechanical properties of ELP have significant impact on cartilage regeneration. The human tropoelastin has been crosslinked with genipin and created a hydrogel with compressive modulus in the range reported for articular cartilage [272]. This sample is then press-fitted into osteochondral defect site in a knee joint of rabbit for in vivo studies. Preliminary results showed no significant inflammation and high level of hyaline articular cartilage formation.

The high cost of producing elastin by recombinant technology or protein synthesis, immunogenicity of naturally derived elastin and low mechanical properties are the hurdles for application of elastin for cartilage repair despite its excellent biological properties.

Fibrin

Fibrin, which is produced by enzymatic cleavage of fibrinogen, is used for cartilage tissue engineering due to its role in natural wound healing [88, 250]. It promotes healing process within the extravascular space. Similar to other natural polymers, products of fibrin degradation are not toxic [250]. Fibrin has been used as a scaffold to deliver chondrocyte [294], mesenchymal stem cell [295] or growth factors [296] for cartilage tissue engineering applications. The natural healing process is optimised by implanting fibrin clot at the defected site [297, 298]. It can, therefore, promote spreading of endogenous blood over the large volume of the lesion site, which otherwise would be occupied with a developing haematoma. Chondrogenesis cells have been incorporated within the network of fibrin hydrogel to further enhance the healing process [251]. Both in vitro and in vivo results confirmed the positive impact of incorporated chondrogenesis cells with fibrin clot for cartilage regeneration [299, 300]. Despite these positive results, due to the low mechanical strengths and immunoresponsive effect of fibrin, this protein has minimal potential to be used directly for cartilage tissue engineering [252, 301, 302].

Silk

Silk fibroin is a typical protein that forms the filament of native silkworm. It has a broad range of applications due to its unique physicochemical properties. Silk mimics many characteristic properties of cartilage extracellular metrics and has potential for cartilage repair. These properties include high mechanical strength, flexibility, low degradation rate and water permeability [254, 303–305]. The

physically crosslinked silk hydrogel is used for chondrogenesis of bone marrow-derived mesenchymal stem cells, in which chondrogenic growth factors are used in culture media [306]. The chondrogenesis of MSCs is further increased by the incorporation of IGF-1 growth factor to silk hydrogel [307]. In vitro cell study on silk hydrogel showed great promise for the application of this type of hydrogel for full-thickness cartilage repair using both chondrocytes and stem cells [308, 309]. A structural protein blend system based on silkworm, silk fibroin and recombinant human tropoelastin has also been developed to form a scaffold with high mechanical strength, controllable degradation behaviour and elasticity [310, 311]. These studies showed human mesenchymal stem cell adhered and proliferated on this scaffold, exhibiting high potential of this protein blend for cartilage repair.

Care must be taken in purification of silk to remove sericin from fibroin silk to inhibit adverse immune response at the host tissue. In vivo studies demonstrated that pure fibroin silk has low immunogenicity and elicits foreign body response. Limited studies claimed that granuloma might form due to abandoned phagocytic response to silk by giant body cells and macrophages [256]. Compared with other biopolymer, however, silk is a desirable natural polymer for cartilage repair.

Chitosan

Chitosan, a positively charged polymer, is a linear polysaccharide that consists of randomly distributed N-acetyl-D-glucosamine and β-(1,4)-linked-D-glucosamine units [312]. It has been broadly used for biomedical applications for the low cost, low toxicity and immunostimulatory effects [260]. Molecular structure of chitosan resembles glycosaminoglycan, which also presents in the molecular structure of GAG. It thus interacts with different articular cartilage growth factors, adhesion proteins and receptors; hence chitosan can stimulate chondrogenesis of cells. In addition, physicochemical and biological properties of chitosan rely on the activity of glucosamine residues from acetylation [313], alkylation [314], carboxymethylation [315] and conjugation of chitosan with methacrylic acid and lactic acid [316, 317]. These techniques have been used to fulfil the characteristics of chitosan-based hydrogels for cartilage repair. The mechanical properties of these chitosan-based hydrogels are increased. Chitosan has been crosslinked with different reagents such as glutaraldehyde [318, 319], 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride [320] and genipin [321–323] to increase its mechanical properties.

Porous chitosan hydrogel has been produced by gas foaming and lyophilisation techniques to create porosity in 3D structure and enhance cell proliferation [261, 321, 324]. For example, Ji et al. simultaneously used gas-foamed and crosslinked chitosan (using either genipin or glutaraldehyde) under higher pressure CO_2 [321, 326]. These pores allowed the nutrient and oxygen transfer, hence the proliferation of fibroblast cells in 3D structure of chitosan hydrogel. In another study, it is demonstrated that chitosan enhances the natural regeneration process of articular cartilage in the rat joint [325]. Polyelectrolytic complex of chondroitin

sulphate and chitosan also provides a good mechanical structure for the adhesion and attachment of chondrocytes and MSC [162, 262]. Chondrocyte cells maintained their round or polygonal morphology and had undergone modest degree of mitosis [326]. Chitosan is used to promote cartilage wound healing as an injectable formulation. As an example, BST-CarGel, a chitosan-based biomaterial, is commercially available for cartilage regeneration [327, 328]. However, due to low mechanical strength, neat chitosan cannot be used for the fabrication of 3D hydrogels for regeneration of full cartilage.

Chondroitin Sulphate

Chondroitin sulphate (CS) is a GAG-based biomaterial composed of a chain of *N*-acetylgalactosamine and glucuronic acid [329]. It has been initially used in articular cartilage repair as a preventive and healing compound [330]. The in vitro [264, 265] and in vivo [266] studies demonstrated that CS promotes the metabolic activity of cartilage, preventing cartilage from degeneration in osteoarthritis.

Controversy results nevertheless reported in the literature for the application of CS hydrogel for cartilage repair. Many studies provided evidence that CS hydrogel inhibits the secretion of cartilage ECM such as collagen precursors [331]. In vitro studies showed that the addition of CS (with concentration of 100 mg/ml) also reduced GAG content in cartilage ECM in long-term culture despite chondrocyte viability [332]. In addition, photo-crosslinkable methacrylate derivative of CS inhibits the synthesis of cartilage ECM compounds [239]. This effect might be due to the presence of negative charge on the CS hydrogel, which attracts free cations within the culture medium. This effect increases the osmolarity within the hydrogel, which has an adverse impact on cell growth within the hydrogel [259].

This issue associated with CS is resolved by using a combination of CS with other hydrogels. For instance, it is found that chondrocyte maintained its phenotype and GAG production was not interfered in the mixture of chitosan with CS [267]. In addition, proteoglycan (related to GAG) production increased in CS-based hydrogel that covalently bonded with collagen type I [268]. Chondrocyte proliferated within this hydrogel and produced proteoglycan. Addition of CS in PEG hydrogel also improved the expression of chondrogenic gene from chondrocytes, enhancing the production of cartilage ECM compared to pure PEG hydrogel [333]. Compared with other biopolymers, CS production cost is high. In addition, its applications may have negative impact on ECM generation for cartilage repair. These data illuminate the limited applications of CS and low potential of using this compound for cartilage repair to date [259].

Hyaluronan (Hyaluronic Acid)

Hyaluronan (hyaluronic acid, HA) is the primary physiological component of the articular cartilage matrix [334]. The physical interaction between the molecule networks of hyaluronan can form extensively long, biodegradable and biocompatible molecules [335]. Theoretically, HA is superior for cartilage repair if it can be implanted in an unmodified form [336]. However, high degradation rate and poor mechanical strength are the major hurdles for its direct application in cartilage repair. The degradation of hyaluronan might lead to chondrolysis under certain conditions [337]. Different approaches, such as esterification [338] and crosslinking [335, 339], have been undertaken to improve its properties. However, these methods showed adverse effect on HA biocompatibility [340].

HA has been used for chondrocyte and stem cell encapsulations to regenerate cartilage. The in vitro and in vivo studies underlined the feasibility of cell proliferation and synthesis of cartilage ECM in a modified form of HA [341, 342] and as a cell carrier for chondrocytes [343, 344] or bone marrow-derived stems cells [345, 346]. Despite all biological and physiochemical issues associated with hyaluronan, Hyalograft C, a hyaluronic acid-based biomaterial, has been commercialised for cartilage repair, and it is in clinical trial phase.

Alginate

Alginate is a binary copolymer of (1 → 4)-linked β-D-mannuronic acid-*co*-α-L-guluronic acid. It can readily form bonds with different divalent metal ions, including calcium, magnesium and barium. This chemical bonding can be used to form alginate hydrogels for different biomedical applications, such as cartilage repair. Superior biological properties, high cell response and low cost are the main advantages of alginate hydrogels. Alginate is used for different cartilage tissue engineering applications such as preserving chondrocyte phenotype, organisation and turnover and differentiation of adipose-derived adult stem cells and bone marrow-derived stem cells for 3D cell growth [176, 243, 257, 258]. In vitro studies showed that chondrocytes, seeded within an alginate hydrogel, synthesised high level of cartilage ECM protein (i.e. collagen type II). The chondrogenesis of cells can be further promoted by the addition of BMP-2 in the structure of alginate hydrogel [347].

