Scaffolds for Cartilage Regeneration: *To Use or Not to Use?*

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Abstract

Joint cartilage has been a significant focus on the field of tissue engineering and regenerative medicine (TERM) since its inception in the 1980s. Represented by only one cell type, cartilage has been a simple tissue that is thought to be straightforward to deal with. After three decades, engineering cartilage has proven to be anything but easy. With the demographic shift in the distribution of world population towards ageing, it is expected that there is a growing need for more effective options for joint restoration and repair. Despite the increasing understanding of the factors governing cartilage development, there is still a lot to do to bridge the gap from bench to bedside. Dedicated methods to regenerate reliable articular cartilage that would be equivalent to the original tissue are still lacking. The use of cells, scaffolds and signalling factors has always been central to the TERM. However, without denying the importance of cells and signalling factors, the question posed in this chapter is whether the answer would come from the methods to use or not to use scaffold for cartilage TERM. This paper presents some efforts in TERM area and proposes a solution that will transpire from the ongoing attempts to understand certain aspects of cartilage development, degeneration and regeneration. While an ideal formulation for cartilage regeneration has yet to be resolved, it is felt that scaffold is still needed for cartilage TERM for years to come.

Keywords

Biomaterial · Cartilage · Chondrocytes · Development · Regeneration · Regenerative medicine · Scaffolds · Tissue engineering

7.1 Introduction

Joint cartilage has been a significant focus in the field of tissue engineering and regenerative medicine (TERM) since its inception in the 1980s. When "tissue engineering" is combined with "regenerative medicine", these two subjects form a broad advanced scientific field. This advanced field is encompassing principles from various disciplines, in which no single subject may deal

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with its all aspects in a meaningful depth. After three decades of research, the TERM is still immature and contributes insignificantly to the actual healthcare settings. Various tissue-engineered medical products (TEMPs) such as cartilage, bone, skin, bladders, small arteries and even a full trachea have been implanted in patients. However, those TEMPs are still considered experimental and not cost-effective. Although some researchers have successfully formed complex tissues or organs, these tissues or organs are still far from being fully reproducible and ready to be implanted into patients. Despite all the uncertainties surrounding these laboratory-grown TEMPs, the TERM field continues to grow.

Represented by only one cell type, cartilage has been a simple tissue that is thought to be straightforward to deal with. After many years, engineering functional cartilage has proven to be anything but easy. With the demographic shift in the distribution of world population towards ageing [1], it is expected that there is a growing need for more effective options for joint restoration and repair. The WHO report outlined some key facts including:

- *The ratio of the world's population over 60 years old will nearly double from 12% in 2015 to 22% in 2050.*
- *The number of people aged between 60 years and above will be more than children younger than five years old by 2020.*
- *Approximately 80% of older people will be living in low- and middle-income countries in 2050.*
- *The leap of population ageing is much faster than in the past.*
- *All countries across the globe will face significant challenges to ensure that their health and social care systems are ready to make the most of this demographic shift.*

The above facts have a direct relation with the readiness of the global healthcare system in managing or dealing with degenerative diseases. Degeneration naturally occurs among the ageing population. Despite the increasing understanding of the factors governing cartilage development and degeneration, there is still a lot to do to bridge the gap from bench to bedside. Dedicated methods to regenerate reliable articular cartilage that would be equivalent to the original tissue are still lacking.

The use of proper cells source, biomaterial scaffolds and signalling factors has always been central to the TERM field. However, without denying the importance of cells and signalling factors, in this chapter, the authors aimed to emphasise on the use of biomaterial scaffolds in regenerating the articular cartilage. Ideal scaffolds for cartilage TERM should meet some requirements related but not limited to safety, biocompatibility, biodegradability and adequate mechanical properties. Numerous studies and characterisations on scaffolds for articular cartilage tissue engineering have been ongoing and evolving in many forms of the physical aspect, ranging from chemically and biologically cross-linked hydrogel, sponge, fibre, micro-/nanoparticles and 3D printing.