The effect of alginate hydrogel on chondrogenesis of chondrocytes has also been studied by comparing the in vitro results from 3D growth in alginate with conventional 2D culture in flask [347], which showed higher expression of collagen type II from chondrocytes seeded within alginate hydrogel compared to control normal 2D growth in the presence of IGF-1 and BMP-1 growth factors. Cai et al. showed that bone marrow-derived mesenchymal stem cells can be well distributed within an alginate hydrogel [348]. After 2 weeks of in vitro culturing that hydrogel is implanted into nude mice, the encapsulated bone marrow-derived mesenchymal stem cells exhibited continued process of induced chondrogenesis in vivo. High

expression of cartilage extracellular matrix proteins (such as collagen type II and aggrecan) confirmed that the alginate-based cartilaginous implants are actively chondrogenic during bone marrow-derived mesenchymal stem cell terminal differentiation.

The main drawbacks in application of alginate for cartilage repair are lack of cell motif sites within its structure and also low and unpredictable degradation behaviour [259]. The former might lead to a low level of chondrogenesis cell adhesion that prevents cell proliferation within the 3D structure. In addition, alginate is negatively charged that inhibits the absorption of proteins to its structure and interferes with the diffusion of biofactors and proteins. Low cell adhesion and protein absorption both might lead to decreasing chondrogenesis cell proliferation within the alginate hydrogels. Previous studies showed that functionalisation of alginate with arginine–glycine–aspartic acid (RGD) sequence enhances the chondrocyte adhesion [349] and thus can address low cell adhesive properties of this biomaterial.

The slow degradation rate of alginate and its unpredictable profile might have adverse effect on the application of this polymer for cartilage repair. Bouhadir et al. addressed this issue by preparing a biodegradable alginate hydrogel using periodate-oxidised alginate. This polymer is crosslinked with calcium ions and the hydrogel degraded within 9 days in PBS solution [350]. In vivo application of this alginate-based hydrogel showed that chondrocyte encapsulated initiated native cartilage formation at the defected site after 7 days of implantation [350]. The most challenging issue in application of alginate for cartilage repair is the extraction of this material from different sources that might have different physicochemical properties, mechanical strengths and degradation rates [259].

14.3.6 Injectable Hydrogels for In Vivo Cartilage Formation

Injectable hydrogels have been used to deliver chondrogenesis cells or drugs to the defected site in a minimally invasive manner to decrease patient morbidity upon the treatment procedure. As shown schematically in Fig. 14.6, a solution that contains a polymer, cells, drug or any combination of these three is injected to the lesion site. The polymeric solution is then crosslinked in situ to form a solid structure at the site. The cells along with the polymeric matrix are surrounded with chondrogenic biological and mechanical signaling that enhances cartilage regeneration. The polymeric matrices are usually degraded gradually, while cartilage ECM is synthesised by chondrogenic cells that are either externally provided or migrated from subchondral regions. Cartilaginous physiochemical environment and delivery of chondrogenic cell/drugs in minimally invasive manner are the main advantages of this technique.

The favourable polymers for injection are those that possess the following characteristics:

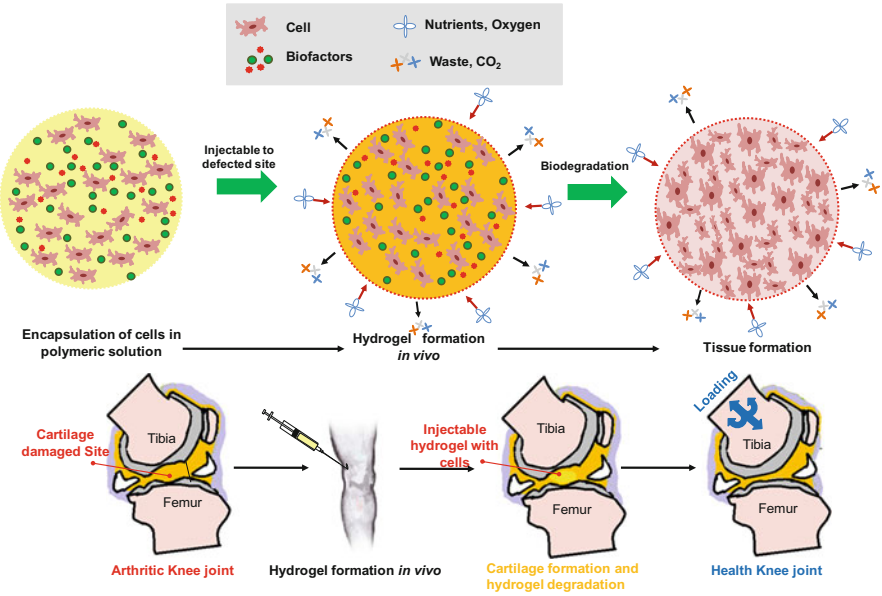

Fig. 14.6 Schematic overview of cartilage tissue engineering, using injectable hydrogels as cell carrier

- Cell adhesive property, biofactor loading capacity and sufficient mechanical strength
- Adjustable gelation properties to facilitate injectability during surgical operation and rapid hydrogel formation in the body to inhibit transfer of formulation to surrounding tissues
- Biocompatibility without toxic chemicals and severe conditions for gelation to promote cell viability

Different strategies have been attempted for in situ gelation such as redox polymerisation, photo-polymerisation, Michael addition, Schiff and enzymatic reactions and physical approaches such as pH induction and thermogelation. The following sections provide an insight into each of these methods.

14.3.6.1 Chemical Reaction

Chemical stimulus induces in situ gelation of polymeric solution by forming chemical changes in the molecular structure of precursors or by formation of covalent bond in polymeric system. The chemical reactions include redox and photo-polymerisation of acrylate-functionalised macromers, Michael addition, Schiff reaction scheme and enzymatic reactions. It is critical to select the conditions that precursors, initiators and catalysts are soluble in aqueous media to develop a biocompatible formulation [351]. A brief summary of injectable chemical stimuli

Table 14.6 Different gelation techniques to crosslink injectable hydrogels for cartilage tissue engineering

Polymer	Gelling strategy	Gelling time	Mechanical/degradation behaviour	Refs.
KGM/collagen–PLA[a]	Redox APS/TEMED	15 min	G' = 0.87–2.15 MPa	[352]
OPF/gelatin[b]	Redox APS/TEMED	8 min	NR	[353]
Chitosan (acrylated)[a]	Redox APS/TEMED	NR	Degrade in 18 days in PBS	[354]
PEG-da/HA[b]	UV photo-crosslink	NR	NR	[355]
PEG-dimethacrylate[a]	UV photo-crosslink	NR	60–590 kPa	[356, 357]
PEG-dimethacrylate/PDS[a]	UV photo-crosslink	NR	40–70 kPa	[358]
PLA–PEG–PLA/AES[a]	UV photo-crosslink	NR	7 kPa–97 kPa by 6 weeks of culture	[359]
Methacrylated hyaluronic acid[ab]	Laser photo-crosslink	NR	5kPa–120 kPa by 6 weeks of culture	[360, 361]
Methacrylated alginate[a]	UV photo-crosslink	NR	NR	[362]
Gelatin-methacrylate[a]	UV photo-crosslink	30 s	0.5 kPa–10 kPa	[363, 364]
Col-PEG with thiolated PEG[b]	Michael addition	30 min	15 kPa–18 kPa	[238]
Chitosan–hyaluronic acid[c]	Schiff reaction	1–4 min	10 kPa–30 kPa	[365]
Hyaluronic acid//H_2O_2[a]	Enzymatic reaction	10–20 s	0.1 kPa–2 kPa	[366]
Tyramine chitosan/HRP/H2O2[a]	Enzymatic reaction	NR	1 kPa–5 kPa, 40 % mass loss in 3 weeks	[367]

NR not reported in the reference
[a]chondrocyte
[b]MSC cell growth
[c]acellular

systems, used for cartilage tissue engineering, is presented in Table 14.6. This section is focused on the properties, type of cell, gelation and degradation behaviour and mechanical properties of this class of hydrogels for cartilage repair.

Redox Polymerisation and Photo-Crosslinking

Acrylate- or methacrylate-functionalised macromers are crosslinked by free radicals, produced from redox reaction in an aqueous solution. Two systems have been used to initiate these reactions that include ammonium persulphate/N,N,

N'N'-tetramethylethylenediamine (APS/TEMED) or APS/L-ascorbic acid (AA) [352, 368]. APS/TEMED redox initiation system is used to crosslink collagen-coated PLA micro-carriers in hydrogel precursor of konjac glucomannan (KGM) for cartilage repair. The composite collagen–PLA/KGM precursor formed hydrogel at 37 °C in 15 min [352]. In vitro studies showed proliferation of chondrocyte cells on this in situ formed hydrogel, demonstrating the potential of this hydrogel for cartilage repair.