On the one hand, a quick search on currently available literature indicated that the following scaffolds are among the most versatile scaffolds which remain viable and relevant in the field of TERM. They include but not limited to:

- Decellularised tissue-derived scaffolds [2–5]
- Chitosan [6–8]
- Platelet-rich plasma scaffold [9]
- Gelatin and poly(lactic-*co*-glycolic acid) (PLGA) [10, 11]
- Hydrogel [12, 13]
- Collagen hydrogel and polyhydroxyalkanoate [14]
- Alginate [15, 16]
- Silk fibroin [17]
- Gelatin/hyaluronic acid [18]
- Poly-ε-caprolactone (PCL) [19]

On the other hand, the scaffold-free approach has been studied equally for cartilage tissue engineering by some researchers in some part of the world which include:

- Chondrocytes and their self-produced extracellular matrix (ECM) [20]
- Glutamic acid-based dendritic peptides [21]
- 3D bioprinting microtissues, spheroid using a high-throughput microwell system [22]
- Cellular spheroids using 3D bioprinting technology (Regenova Bio 3D Printer) [23]
- "Osteo-chondro" constructs using a scaffold-free bioprinter [24]
- Cell sheet technology [25]

Information given in this chapter is not meant to be comprehensive but to present some efforts in TERM and proposes a solution that will transpire from the ongoing attempts to understand certain aspects of cartilage development, degeneration and regeneration. The question is whether the answer would come from the methods to use or not to use scaffolds for cartilage regeneration.

7.2 Cartilage Structure and Function

Cartilage (*chondral*) is made up of one cell type, i.e. chondrocyte (*chondros* = cartilage; *cyte* = cell). By physical properties, cartilage is categorised as a supporting connective tissue. Cartilage and bone, another supporting connective tissue type, work together and make up the human skeleton to protect soft tissues and organs and support the weight of part or all of the body. Supporting connective tissues vary from connective tissue proper (e.g. adipose tissue and tendon) and fluid connective tissue (e.g. blood and lymph). They have a lesser diverse cell population and a matrix containing much more densely packed fibres than the connective tissue proper and the fluid connective tissue. The ECM of cartilage is a gel with characteristics that vary with the predominant type of fibre [26].

The ECM of cartilage is a firm gel that contains polysaccharide derivatives known as chondroitin sulphate. Chondroitin sulphates and proteins form complexes producing proteoglycans in the ground substance. The only cells in the cartilage ECM, i.e. chondrocytes, occupy small chambers known as lacunae. The proteoglycans of the ECM, as well as the type and abundance of extracellular fibres, determine the physical characteristics of cartilage [27].

Unlike bone and other connective tissues, cartilage is avascular, aneural and alymphatic, so all nutrients and waste products exchange take place by diffusion through the ECM. Because of this situation, cartilage cannot heal efficiently. There is no blood vessels growth in cartilage because chondrocytes produce a chemical known as an antiangiogenetic factor that inhibits their formation. Other angiogenesis inhibitors have also been identified and developed as drugs to treat cancer. The inhibitors discourage the formation of new blood vessels to tumours, thus decelerating the growth [28].

Cartilage is separated from its surrounding tissues by a fibrous perichondrium. The perichondrium consists of two distinct layers, i.e. an outer fibrous layer comprising dense irregular connective tissue and an inner layer consisting the cellular component. The fibrous region gives mechanical support and protection. The layer also attaches the cartilage to other structures. The cellular layer is essential to cartilage growth and maintenance. The presence of blood vessels in the perichondrium is essential in order to provide oxygen and nutrients to the underlying chondrocytes [29].

The three main types of cartilage in the human body are hyaline, elastic and fibrocartilage. Hyaline cartilage (*hyalos* = glass) is the most common type of cartilage. The examples of hyaline cartilage in adults include the nasal cartilages, the connections between the ribs and the sternum, the supporting C-shaped rings cartilages along the trachea and the articular cartilages, which cover the end of bone surfaces within many synovial joints, e.g. the elbow and knee. A dense perichondrium surrounds hyaline cartilages except inside the synovial joint cavities. Hyaline cartilage is a tough tissue but relatively flexible because its ECM has tightly packed collagen fibres. Since these fibres are not in large bundles and do not stain darkly, they are not always seen under the light microscope [26].

Elastic cartilage is exceptionally resilient and flexible because it has numerous elastic fibres.

These cartilages usually have a yellowish colour macroscopically. Examples of elastic cartilage include the auricle or, the external flap of the outer ear, the epiglottis at the opening of the windpipe which prevents food and liquids from entering the trachea when swallowing, the auditory passageway and the cuneiform cartilages in the larynx or voice box [27].

Fibrocartilage is a sturdy and extremely durable tissue because it contains little ground substance and its ECM is dominated by densely interwoven collagen fibres. This tissue can be found as fibrocartilage pads, e.g. in the intervertebral discs which lie between the spinal vertebrae, around tendons and within or around joints and between the pubic bones of the pelvis. In these positions, fibrocartilage absorbs shocks, resists compression, limits movement and helps prevent damaging bone-to-bone contact [28].

7.3 Cartilage Development, Degeneration and Regeneration

In embryogenesis, the skeletal system is derived from the mesodermal layer. Cartilage development (or also known as chondrogenesis or chondrification) is a process by which cartilage is formed from condensed mesenchyme tissue. The mesenchymal cells will differentiate into chondrocytes and begin secreting molecules and substances to form the cartilaginous ECM. Early in foetal development, a major part of the skeleton is cartilaginous in nature. This temporary cartilage is replaced gradually by bone through endochondral ossification, which usually ends at puberty. Nonetheless, the cartilage in the joints remains unossified throughout life and is, therefore, permanent.

Cartilage develops through interstitial and appositional growth. Interstitial growth expands the cartilage from inside. Chondrocytes in the cartilage ECM divide and the daughter cells produce additional ECM. Interstitial growth is an essential process during cartilage development. The process begins early during embryonic development and continues through adolescence. Appositional growth increases the size of the cartilage gradually by adding to its outer surface. During this process, the inner layer cells of the perichondrium divide repeatedly and become chondroblasts [26].

Chondroblasts are immature chondrocytes. The cells begin producing the cartilage ECM. As they are surrounded by and embedded in a new ECM, the chondroblasts differentiate into mature chondrocytes. They now become part of the cartilage and continue to grow. Both interstitial and appositional growth occurs during cartilage developmental stage, but interstitial growth contributes more to the mass of adult cartilage. Neither interstitial nor appositional cartilage growth occurs in healthy adults. However, appositional growth may take place in rare conditions, e.g. after the cartilage has been damaged or stimulated by growth hormone from the pituitary gland excessively. Insignificant cartilage damage can be regenerated and repaired by appositional growth at the affected surface. If the damage has become more severe than the above condition, a dense fibrous patch will develop and substitute the injured portion of the cartilage [29].

In the human body, there are several complex joints, including the knee joints that consist of both hyaline cartilage and fibrocartilage. The hyaline cartilage articulates the end of bone surfaces, while the fibrocartilage pads the joint to prevent friction between bones during movement. Any injuries to these pads can interfere with regular movements because they do not heal spontaneously. After repeated or severe damage, joint mobility is significantly reduced. Although surgery may be prescribed to overcome joint mobility issue, it usually gives only a temporary or incomplete repair. Unlike cartilage, complete bone regeneration and repair can be achieved even after severe damage to the structure [26, 27]. It is because the bone is rich in vascularisation, but the cartilage is not.

A compelling argument in TERM field is that *is developmental process* equivalent with *regeneration*? In a recent review article on cellular senescence in development, regeneration and disease, Muriel et al. [30] indicated that although many studies have exposed beneficial effects of

senescence, especially in the context of embryonic development, tissue repair and regeneration and cellular reprogramming, the understanding of the biological functions of the senescence process is still lacking. Perhaps a thorough comparison of senescent cells in each stage will help to understand their real biological significance.

Myohara [31] has suggested previously that comparisons between development (or embryogenesis) and regeneration can give information about the steps essential to regeneration. The knowledge would help the scientist to gain better insight into how much reactivation of developmental processes might help improve regeneration capacity in higher vertebrates. By using an example of the in vivo osteogenesis potential of mesenchymal-like cells derived from human embryonic stem cells (hESC-MCs) study, Kuhn et al. [32] suggested that the implanted hESC-MCs differentiated to chondrocytes and bone-forming cells and tissue via an endochondral ossification pathway. Interestingly, no osteogenic or chondrogenic differentiation protocols were introduced to the cells before implantation. According to Kuhn et al. [32], this developmental-like bone regeneration study represents a crucial step forward for tissue engineering because of the reproducibility of new bone formation without preimplantation differentiation to osteo- or chondroprogenitors or having to over-commit the hESC-MCs to a particular lineage before implantation.