In addition, OPF macromer is crosslinked with redox initiation system to form injectable hydrogels for cartilage regeneration [217, 353]. Park et al. loaded TGF-β1 growth factor into the structure of OPF hydrogel crosslinked with PEG-da using redox initiation system of APS/TEMED [353]. In their study the biological properties of injectable hydrogel were enhanced by addition of gelatin microspheres that were crosslinked with GA. It is observed that MSCs that are encapsulated within OPF/gelatin maintained viable and synthesised cartilaginous ECM proteins [353]. In another study, injectable OPF/gelatin hydrogel is used for coculture of osteogenic and chondrogenic cells to adjacent subchondral bone condition [217]. In vitro studies showed the synthesis of GAG, collagen type II and aggrecan by encapsulated MSC in OPF/gelatin hydrogel. These data suggested that OPF/gelatin and the developed crosslinking strategy may have potential for cartilage tissue engineering [217, 353].

In addition to synthetic polymers, such as OPF, naturally derived polymers such as chitosan are also crosslinked with redox initiation system. Acrylate-functionalised chitosan hydrogel is crosslinked with redox initiation system of APS/TEMED and used for cartilage repair [354]. Encapsulated chondrocytes within this hydrogel maintained their spherical phenotype. The hydrogel is completely degraded in 8 days, using lysozyme to mimic physiological environment [354].

Redox polymerisation is considered as a biologically benign process. The generation of free radical ions, however, might have negative impact on cells, biofactors and surrounding tissues. The biological consequences of releasing free radicals are a major concern of using this strategy. Further research is required to be conducted to fully understand their effects prior to broad applications in cartilage and other tissue engineering [369].

Different biomaterials are photo-crosslinked under ultraviolet (UV), visible and laser light sources. Photo-initiators are used to release free radicals to initiate the crosslinking. Irgacure 2959 and eosin/triethanolamine are the most widely used photo-initiator in biomedical applications which are FDA approved and commercially available. Both synthetic and naturally derived polymers can be photo-crosslinked to form hydrogel. Synthetic macromers, such as PEG-da, PEG-dimethacrylate/star-shaped poly(dimethylsiloxane) methacrylate, methacrylate end-capped PLA–PEG–PLA, mono-2-(acryloyloxy)-ethyl succinate grafter poly(vinyl alcohol) and OPF/PEG diacrylate, are photo-crosslinked for biomedical applications. In addition, naturally derived copolymers, including methacrylated chondroitin sulphate, methacrylated HA, PLA methacrylated HA, methacrylated

glycol chitosan, heparin methacrylamide, methacrylated alginate and methacrylated alginate, are photo-crosslinked to form in situ injectable hydrogels.

PEG is functionalised with methacrylate groups to form photo-crosslinkable hydrogel, suitable for cartilage tissue engineering. Chondrocyte embedded within the 3D structure of this hydrogel induces chondrogenic ECM production. Mesenchymal stem cell [355] and also embryonic cell [210] are encapsulated in this photo-crosslinkable hydrogel. By using growth factor (β-TGF), cartilage is formed in both of these two studies [210, 355, 370]. A solution of PEG-da and HA with TGF-β3 and Irgacure 2959 is used to deliver MSC in vivo [355]. After subcutaneous injection, the skin of mice is exposed to UV light. PEG-da macromers are photo-crosslinked, leading to formation of in situ hydrogel. The encapsulated MSC synthesised collagen type II and proteoglycan. This result showed the chondrogenesis properties of PEG-da/HA injectable hydrogel that can be used for cartilage regeneration [355]. PEG-dimethacrylate is also photo-crosslinked by UV radiation [356, 357]. Chondrocyte is encapsulated within this injectable hydrogel, and upon in situ gelation, GAG and collagen type II are produced.

The compressive modulus of this hydrogel is varied in the range of 60–590 kPa by changing the concentration of macromer in injectable precursor [356, 357]. The modulus of hydrogels affects both anabolic and catabolic activity of encapsulated cells [356, 357]. For instance, GAG is synthesised in softer hydrogel fabricated from PEG-dimethacrylate, while in hydrogels with a higher mechanical strength, collagen type II is mainly produced by encapsulated chondrocyte cells. RGD is incorporated into the 3D structures of PEG-dimethacrylate-based hydrogels to enhance cell proliferation. PEG-dimethacrylate and star-shaped poly (dimethylsiloxane) (PDS) methacrylate are crosslinked with acryloyl-PEG-RGD by photo-polymerisation. The compressive modulus of this hydrogel varies in a range of 40–70 kPa, depending on the composition of injectable precursor. Despite favourable biological performance of PEG-based hydrogels,application in cartilage repair is limited due to the lack of biodegradability [358].

Methacrylated end-capped PLA–PEG–PLA can be photo-crosslinked by forming IPN hydrogel with mono-2-(acryloyloxy)-ethyl succinate (AES)-grafted PVA. This IPN hydrogel is fabricated to achieve desirable mechanical, biological and gelation properties for cartilage tissue engineering [359]. This study showed that when chondrocytes are encapsulated in this hydrogel, cartilage ECM is formed within 6-week culture. The entanglement of cartilage ECM within the structure of hydrogel dramatically enhanced the compressive modulus of construct from 7 to 97 kPa [359].

Naturally derived macromer of chondroitin sulphate, functionalised with methacrylate groups, is synthesised to form hydrogel with UV, using Irgacure 2959 as photo-initiator [371]. High cell viability is detected for encapsulated chondrocytes during the in situ gelation. In the presence of suitable enzyme (chondroitinase with concentration of 0.8 mg/ml), the hydrogel is degraded within 24 h, whereas in the absence of this enzyme, the hydrogel retains its structure up to 7 days [371]. IPN hydrogel of methacrylated chondroitin sulphate and PEG-dimethacrylate is also

formed for cartilage regeneration [239]. The compressive modulus of this hydrogel is controlled by changing the concentration of polymers [239].

Methacrylated hyaluronic acid is crosslinked using eosin/triethanolamine as photo-initiator [360]. The crosslinking process is activated using argon laser at 514 nm. Eosin is excited by laser to the triplet state, and then triethanolamine releases an electron to produce the free radical anion of eosin and radical cation of ethanolamine. This free radical cation can polymerise methacrylated HA. Laser beam can reach deep tissue; therefore, it could have more applications for cartilage tissue engineering compared to visible light and UV [360]. In vitro and in vivo study confirmed that chondrocyte maintains its spherical phenotype when encapsulated within this system [360], and chondrogenic proteins, such as collagen type II, are synthesised [361]. In vitro studies showed that in 6 weeks, chondrogenesis is induced in the presence of TGF-β3 using this hydrogel with encapsulated MSC cells. The formation of cartilage ECM and synthesis of GAG and collagen type II by MSCs resulted in an increased compressive modulus from 5 kPa to 120 kPa [361].

Alginate is functionalised with methacrylate groups to form photo-crosslinkable biopolymer. The effect of concentration of Irgacure 2595, the photo-crosslinker reagent, on the physical properties of this hydrogel is examined, which included swelling behaviour, compressive modulus and degradation behaviour. After 3 weeks, chondrocyte maintained its spherical phenotype [362]. In addition to alginate and hyaluronic acid, gelatin-methacrylate (Gel-ma) that creates hydrogel under UV within 30 s has been attempted for cartilage repair [363]. The compressive modulus of this hydrogel varied from 0.5 to 10 kPa based on the concentration of polymer [364]. Although adverse biological effect has not been observed in using the hydrogel formation strategy, the impact of UV radiation for in situ delivery for non-invasive operation and surrounding tissues is still questionable due to free radical formation [369].

Michael Addition

Michael addition is the 1,4-addition of nucleophiles to α,β-unsaturated ketones or esters (electrophiles) [369]. Amine- and thiol-functionalised macromers are the main nucleophiles, whereas acrylate-, methacrylate- and methacrylamide-functionalised macromers are the main electrophiles in Michael addition. The combination of these two in polymer solution is required for in situ gelation [369]. This reaction scheme is favourable for biomedical applications such as cartilage repair due to its high gelation efficiency in physiological condition without any side products. Different combinations of synthetic and naturally derived polymers are used to form in situ gelled systems for cartilage repair, using Michael addition.