Nevertheless, from the analyses conducted on annelids or segmented worms, Myohara [31] stated that the alkaline phosphatase (ALP) expression patterns and central nervous system (CNS) development differ between embryogenesis and the regeneration. Although annelids are invertebrates, the results serve as an indication that regeneration is not a simple replication of embryogenesis but involves different regulatory mechanisms, especially in higher vertebrates. In another study on a stepwise model system for limb regeneration, Tetsuya et al. [33] suggested that although the later phase of limb regeneration is equivalent to its development, the early phase involving blastema genesis is unique to regeneration that perhaps would enhance regenerative processes in humans. There are many other examples, but the above initiatives give a basis for the exposition of unique and crucial mechanisms to regeneration which remains underexplored in cartilage tissue engineering.

7.4 Cartilage Disorders and Management

Findings of a Global Burden of Disease (GBD) 2017 study show that human life expectancy is 73 years, but healthy life expectancy is only 63 years [34]. From the two figures, on average, 10 years of life were spent in poor health globally. Another GBD study indicated that musculoskeletal injury and degeneration are leading causes of disability in 2010, with osteoarthritis (OA) as the most common cause of disability in older adults [35]. With a demographic shift in the distribution of world population towards ageing as per stated in the [1] report, it is expected that there is a growing need for more effective options for joint restoration and repair [1].

Osteoarthritis is a long-term chronic disease characterised by the deterioration of the cartilage in joints. Other than related to ageing, OA is also associated with various modifiable and non-modifiable risk factors, e.g. obesity, lack of exercise, bone density, occupational injury, trauma, gender and genetic predisposition (Table 7.1). These examples are based on the assessment in the context of the Malaysian population. The OA symptoms include joint pain, stiffness, joint swelling and decreased range of motion. If the vertebrae or backbone is affected, numbness and weakness of the arms and legs will indeed affect work and alter daily activities.

Table 7.1 Risk factors

Non-modifiable	Modifiable
Advancing age	Body mass index (BMI)
Female	>25 kg/m^2
Genetic	Previous knee injury
Heberden's nodes in hand OA	Joint malalignment

Adopted from the Malaysia Health Technology Assessment Section, MaHTAS [36]

Osteoarthritis can be classified into primary (idiopathic) and secondary OA based on the joint involved, i.e. hand, hip or knee, or by aetiology. The primary OA includes generalised OA, a condition associated with Heberden's nodes and polyarticular disease which occurs mainly in the hand, with a female preponderance and has a high prevalence in first-degree relatives. As for the secondary OA, it can be due to several factors: (1) metabolic disorders such as acromegaly, haemochromatosis and chondrocalcinosis; (2) anatomic such as slipped femoral epiphysis, Legg-Perthes disease, congenital dislocation of the hip, leg length inequality, hypermobility syndromes and avascular necrosis; (3) trauma such as joint injury and fracture through a joint or osteonecrosis; and (4) inflammatory such as rheumatoid arthritis, psoriatic arthropathy and septic arthritis.

As indicated in the earlier section, mature cartilage tissue has minimal capacity for self-repair. If the cartilage is injured and left untreated, it can lead to early degeneration and progress into OA. As far as this paper is written, there is no known cure for OA. Pharmacotherapy, physical rehabilitation, strengthening exercise, interventional therapy, complementary medicine and surgery help to improve patient's outcome. However, the available therapies do not treat or address the underlying issues. Although current surgical interventions to cartilage repair are clinically useful, they are unable to restore the structurally and functionally normal articular cartilage surface. In the case of Malaysia, the algorithm on the management of knee and hip OA is summarised in Fig. 7.1.

As of 2013, because of the lack of available evidence, the Clinical Practice Guidelines (CPG) and Quick Reference (QR) for the Management of Osteoarthritis (Second Edition) issued by the Ministry of Health (MOH) Malaysia were unable to recommend the use of intraarticular stem cells, autologous chondrocyte implantation, platelet-rich plasma or even any recent advances in orthopaedic tissue engineering approaches in the treatment of OA [36, 37]. It was indicated in the 2013 CPG document that it would be reviewed if new evidence in the treatment of OA becomes available, which is not the case, as of 2019. It is felt that the outcome of TERM research, if successful, may have an impact on the Malaysian CPG. Relevant scientific evidence for OA management will be disclosed based on the best cartilage TERM approaches. The information perhaps can shed some light and give some insight into OA holistic healthcare model and be included in the CPG, MOH Malaysia, as one of the viable benchmarks for OA management.