Thiolated hyaluronic acid and PEG vinylsulfone (PEG-VS) macromers are in situ crosslinked within 14 min, using Michael addition scheme. Encapsulated chondrocyte maintained their phenotype after 21 days, while this hydrogel is

degraded within this period. GAG and collagen type II are produced when using these cell-laden hydrogel underlining their high potential for cartilage repair [372]. To accelerate the in situ gelation of hyaluronic acid-based hydrogels, poly (ethylene glycol)-bis-maleimide (PEG-BM) is used. This system exhibited in situ gelation within approximately 1 min through Michael addition scheme.

Heparin-based hydrogels are formed, using thiolated heparin and Michael addition scheme [373]. Thiolated heparin and PEG-da are in situ gelled at physiological condition using Michael addition. This hydrogel promotes the in vitro re-differentiation of encapsulated dedifferentiated rabbit chondrocytes. In vivo studies are conducted by transplanting the hydrogel in the subcutaneous dorsum of mice, which showed the proliferation of chondrocyte in vivo and formation of cartilage without using any biofactors [373].

Semi-interpenetrated network (SIPN) scaffold is also fabricated by incorporating in situ gelled heparin-based precursors (thiolated heparin–PEG-da system) and poly(L-lactide-*co*-ε-caprolactone) (PLCSL) scaffold. BMP-2 and chondrocytes are encapsulated within this system and is locally delivered to the defected cartilage site to promote the formation of calcified fibrocartilaginous transition region. This hydrogel enhanced the integrity of regenerated cartilage and secured its attachment to the surrounding tissue [374]. In addition, thiolated gelatin is crosslinked using PEG-da through Michael addition scheme. The gelation time is in between 3 and 5 min depending on the concentration of macromers in the precursor system. Bone marrow-derived MSCs are encapsulated within this hydrogel and exhibited a high level of viability. This system has been used for different cartilage regeneration applications [375]. More complicated systems are designed by conjugation of synthetic and naturally derived copolymers to closely tune the gelation, degradation and mechanical properties of this group of hydrogels.

Collagen-mimetic peptide (Col-P), which contains a GFOGER sequence (responsible to maintain chondrocyte phenotype), is conjugated with poly(ethylene glycol) tetraacrylate to form Col-P-*co*-PEG macromer. This macromer could form gel in 30 min upon mixing with thiolated PEG in physiological environment (37 °C and 7.4 pH) [238]. The encapsulated MSCs showed a higher degree of chondrogenesis compared with the thiolated PEG hydrogel without the addition of peptide sequence (no conjugation of Col-P). The compressive modulus of hydrogels is tuned from 15 to 18 kPa by changing the concentration of macromers within the precursor solution. The softer gel induced a higher degree of chondrogenesis differentiation of MSCs compared to the stiffer gel [238], due to a lower mass transfer rate in the latter system.

Schiff Reaction

Schiff-based reaction schemes are based on the chemical reactions between an amine and aldehyde groups. It is one of the promising schemes in the formation of in situ gelled hydrogels due to high yield in physiological condition [365]. *N*-Succinyl-chitosan and aldehyde hyaluronic acid macromers are synthesised and

chemically conjugated to form an injectable precursor. The in situ gelation time of this system in physiological environment varied from 1 to 4 min [365]. The crosslinking density within the macromers affects the mechanical properties within 10 kPa to 30 kPa, and the degradation time varies between 1 and 30 days. In vitro studies showed that the encapsulated chondrocytes retained their phenotype, when encapsulated in this hydrogel [365]. Most of the injectable hydrogels, formed with this reaction scheme, suffered from low mechanical properties. This class of hydrogels, therefore, is not widely used for cartilage tissue engineering.

Enzymatic Reaction

Enzymes can form new bonds or can cleave specific bonds in a polymer to induce gelation. Due to the specificity of enzymatic reactions, the other chemical moieties in polymers are not interfered. Therefore, enzymatic gelation is a rational platform with low risk of side products. Enzymes such as horseradish peroxidase (HRP) [376], phosphatase [377], tyrosinase [378], thermolysin [379], α-galactosidase [380] and esterase [381] have been used to prepare hydrogels through enzymatic reactions. The gelation occurred by crosslinking polymer networks or by decreasing the solubility of polymer in aqueous solution[2].

HRP is the most widely used enzyme for the preparation of hydrogel for cartilage tissue regeneration. Tyramine-*co*-hyaluronic acid (THA) macromer is synthesised which undergoes instant gelation in the presence of HRP. For in vivo studies, THA is dissolved in H_2O_2 and subcutaneously injected into rats, followed by separate injection of HRP [366]. This polymer instantly formed hydrogel after the addition of HRP. The gelation time and the mechanical strength of the resulted hydrogel are controlled by changing the concentration of HRP. The gelation time is less than 10 s when HRP concentration is from 1.3 to 2.2 unit/ml. This hydrogel exhibited compressive modulus of 0.1–2 kPa [366].

Chitosan is also functionalised with tyramine groups [367], which are crosslinked with HRP/H_2O_2 system to form hydrogel. The compressive modulus of this hydrogel varies in the range of 1–5 kPa by changing the concentration of functionalised chitosan from 1 to 2 wt%. In the presence of lysozyme, chitosan hydrogel is rapidly degraded and loses 60 % of its weight after 3 weeks. The chondrocyte viability in this hydrogel is high and their phenotype is maintained after 2 weeks of encapsulation [367].

Enzymes are involved in the gelation process. After the formation of gels, the enzymes act as impurities that might have biological impact on encapsulated cells and also the host tissue. Additionally, the denaturation of an enzyme in the body may induce immunogenicity. Due to these reasons, the application of this group of in situ gelled precursors for cartilage tissue engineering is limited [369].

[2]Hydrogel is formed through condensation process upon decrease of solubility of polymer in aqueous solution.

14.3.6.2 Physically Induced Gelation

Hydrogel can be formed via physical stimulation of polymers to create interaction between molecules. Thermo- and pH-responsive stimulators are the most common approach for the fabrication of hydrogels. In physical stimuli, intermolecular interactions such as van der Waals, hydrogen and covalent bonding result in phase transition behaviour. Physically induced gelation scheme, therefore, provides a mild condition for delivery of chondrogenesis cells to the defected site, which might exhibit low cytotoxicity and enhance cell viability and regenerate cartilage at the defected site. This section provides an overview for different types of physically induced gelation systems (pH and thermoresponsive) that have been attempted for cartilage repair.

pH-Induced Gelation

Changes in pH can induce in situ gelation of polymers. Different synthetic and naturally derived polymers can be functionalised with carboxylic acid-derived monomers, such as methacrylic acid (MAA [382, 383] and AA [384–386]), to synthesise copolymers that can undergo gelation by variation of pH [387]. The functionalised polymer with MAA and AA exhibited pKa value of approximately 5 and 4.8, respectively [387]. It is, therefore, feasible to incorporate the cells into the solution, when the pH is below the pKa. The hydrogel is formed by increasing the pH above this value. However, cells and growth factors tolerate physiological pH (6–7.5) and cannot maintain viable out of this range for a long period. Care must be taken in using pH-sensitive hydrogels to ensure having less impact on cell viability. For this reason, pH-sensitive hydrogels have not been used broadly for tissue engineering of cartilage.

Thermogelation

Temperature variation has an impact on the molecular conformation of thermoresponsive polymers. Two types of these polymers are identified, (i) lower critical solution temperature (LCST) and (ii) upper critical solution temperature (UCST), shown schematically in Fig. 14.7. In the former, the polymer maintains miscible only at any temperature below the LCST. In the latter, however, the opposite phenomenon occurs, in which the polymer is only miscible above the UCST. Thermogelation is a promising and clean approach for hydrogel formation and development of an injectable formulation due to the absence of any chemical, reagent and enzyme that might exhibit an adverse effect on cell viability and performance. Table 14.7 summarises different thermoresponsive injectable hydrogels that are synthesised for cartilage regeneration. These injectable systems are discussed more in depth in this section.

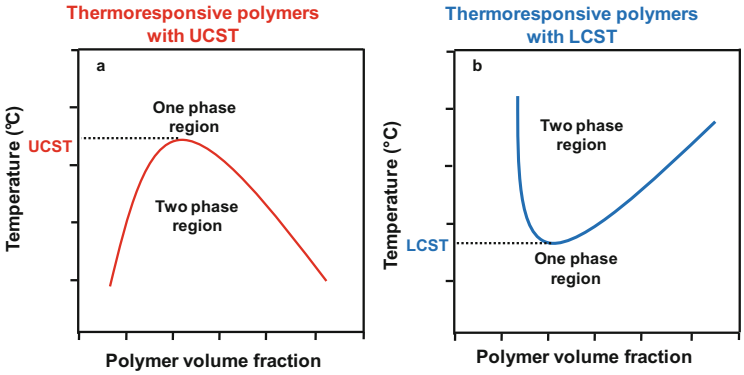

Fig. 14.7 Schematic illustration of phase transition behaviour for thermosensitive polymers with upper critical solution temperature (UCST) (**a**) and lower critical solution temperature (LCST) (**b**)

The polymers that possess LCST below the body fluid are favourable for tissue engineering. In this case, the biofactors and cells are incorporated into the solution at low temperature with low risk of denaturation and minimal impact on cell viability. Poly(N-isopropylacrylamide) (PNIPAAm), Pluronic®, elastin-like polypeptides (ELPs), chitosan-derived polymers, functionalised hyaluronic acid and heparin are the main synthetic and naturally derived polymers used to form thermoresponsive precursor for cartilage tissue engineering.

The LCST of PNIPAAm (32 °C) is close to physiological temperature (37 °C), and it can be readily tuned by adding hydrophilic and hydrophobic side chains to its molecular structure. PNIPAAm is a biocompatible polymer with very high rate of phase transition behaviour. Therefore, PNIPAAm is the most commonly used thermoresponsive polymer for tissue engineering applications. PNIPAAm-based copolymers form gel through coil-to-globe phase transition process [403]. Hydrogel formations are initiated by increasing the temperature above the LCST of copolymer [404]. For fabrication of biodegradable PNIPAAm-based hydrogels, it is critical to conjugate hydrophilic moieties, such as acrylic acid or PEG, and a hydrophobic segment to PNIPAAm-based macromers [387, 388]. The mechanical properties of PNIPAAm-based copolymers are increased by conjugating hydrophobic moieties such as n-alkyl acrylate and poly(lactide) macromers [387]. The incorporation of hydrophobic macromer resulted in increasing compressive modulus [387].

Yun et al. synthesised more biocompatible PNIPAAm-based copolymer by the incorporation of acrylic acid (AAc) segments to PNIPAAm molecule to form P (NIPAAm-*co*-AAc). TGF-β3 growth factor is encapsulated within this hydrogel to promote cartilage formation. In vitro cell study showed that chondrocytes retained their phenotype after 8 weeks. A solution of precursor, chondrocyte and TGF-β3 growth factor is injected subcutaneously into nude mice and formed gel at the injected location. After 8 weeks, the regenerated cartilage acquired normal histological and physiochemical properties [389].

Table 14.7 Different thermoresponsive hydrogels for cartilage regeneration

Polymer precursor	Gelling temperature	Degradation behaviour	Mechanical properties	Refs.
P(NIPAAm-*co*-propylacrylic acid)[a]	37 °C	NR	1 kPa (Comp Mod)	[387, 388]
P(NIPAAm-propylacrylic acid-butyl acrylate)[a]	>37 °C	NR	2 kPa (Comp Mod)	[387, 388]
P(NIPAAm-*co*-acrylic acid)[+]	~37 °C	Retain for 8 weeks	NR	[389]
P(NIPAAm-PLDLA-dextran)[+]	32 °C	Retain for 4 weeks	NR	[390]
PNIPAAm-g-methylcellulose[+]	~32 °C	Retain for 4 weeks	NR	[391, 392]
DNA-modified ELP[+]	~35 °C	NR	$G^d = 80$ pa	[287, 393]
P(NIPAAm-AAc-NAS-HEMAPLA)[b]	18 °C–26 °C	85 wt% in 21 days	~0.5 MPa (Tensile Mod)	[394]
Chitosan/GP[+]	37 °C	Retain in 3 weeks	NR	[395, 396]
Chitosan/GP/starch[+]	37 ± 2 °C	67 % loss in 56 days	NR	[183]
Chitosan/PPO–PEO[+]	25 °C	Retained in 28 days	NR	[397]
Chitosan/PNIPAAm[d]	<37 °C	NR	NR	[398]
Chitosan/PNIPAAm/HA[+]	30 °C	NR	NR	[399]
PEO–PPO–PEO[+]	37 °C	Fast dissolution rate	Very weak	[400–402]

NR not reported in the study
[a]Acellular matrix
[b]chondrocyte
[c]smooth muscle cells
[d]MSCs

A porous structure of poly(NIPAAm)-based hydrogel is formed by the incorporation of PLDLA and dextran (conjugation via chemical bonding) to form a copolymer with LCST at32 °C. The chondrogenic ECM protein, such as collagen type II, is formed after 4 weeks of encapsulation of chondrocyte within this hydrogel. To assess the phenotype of chondrocyte after culturing within this hydrogel, they are detached from the surface by decreasing the temperature from 37 °C to below 32 °C (LCST of hydrogel). The detached chondrocyte exhibited a round shape which confirmed that its phenotype is maintained during this period.

Poly(NIPAAm)-*g*-methylcellulose copolymer is also synthesised to form hydrogel at 32 °C [391, 392]. ATDC5, a chondrogenic cell line, is encapsulated in this hydrogel to assess its chondrogenesis properties. The in vitro studies confirmed the high potential of this injectable hydrogel for cartilage tissue engineering due to the low degree of cytotoxicity and high cell proliferation within this hydrogel [391, 392].

Another thermoresponsive hydrogel is produced by the conjugation of chitosan with PNIPAAm. This copolymer is water soluble with the LCST less than 37 °C [398]. MSCs are mixed with this copolymer solution and injected for assessing the application of hydrogel for cartilage tissue engineering. The hydrogel is formed in the body temperature and promising results were acquired; chondrocytic markers were produced including GAGs and collagen type II at the injected site [398].

HA is also conjugated with PNIPAAm to form thermoresponsive poly (NIPAAm-co-HA) hydrogel. Preliminary studies on this hydrogel showed continuous and sustained release of fluorescein isothiocyanate (FITC)-labelled bovine serum albumin up to 60 h by in vivo subcutaneous injection on the dorsal surface of rabbit. These data demonstrated the potential of thermosensitive hydrogel for cartilage tissue engineering [405].

Chitosan/PNIPAAm copolymer is grafted with hyaluronic acid (HA) to further mimic the properties of natural cartilage ECM. Chitosan/PNIPAAm/HA hydrogel formed injectable hydrogel above 5 wt% at 30 °C. In vitro cell study with this hydrogel showed that chondrocyte maintains its morphology and posed a high degree of proliferation and differentiation [399].

These studies underlined that most of PNIPAAm copolymers conjugated with natural or synthetic polymers are suitable for chondrogenesis cells to produce cartilage ECM. They might be favourable systems for developing an injectable formulation; however, it is still in its infancy, and further research is required to select a polymer with desirable injection properties, stability, degradation rate and mechanical strength for regenerating cartilage.

Synthetic and naturally derived polymers, such as block copolymers of ethylene oxide (PEO) and propylene oxide (PPO), elastin-like polypeptides (ELPs) and chitosan with thermoresponsive properties, were used to form injectable hydrogels for cartilage tissue engineering. Block copolymer of silk and elastin peptide sequence (SELP) were used as acellular therapy for cartilage tissue repair in a rabbit model of an osteochondral defect [406, 407]. In this study SELP was injected into the osteochondral defect site on the femoral condyles of rabbit knee and crosslinked in vivo [407]. SELP hydrogels were also used for cartilage tissue engineering, using mesenchymal stem cell (MSC). Molecular structure of SELP copolymer is changed to achieve a thermosensitive copolymer, named SELP-47 K. This copolymer undergoes gelation at 37 °C. MSCs are encapsulated within SELP-47 K hydrogel in the presence and absence of chondrogenic TGF-β3 growth factor. After 28 days, cartilaginous matrix components (sGAG and collagen type II) are formed by MSC encapsulated in SEL-47 K hydrogel in both cases (in the presence and absence of TGF-β3) [393]. This result showed that ELP can act as an engineered bioactive molecule to stimulate chondrogenesis pathway in MSC.

A thermosensitive chitosan-based hydrogel was fabricated, using β-glycerol phosphate disodium salt (GP) as sol–gel initiator at physiological pH and temperature [395]. GP showed an osteogenic effect when added to bone marrow stromal cells. In vitro study of chitosan/GP hydrogel showed its capability to deliver an osteogenic mixture of β-transforming growth factor (β-TGF) and encapsulated chondrocyte over 3 weeks [396]. The use of chitosan as a cationic polysaccharide

in this mixture led to the adhesion of hydrogel to tissue surface which normally bear net anionic characteristic. In addition, chitosan/GP mixed with chondrocyte is used as cytocompatible space filler [408]. BioSyntech Inc. (Laval, Quebec, Canada) developed the thermoresponsive (chitosan/GP) solution, which is in clinical trial for cartilage tissue engineering [409].

The properties of chitosan/GP hydrogel were further improved by the incorporation of starch to the thermoresponsive mixture [183]. The injectable chitosan/GP/starch mixture had a gelation temperature of 37 ± 2 °C. The hydrogel also exhibited microporous structure with 67 % of degradation within 56 days in PBS solution containing 0.02 mg/ml of lysozyme. Chondrocyte remained its phenotype after 14 days of culturing within this hydrogel [183].