7.5 Cartilage Tissue Engineering

7.5.1 Cells Source

Cells can be taken from autologous, allogeneic or xenogeneic cells sources. Autologous cells are harvested from the same individual (donor = recipient), while allogeneic and xenogeneic cells are harvested from a different person and a different species, respectively. The types of cell can be divided into differentiated and undifferentiated cells. These two cell types vary in that the differentiated cells (or also known as adult progenitor cells, specialised cells or committed cells) perform a specific function in the tissue, while the undifferentiated cells are uncommitted cells (or also known as stem cells) that will remain uncommitted until appropriate signals stimulate the stem cells to differentiate into committed cells.

It has been well-documented that the triggering needs for stem cells in TERM are because of the inadequate supply of committed cells so far. Other unresolved issues include morbidity at the harvested donor site as well as lack proliferative and biosynthetic activities of the committed cells. Stem cells have been known for their ability to self-renew and to divide actively in the monolayer in vitro culture. Stem cells can differentiate into multiple specialised cell types in the body. This criterion makes them as a suitable candidate for tissue regeneration and repair, especially for tissues that are unable to regenerate spontaneously after injuries.

Stem cells can be isolated from a human embryo, foetal or relevant adult tissues. Other

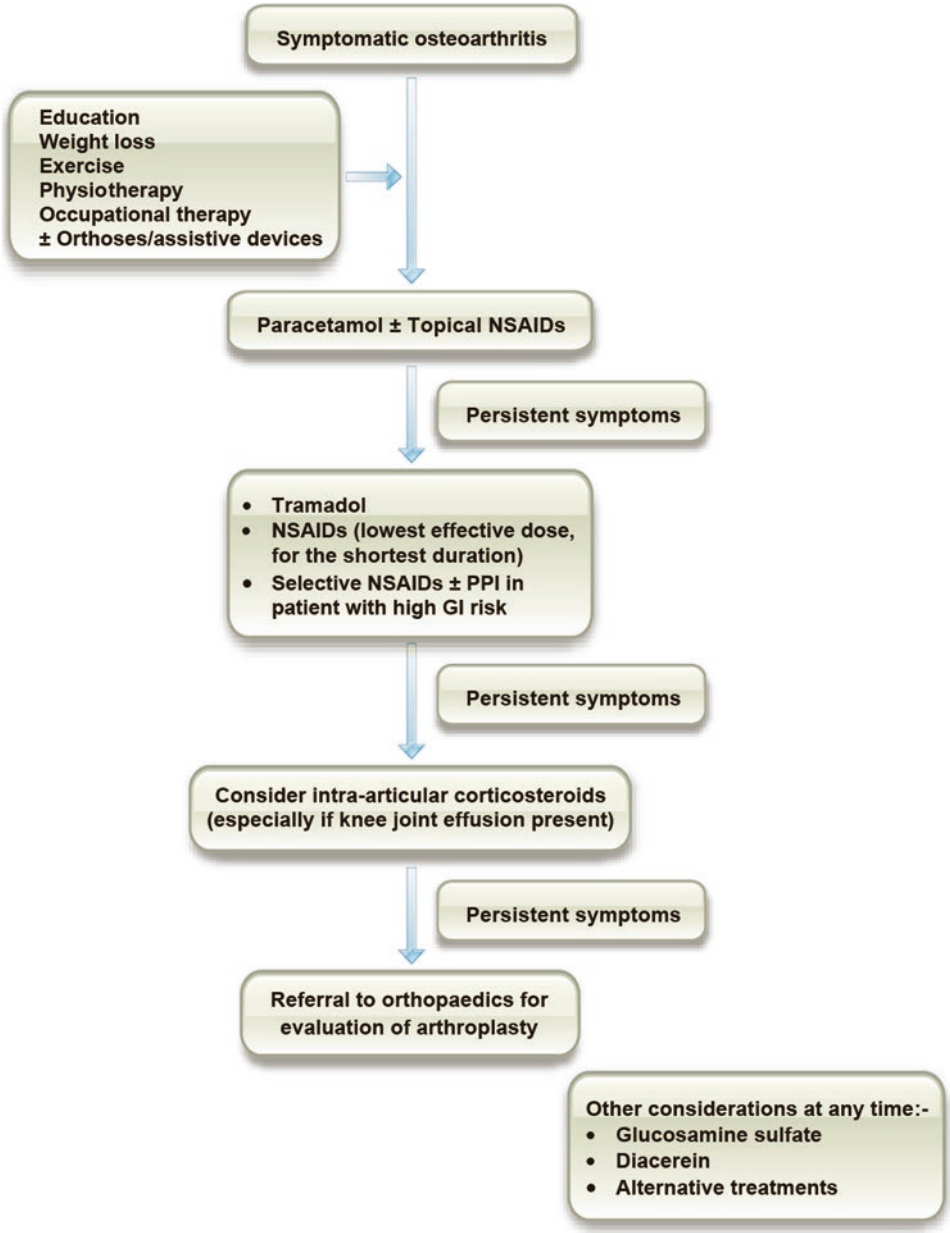

Fig. 7.1 Algorithm on the management of knee and hip osteoarthritis based on the CPG and QR, Management of OA, MOH Malaysia (Adopted from Refs. [36, 37])

than isolating cells from the inner cell mass of the blastocyst, the pluripotent embryonic stem cells (ESCs) can also be harvested from foetal tissue from terminated pregnancies. To date, TERM researchers are still investigating whether the differentiated cells and the undifferentiated stem cells (from adult tissues) have equivalent potential to that of the ESCs [12, 19, 38]. In terms of development potential, ESCs have been reported to have a more significant differentiation potential than the differentiated cells and adult stem cells (ASCs) [39]. While the ESCs can differentiate into almost every cells lineage, the ASCs may only develop into limited cell types. However, the

ASCs have shown to have greater plasticity than they were initially thought [9, 40]. The remaining challenge is that *which cells source holds advantages for tissue regeneration*?

From the above arguments, both the differentiated cells and the ASCs hold a unique advantage. In a fully autologous system, a patient's cells will be harvested, cultured and reimplanted or transplanted back into the same patient. It can be appreciated that there shall be no issues on immune rejection since the autologous cells are compatible with the patient's own body. Nevertheless, for ESCs, the recipient may require lifelong immune-suppressive drugs to overcome rejection of the newly transplanted cells. The differentiated cells and ASCs are adult tissues and obtained with consent from the patient. Technically, there may be little if any ethical issue on the ASCs therapies compared to the ESCs.