In addition, injectable chitosan-based hydrogel was developed by fabricating interpenetrating polymeric network of chitosan with other natural polymers such as alginate and hyaluronate, hyaluronic acid and heparin, which enhanced the production of collagen type II and increased the rate of cellular proliferation after 2 weeks of cell growth [410, 411]. Combining chitosan with other biomaterials, however, does not necessarily result in favourable effects. For example, the combination of chitosan with chondroitin sulphate and dermatan sulphates showed adverse effect on production of collagen type II and morphology of chondrocyte cells [412].

Alternatively, chitosan-based thermosensitive hydrogel is prepared by graft copolymerisation with Pluronic® (block copolymer based on ethylene oxide and propylene oxide) (PPO–PEO). This thermosensitive hydrogel (chitosan/PPO–PEO) is used for cartilage tissue engineering and had sol–gel transition temperature of 25 °C. Chondrocyte proliferation and GAG production are significantly increased after 28 days by using chitosan/PPO–PEO in situ gelled precursor [397].

Copolymer of hyaluronic acid conjugated with heparin and Pluronic® (block copolymer based on ethylene oxide and propylene oxide) (PPO–PEO) is synthesised to form in situ gelled at 37 °C in less than 10 min. TGF-β3 is successfully loaded within the structure of hydrogel and continuously released from hydrogel for 20 days. This resulted in formation of cartilage ECM at full-thickness cartilage defect of rabbit knee [413].

HA is also conjugated with PNIPAAm to form thermosensitive hydrogel p (NIPAAm-*co*-HA). Preliminary studies on this hydrogel showed continuous and sustained release of FITC-labelled bovine serum albumin up to 60 h by in vivo subcutaneous injection on the dorsal surface of rabbit. This demonstrated the potential of this thermosensitive hydrogel for cartilage tissue engineering [405].

Therapeutic agents such cartilaginous bioactive compounds as BMP-2 were also delivered to the cartilage lesion site with a HA-based injectable hydrogels to control the release of bioactive compounds [414]. These HA-based hydrogels are conjugated with a heparin sulphate proteoglycan and are used to deliver BMP-2. The controlled and slow release of BMP-2 and the presence of proteoglycan within the structure of this hydrogel had an anabolic influence on articular cartilage in an osteoarthritis model. The synthesis of proteoglycans and other cartilaginous ECMs is stimulated upon the injection of this hydrogel at the lesion site [414].

14.3.7 Products Approached Clinical Trial for Cartilage Repair

Current cell-based therapies for cartilage repair fall into three categories: (1) cell or tissue implementation [415–418], (2) cartilage or bone grafts [419, 420] and (3) bone marrow stimulation through either abrasion, arthroplasty, Pridie drilling or microfracture [96, 421, 422]. Promising clinical results have been reported for most of these treatments [327]. Based on the preferred treatment by the surgeon, different classes of biomaterials can be selected. The major available products for cartilage repair are BST-CarGel (e.g. filler in bone marrow stimulation) and Hyalograft C, CaReS, TruFit, NeoCart, Pluronic and PEG-da-based injectable hydrogel. A brief summary of these treatments is provided in Table 14.8. This section aims to provide insight about the properties, type of cell and results of animal and clinical studies.

14.3.7.1 BST-CarGel

BST-CarGel is developed to stabilise blood clot at the defected site by dispersing biological adhesive solution at the wound site. This technique involves the use of an aqueous solution of chitosan in glycerol phosphate buffer at pH 7 [396]. Intrinsic biocompatible and biodegradable properties of components and neutral pH of this solution make it biocompatible, biodegradable and adhesive to native tissue. In surgical applications, BST-CarGel is mixed with blood with a ratio of 1:3 to generate normal clot with reinforced properties and impede clot retraction. BST-CarGel is injected (normally with 18-G needle) at the lesion site in liquid form and solidifies in 10 min [423, 424]. The cationic nature of chitosan increases the adhesive property of the mixture to cartilage lesion and thus ensures longer clot residency at the cartilage defected site. This method provides critical blood components, red cells and nutrients above the bone marrow wound site (holes or microfracture) to ensure initiation and activation of tissue regeneration and repair [327].

Animal studies have been conducted using BST-CarGel [424, 440, 441]. Skeletally mature rabbit (8–15 months old) are used to assess the characteristics of regenerated cartilage after implementation of BST-CarGel. It is observed that BST-CarGel stimulates the proliferation of stromal cells through the gel towards the lesion site. Vascularisation within the subchondral bone is also significantly increased which indicates positive impact for cartilage regeneration. The BST-CarGel, which is mainly chitosan, is degraded via neutrophil phagocytosis after approximately 1 month [440].

In another animal study, 3–6-year-old sheep models are used, in which BST-CarGel is injected in a surgically prepared 1-cm^2 condylar and trochlear defects. The cartilage regeneration in this defected side is compared with a negative control (microfracture only without any filler) after 6 months of implantation. It is

Table 14.8 General information of commercial scaffolds/hydrogels for cartilage tissue engineering applications

Commercial product	Application/method of delivery	Mechanical/ gelling properties	Rehabilitation program	Opinion	Ref.
Chitosan (BST-CarGel)	Blood clot formation, 10 s of mixing and then injection with 18-G needle	Solidifies in 10 min	The knee is immobilised for 24 h with soft braces. Soft braces are recommended for the first 2 weeks. The joint is non-load bearing for at least 6 weeks post surgery by using crutches. It is possible to have a full load bearing in 9–11 weeks after the surgery. The patients are not allowed to participate in any intense sport activity within the first year after the surgery	Safe, no unpredicted effect based on blood analysis and physical examination and better level of pain, stiffness and function based on WOMAC questionnaires	[327, 328, 396, 423, 424]
Benzilic acid of hyaluronic acid (Hyalograft C)	Full articular cartilage replacement, arthroscopic and mini-arthrotomy implantation	Degrades in 4 months, insoluble in water	Immobilised for 24 h after surgery with braces and non-load bearing for 4 weeks. Gradually proceed to full load bearing within 10–12 weeks. No intense sport activity at least in the first year of surgery	It can provide excellent outcome for the active and young only	[342, 425–430]
Collagen type I (CaReS)	Full articular cartilage replacement, open surgical process to fill the cartilage defected site	Not reported	Knee joint immobilises for 48 h (<10 ° of flexion). From the third day after surgery, knee flexion is limited to 30 ° for 21 days and thereafter to 60 ° for another 21 days Bicycle training after 6 weeks from surgery and muscle enhancement training after	IKDC score in all patients significantly increased; pain significantly is decreased. Surgeons rated 61 % of the outcomes as very good	[[165], [431, 432]]

			12 weeks. No intense sport or physical activity for 1 year		
PLGA/calcium sulphate (TruFit)	The defected site is drilled, and the plugs are inserted at the defected site to promote cancellous bone and cartilage formation	Degradation in 9 months	No braces are used as support, 0–60 ° of passive flexion, for the first 4 weeks after operation, partial load bearing, between 4 and 6 complete range of movement and cycling and swimming to strengthen the joint	80 % improvement in KOOS score after 12 months; after this time, the patients' conditions are worsening because of pain and joint functionality. Repeated operation is required in 70 % of cases after 18 months	[73, 389, 433–437]
Collagen type I (NeoCart)	Full articular cartilage replacement, open surgical process to fill the cartilage defected site	Not very robust, might damage before implantation	For approximately 10 days, the joint is immobilised; for 6 weeks after surgery, non-load bearing on the surgical knee; for 12 weeks muscle enhancement training. Within the first year after surgery, no intense sport physical activity	Improvement based on VAS scale and level of pain, approximately 85 % success rate in increasing knee function	[438, 439]

Therapeutic strategies include (1) cell or tissue implementation, (2) cartilage or bone grafts and (3) bone marrow stimulation

observed that there is a high level of adhesion between the BST-CarGel/blood clot, bone and cartilage. The volume and quality of hyaline cartilage regenerated at the defected site are also increased. In addition, relatively mature articular cartilage is observed in the sheep model [424, 441].

Depending on the size and the location of the lesion, there are two approaches that surgeons can use to implant BST-CarGel: (1) mini-open and (2) arthroscopic approaches [327] in clinical applications. In the former a mini-arthrotomy is used to facilitate visualisation of the lesion and delivery of BST-CarGel. Arthroscopic method is feasible only when the entire lesion site is within arthroscopic field of view. It is critical to deliver the mixture of BST-CarGel and blood over individual bone marrow stimulation channels (holes or microfractures) and then over the remaining lesion. In most of the cases, therefore, incision is required to implant BST-CarGel at the exact lesion site.