7.5.2 Signalling Factors

The governing principle of this part is that cell fate is influenced by cells' interactions with components of their microenvironment. Cell fate is believed to have a strong association with culture conditions. Cell differentiation requires optimum physiological conditions such as temperature, pH, oxygen, 3D environment and adequate cell-to-cell contact. Biochemical factors (e.g. nutrients and growth factors) and physical stimulation (e.g. compression and tension) are essential to direct proper cell growth and differentiation. Insufficient signalling factors will lead to loss of specific function, cells senescence or ageing and, eventually, cell death. The signalling factors may include soluble and immobilised factors, the ECM (*see* biomaterial scaffolds) and signals presented by adjacent cells. In cell culture basis, defined culture media induce cell differentiation by providing vital regulatory factors.

Dynamic culture system such as bioreactors improves cell seeding and functional tissue development by providing mixing, mass transport and biophysical stimulation. This microenvironment simulation is critical for proper expansion of cells in vitro and particularly significant for both primary and translational research in TERM.

Gene transfer approaches have been introduced for TERM applications due to inefficiencies of protein delivery in vitro ([41]; Md Ali@ [42]). The difficulties of protein delivery include short biological half-life, ineffective localisation, rapid withdrawal from the application site, the higher dosage required, unwanted side effects and very costly. In overcoming these issues, gene transfer offers more efficient management of protein delivery through independent protein regulation [43]. The advantages of gene transfer include the ability to sustain and regulate the endogenous synthesis of a gene product, efficient localisation and higher biological potency with multiple gene transfer [44]. In practical, gene transfer can be done in situ with minimal scaffolds requirement.

Genetic engineering is one of the most significant discoveries in modern science nowadays. Its applications (e.g. cloning and recombinant technology) enable us to synthesise growth factors or its gene and hormones (e.g. insulin that was taken from pig previously) for both research and clinical treatments. Gene transfer involves cloning and thus part of genetic engineering. If the combination of gene transfer and tissue engineering approaches is successful, a simple, cost-effective, expedited tissue restoration may be achieved using a single intraoperative procedure, as indicated in Fig. 7.2.

Figure 7.2 illustrates the hypothetical impression to use the gene transfection procedure using the identified vector into the harvested mesenchymal stem cells for osteochondral treatment. The transfecting cells will be then incorporated with a suitable biomaterial scaffold and transplanted into the defect. It is anticipated that the resulting cells-scaffold complex will be able to regenerate and achieve full tissue reparation. It is also believed that this single intraoperative procedure will reduce harm to the patient [46].

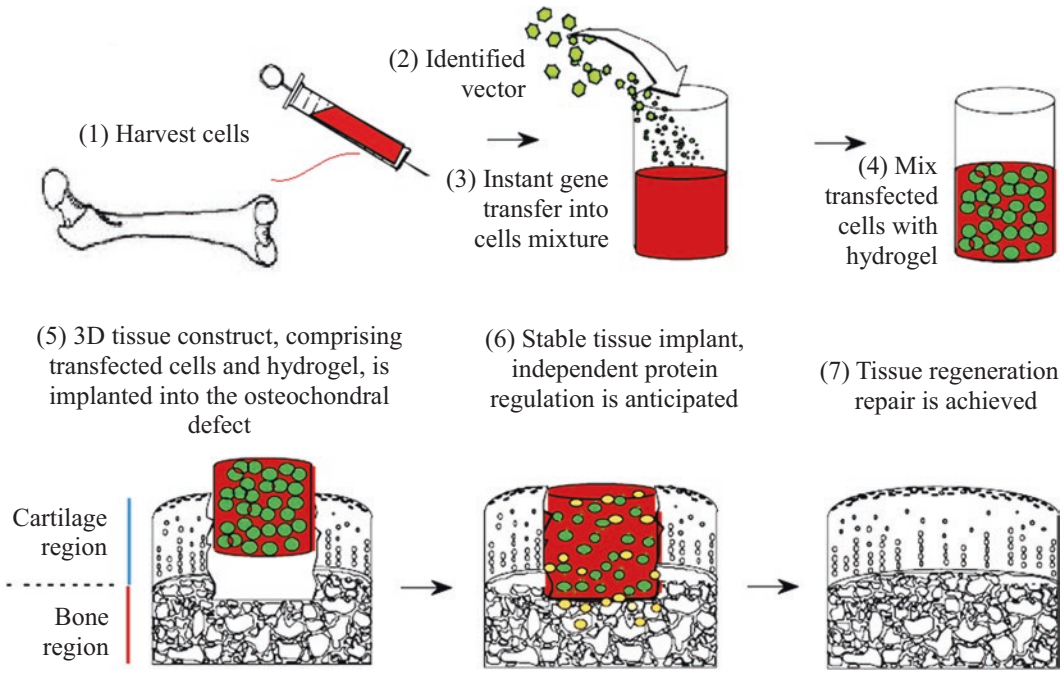

Fig. 7.2 A stepwise gene transfer approach for cartilage TERM based on the osteochondral defect model (Adopted and adapted from Ref. [45])

7.5.3 Biomaterial Scaffolds

The use of cells and growth factors are quite specific in TERM experiments. However, the use of biomaterial scaffolds may vary depending on the needs or design of a tissue. It is believed that "nature" is the best designer for tissue or organ development. It has never been easy to manufacture scaffolds since the suitable design for biomaterial scaffolds should bear a resemblance to the actual extracellular matrix of the tissue [47].

Biomaterial scaffolds can be either natural or synthetic. The natural and synthetic biomaterials can be used individually or in combination to produce functional scaffolds. Suitable scaffolds will direct cell growth and regenerate 3D tissue [48]. The naturally derived biomaterials include protein- and polysaccharide-based materials. Proteins and polysaccharides hold significant advantages and meet the requirements for TERM applications based on their multitude of functions in the human body. Natural biomaterials usually have suitable sites for cellular adhesion and biocompatible to the human body. However, the composition of natural biomaterials can be varied and uncertain. The purity of the protein-based biomaterials (e.g. collagen, silk and fibrin) or polysaccharide-based biomaterials (e.g. agarose, alginate, hyaluronan and chitosan-based scaffolds) must be appropriately identified to avoid potential post-implantation activation of the immune response. In terms of mechanical properties, usually the naturally derived scaffolds lack mechanical strength [49] and thus need to be optimised accordingly.