In 2003–2004 Health Canada's Special Access Program for medical device assessed the BST-CarGel for cartilage repair in human cases. In this study, 33 patients (22 males and 11 females) are treated with BST-CarGel. The treatment is carried out on a case-by-case basis, and it is not legally contemplated as a clinical trial due to the absence of negative control. The outcomes of this study are not reliable as there is no control on the examination of patients and lesion [327]. For example, the size of the lesion ranged from 0.5 cm^2 to 12 cm^2; BST-CarGel is delivered for 22 patients by arthroscopy and for 11 patients by mini-arthrotomy. Nevertheless, the treated patients suffered from both traumatic and degenerative lesions.

It is concluded that BST-CarGel treatment is safe as no complication is observed from physical examination of 33 patients and their blood tests. The Western Ontario and McMaster (WOMAC) Universities Osteoarthritis Index [328] questionnaires are administrated preoperatively and postoperatively after 3, 6 and 12 months. After 12 months postoperatively, pain, stiffness and function index value (based on WOMAC questionnaires) are significantly decreased underlining clinical benefit of using BST-CarGel [327].

The lesion grade (depth), location, size and status of the opposing chondral surface are factors that play a critical role in selecting BST-CarGel for cartilage repair. BST-CarGel has not yet been approved for sale in any country, and further investigations and clinical trials are still required.

14.3.7.2 Hyalograft C

Hyaluronic acid (HA)-based scaffolds such as Hyaff 11 have been used for cartilage tissue engineering applications. Hyaff 11 is made of linear derivative of HA modified by complete esterification of the carboxyl functions of glucuronic acid with benzyl group [338]. HA-containing scaffolds are biocompatible and support cell–cell interaction, cluster formation and extracellular matrix production for cartilage tissue engineering [442]. Hyaff 11 is a 3D fibrous scaffold that is

fabricated from 20-μm thick fibres that are insoluble in water [338, 443]. This scaffold undergoes a hydrolysis process, releasing benzyl alcohol and hyaluronan [427].

Hyalograft C is a tissue engineering graft, comprised of autologous chondrocyte, grown on Hyaff 11 scaffolds. This product was attempted for treatment of knee cartilage defects caused by either trauma or osteochondritis diseases in 1999 [429, 444]. In most cases arthroscopic techniques have been used for implantation of Hyalograft C due to the easy handling properties of Hyaff 11 [445]. Hyalograft C has been approved by the Food and Drug Administration (FDA) and is successfully used for the treatment of different articular cartilage defects in more than 3600 patients [428–430].

Several clinical studies are conducted to assess the therapeutic effect of Hyalograft C for articular cartilage treatment [425, 429, 444]. In one clinical study, 53 patients (31 males and 22 females) with mean age of 32 ± 12 years are contemplated for the treatment with Hyalograft C. The average size of cartilage defect is 4.4 ± 1.9 cm^2 in these patients who are examined for 7 years. Based on the cartilage lesion site, either arthroscopic or mini-arthrotomy (medial or lateral parapatellar arthrotomy) techniques have been used to access the lesion site of patients. The procedure for the preparation of implant involves several steps. For enzymatic isolation of autologous chondrocytes, articular cartilage is harvested from non-weight-bearing area of the knee joint. The chondrocytes are expanded on conventional monolayer cell culture flasks for 2 weeks and then seeded on Hyaff 11 and cultured for another 2 weeks in vitro. Before the implantation of construct, the lesion site is debrided, and cartilage defect site is prepared by complete excision of all nonviable tissues [446]. The defected cartilage site is cleaned to the depth of 2 mm to avoid disrupting the subchondral plate. Subsequently, the knee joint is drained of fluid to assist graft delivery and visualisation. Afterwards, the implant with the required size and shape is delivered either with arthroscopic or mini-arthrotomy implantation. In most of the cases, except for patellar and selected trochlear lesions, there is no need for open surgery, and the Hyalograft C implant can be delivered with arthroscopy [425, 428]. Due to the intrinsic adhesive properties of Hyaff 11, there is no need to use additional fixation devices such as tissue glues. This approach eradicates the use of inorganic chemicals in implantation, which promotes the biocompatibility of implants [425]. Hyalograft C provides required support for in vivo cartilage growth during the rehabilitation procedure, and it is completely dissolved and degraded after 4 months post implantation [426].

Based on different clinical tests, Hyalograft C autograft can provide excellent outcome for the repair of deep articular cartilage defects for young and active patients who suffered from singular defects, with good knee alignment and stable joint [444, 447–449]. The effect for other patients is questionable, and it appears that the patients with secondary indications may require further surgeries due to the continuation of pain and swelling after 6-month to 5-year implantation of Hyalograft C [444]. Therefore, further follow-up studies are required to determine the long-term effect of cartilage defect treatment with Hyalograft C.

14.3.7.3 CaReS

Cartilage Regeneration System (CaReS) is a collagen type I hydrogel, prepared from rat tail tendon [432]. In this process, chondrocytes are isolated from the autologous cartilage biopsy specimen. Subsequently, the isolated cells are suspended in a double-buffered 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)[3] solution and gently mixed with type I collagen with the volume ratio of 1:1 (HEPES to collagen). The final mixture (chondrocyte and collagen type I) is then allowed to form hydrogel when they are incubated at 37 °C. Generally, the cell-seeded implants are cultured in autologous serum for a period of 10–13 days [431]. Quality control analysis is conducted to determine cell viability of higher than 80 % and expression of collagen type II for ensuring chondrogenesis from the seeded cells [431, 432].

The therapeutic effect of clinical treatment with CaReS is studied from 2003 to 2008 on 116 patients (67 males and 49 females) with average age of 32.5 ± 8.9 years. The patients suffered from cartilage defects as the result of either trauma degeneration (84 patients) or osteochondritis dissecans (32 patients) with an average defected size of 5.37 ± 2.7 cm^2 (35 patients with lesion size less than 4 cm^2 and 81 above this value) [431]. Chondral defect site is at femoral condyle, patella/trochlea or tibial plateau sites.

Depending on the cartilage defected site, medial or lateral parapatellar arthrotomy has been used to access the lesion site [431]. The defected cartilage site is debrided down to the subchondral bone. The base of the cartilage defect site is then coated with fibrin glue. After which the CaReS implants (with 1-mm wider diameter than the defected site) are push-fitted into the trimmed chondral defected site. Generally, a hydrogel is fabricated with twice the height of the defected cartilage. It is then fitted into the defected site by squeezing the hydrogels and removing 50 % of its water content.

The functional outcomes of the treatment procedure with CaReS are evaluated using the International Cartilage Repair Society (ICRS) and the International Knee Documentation Committee (IKDC) scales. ICRS is a four-level scale, used to categorise functionality and status of cartilage and pain level, while IKDC is a subjective knee assessment questionnaire. At mean follow-up time (30.2 months), IKDC score is significantly ($p < 0.001$) improved compared to preoperative condition (from 42.2 ± 13.8 to 70.5 ± 18.7). The pain level is also significantly decreased ($p < 0.001$) from 6.7 ± 2.2 to 3.2 ± 3.1 from preoperative condition to latest follow-up time. The IKDC results and pain comprehensive reduction level in patients demonstrated the benefit of CaReS for the treatments of traumatic or degenerative articular cartilage defects. During the surgery and in particular the debridement of the subchondral bone, bone marrow space is opened, which provides access for chondroprogenitor cells to CaReS hydrogel (culture with autologous chondrocyte). Therefore, cells can migrate to the defected site and produce

[3]HEPES is a buffer solution, widely used in vitro cell culturing to maintain neutral pH in media.

cartilage extracellular matrix. It is suggested that autologous chondrocytes cultured on CaReS hydrogel are not the exclusive cell source for cartilage formation [431].

14.3.7.4 TruFit

TruFit bone (or cartilage) substitute plugs (Smith & Nephew, Andover, MA) are cylindrical-shaped scaffolds comprised of PLGA and calcium sulphate that are mechanically stable [433]. These scaffolds are commercially available with deferent diameters (7, 9 and 11 mm) [434] and are clinically used to promote cartilage and bone regeneration to restore osteochondral defects [435]. The scaffolds are designed to degrade within a year after implantation (approximately 9 months) [73]. They are used for back filling femoral donor site during mosaicplasty or for primary repair of full-thickness articular cartilage of the femoral condyle lesions [436]. During the operation, the defected sites are drilled and the plugs (TruFit scaffolds) inserted into the drilled site. TruFit scaffolds promote cancellous bone formation due to the presence of osteoinductive calcium sulphate components in its structure with regeneration of fibrocartilage on the surface [450]. It is claimed that this material promotes the regeneration of articular cartilage in full-thickness chondral defect by supporting chondrogenesis cell migration from the surrounding tissue to the defected site [433]. Different in vitro animal and human studies support the positive impact of using TruFit plugs for cartilage regeneration.

TruFit implants can be bioabsorbed in vivo within 1 year after implantation to create space for further regeneration of cartilage ECM [73]. A mini-open surgery at the defected site is performed to deliver the implant TruFit plugs to the osteochondral lesion site. The defected site is then shaved, and the edges of the damaged cartilage are trimmed back to stable healthy location. The scaffolds are then inserted into the defected site to promote osteochondral tissue regeneration [433]. Single or multiple holes (based on the decision made by the surgeon to use single plug or multiple plugs) are drilled with the depth from 8 to 12 mm [437]. It is critical that the diameter of drilled holes and plugs is matched with the size of the lesion site. The plug is then filled with implant and surface of implant is adjusted with healthy cartilage. It is reported that this tamping process might deform the implant; therefore, a bridge distance of 1–2 mm is contemplated while using multiple plugs [437]. It is important that the edges of implants are in direct contact with the surrounding chondral surface to induce normal cartilage regeneration. This strategy enables natural migration of chondrogenesis cells to the site and thereafter regeneration of articular cartilage [437].

The functional outcome of treatment with TruFit is assessed with clinical outcome score (KOOS). The study is conducted on 10 patients (15–50 years old) with 20 % grade III and 80 % grade IV cartilage defect and an average lesion size of 2.65 cm^2. After 1 year, 80 % of patients (8 out of 10) had improvement based on clinical outcome score (KOOS). The clinical outcomes of the operation for the other two patients are poor according to the implemented clinical outcome score. Subsequent monitoring (in 18 months and 24 months) revealed that the clinical

outcome score of patients is reduced significantly. Pain and inflammation are increased in patients and joint functionality is significantly decreased. The implants are removed from 7 patients (7 out of 8). Histological analysis showed the formation of hyaline cartilage on the implants, but a bony cyst is also formed within the regenerated cartilage rather than bony restoration [437].

In a recent clinical study, 15 patients with almost similar lesion grade and an average size of 2 cm^2 are treated with TruFit plugs. After 1 year, same as the previous clinical study, 80 % of patients are improved. Three patients out of 15 have undergone reoperation in less than 1 year. The study is undergoing to evaluate the efficacy of TruFit in the long term [450].

14.3.7.5 NeoCart

NeoCart implant (Histogenics Corporation, Waltham, Massachusetts) is a 3D biodegradable bovine collagen type I scaffold. For cartilage tissue engineering application, NeoCart is seeded with autologous chondrocytes followed by in vitro cultured in bioreactor to promote cartilage regeneration. The resulting product is a chondrogenic active implant, which is rich in proteoglycan and glycosaminoglycan compounds. Preclinical trials and in vitro studies demonstrated that NeoCart implantation at lesion site leads to hyaline cartilage repair at full-thickness defect sites. It is proposed that NeoCart can be used for the treatment of full-thickness (grade III) cartilage lesion of femoral condyle. The treatment process comprised of five main steps, namely, biopsy, cell isolation, cell expansion, 3D culture in bioreactor and implantation [438].

Different in vitro and animal studies are performed to investigate the efficacy of this method for articular cartilage treatment [438]. The development process of NeoCart commenced from the isolation of chondrocyte cells from biopsy specimen taken from the patient. For harvesting cells, approximately 200–400 mg of tissue is taken from non-weight-bearing portion of femoral condyle or from the femoral notch of the lateral knee. During this process, the subchondral bone is not interfered. The biopsy specimen is then processed in laboratory to isolate chondrocyte cells. The cells are then seeded on 3D collagen type I hydrogel and culture in bioreactor. The operation condition, such as hydrostatic pressure, inside the bioreactor is tuned to stimulate chondrocytes to synthesise cartilage glycoprotein [439, 451]. After the levels of produced glycosaminoglycan and proteoglycan approached to a defined value, the scaffolds are removed from the bioreactor and further cultured in static condition. The average culture time (i.e. dynamic and static) is 67 ± 18 days. The cartilage lesion site is then shaved and trimmed before the implantation of the NeoCart. After this step the implant is cut to fit in the defected site and implanted by an open surgery. The surgical knee is then immobilised for 10 ± 2 days after the surgery [438] followed by a passive range of motion to strengthen the joint and surrounding muscles. For 6 weeks after implantation, the surgical knee is non-load bearing with restriction only to rehabilitation similar to femoral condyle microstructure and ACI-type protocols.

The efficacy of NeoCart for the treatment of full-thickness articular cartilage defect is studied on ten patients with grade III cartilage lesion of femoral condyle with an average defected size of 2.2 cm^2. The patients are in the range of 18–55 years old. In two cases, the implants are damaged due to intraoperative motion testings and the procedure is not preceded. The other patients had an isolated grade III chondral injury to the weight-bearing region either in the medial or lateral condyle. Based on visual analogue scale and pain scores, there are significant improvements in all 8 patients. The results of average range of motion evaluation and also knee function assessment with IKDC scale, however, indicated that 7 out of 8 patients had improvement after operation using this implant [438].

14.3.7.6 Pluronic and PEG-da Systems

Pluronic®[4] is a commercially available polymeric system that undergoes physical crosslinking at physiological temperature (37 °C). This biomaterial is used as an injectable system for cartilage regeneration. Chondrocyte suspended in pluronic solution is used for mandibular condylar reconstruction [452]. Pluronic® solution is advantaged by its mild gelation and favourable biocompatibility properties. However, the application of pluronic solution in cartilage tissue engineering is limited due to its very poor mechanical strength, quick dissolution and high permeability [400]. A combination of Pluronic® with other injectable polymers has been used. These formulations include Pluronic®/PEG/NIPAAm, Pluronic®/poly(lactic acid/glycolic acid) (PLGA)[401, 402], Pluronic®/chitosan and Pluronic®/HAPromising [397].

Pluronic®/HA known as a X-HA is used at articular defect model of full-thickness defect of rabbit [413]. Adipose-derived stem cells are stimulated to undergo chondrogenic differentiation, using X-HA hydrogel, loaded with TGF-β. Copolymer of hyaluronic acid conjugated with heparin and Pluronic ® is synthesised to form the hydrogel at 37 °C in less than 10 min. TGF-β3 is successfully loaded within the structure of this hydrogel and could be released continuously in 20 days, which promoted cartilage ECM formation at full-thickness cartilage defect of rabbit knee [413]. Due to superior biological properties of naturally derived polymers, different protein-based polymers such as elastin-like polypeptides are also used for fabrication of hydrogel.

A PEG-da-based injectable hydrogel achieved clinical trial for the treatment of cartilage defects [453]. This PEG-da-based hydrogel is designed to support cartilage matrix production with easy surgical applications. In this approach, to bond the PEG-da-based hydrogel with the cartilage, chondroitin sulphate adhesive is first applied to the lesion site, and the joint is filled with polymer solution. The polymer solution contains 100 mg/ml PEG-da, 5 mg/ml hyaluronic acid and 0.5 mg/ml of Irgacure as photo-initiator in PBS [453]. A specially designed cone is used to apply

[4]Pluronic® is triblock copolymer of PEO–PPO–PEO.

light to the polymer solution to induce the gelation at a constant rate. Fifteen patients with symptomatic cartilage defects are treated with this injectable hydrogel after microfracture, and three patients are treated just with microfracture as control. After 6 months of surgery, no adverse effect is reported and the use of adhesive photo-crosslinked polymer allowed for greater filling of the defected site [453]. In addition, the patients who are treated with the injectable hydrogels reported a decrease in overall pain severity overtime

14.4 Conclusion

Articular cartilage is highly resilient connective tissue with unique mechanical properties which facilitate the mobility of the human body. The avascular structure and low metabolic activity of this tissue resulted in limited capability of this tissue for self-regeneration and repair. Early treatment approaches lead to unpredictable outcomes which are substantially interrelated with age, gender and physical conditions of patients. Tissue engineering approaches are therefore deemed to be more efficient for in vitro or in vivo regeneration of cartilage. Clinical studies on the commercially available biomaterials for cartilage tissue engineering showed that in the most of cases, the long-term final outcomes are unpredictable. The success rate in the best cases based on current results is 80 % for TruFit and 85 % for NeoCart. Many available treatments involve open surgery for transplantation of implant. The massive invasion in the joint has adverse impacts on the healing process of surgical knees. As a result, complicated rehabilitation protocols are generally recommended to patients after surgeries. The side effects of open surgeries and also not fully satisfied rehabilitation process in patients result in the failure of many treatment cases. Additionally, the defected cartilage may include an irregular shape that cannot be repaired by standard-sized scaffolds. Therefore, less invasive approaches such as injectable systems for delivery of cartilaginous construct to the defected site are favoured to tackle the current problems in cartilage tissue engineering. Injectable hydrogels have been contemplated as a non-invasive means for cartilage tissue engineering applicable for regular and irregular shaped cartilage lesions.

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