Polymer-, peptide- and ceramic-based biomaterials are the most common synthetic biomaterials used in TERM. As an alternative to the natural biomaterials, these synthetic biomaterials have well-defined chemicals and biomechanical compositions. The synthetic biomaterial scaffolds can be tailor-made to meet specifications at the injury or implantation site. The properties are essential to determine cell differentiation and facilitate reproducibility of the scaffolds in that the mechanical properties, shape and degradation

rate can be controlled based on the intended requirement. In drug developments, the specific degradation rate is more critical as it controls the release (rate) of drugs incorporated into scaffolds. Unlike natural biomaterials, the synthetic biomaterials lack sites for cell adhesion. The sites must be altered chemically to allow appropriate signals for cell adhesion and proliferation.

The suitability for in vivo implantation is subjected to the biocompatibility of the materials [50]. Therefore, biocompatibility assessment of the materials and its by-product is essential to avoid any harms or complications such as unwanted immune responses that may be triggered in the host-recipient after implantation [51]. Biocompatibility testing can be done based on the US Food and Drug Administration (FDA) guideline to ensure a thorough safety assessment. Other than safety issues, the origin of the materials should be observed and must not contain prohibited materials.

7.5.4 Scaffold-Based and Scaffold-Free Approaches: *Current Trend and Way Forward*

It can be appreciated that the current methods in TERM employed two different yet interrelated strategies, i.e. scaffold-based and scaffold-free approaches. A systematic search on cartilage tissue engineering study between 1994 and 2017 using Web of Science (WoS) and Scopus databases yielded 4071 articles after the removal of duplicate items in both databases amounting to 1393 articles. All data were extracted between January and March 2018, and the thematic analysis was completed on 30 May 2019. After the exclusion of 189 non-English articles, 1361 non-original research articles, 138 unavailable full-text articles and 594 indirectly related articles, a total of 1789 articles included for the analyses with 1645 articles are directly related to "biomaterials". Although Martin-Martin et al. [52] suggested that in all areas, Google Scholar database citation data is *a superset of WoS and Scopus, with substantial additional coverage*, the selection of the two later databases is enough for the review of this paper.

Out of 1645 articles, 706 studies involved natural biomaterials, 363 studies used synthetic biomaterials, 242 studies used combination of the natural-synthetic biomaterials, 183 studies aimed at scaffold-free approach, 115 studies did not specify the types of biomaterials or scaffold they used and 36 studies used either natural or synthetic biomaterials in their articles (Fig. 7.3). From the results, the scaffold-based approach (89%) is more popular than the scaffold-free approach (11%) across the TERM field worldwide. Nonetheless, Ovsianikov et al. [53] opined that the rapidly emerging synergetic TERM strat-

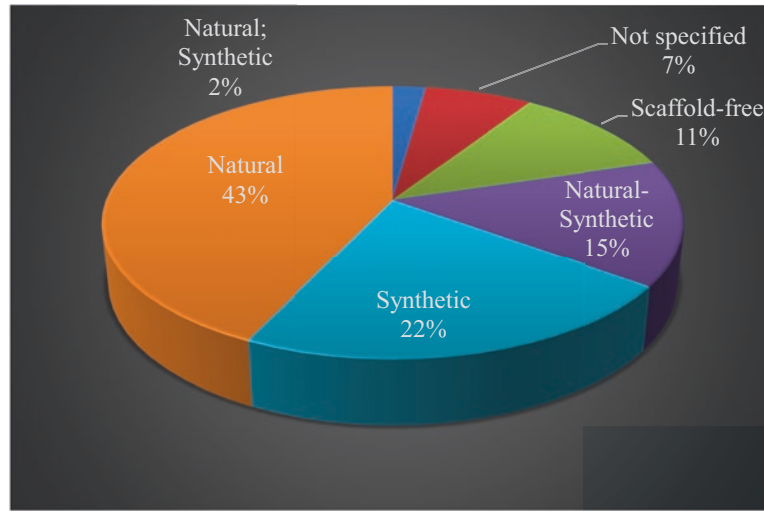

Fig. 7.3 The distribution of scaffold-based and scaffold-free approach based on 1645 articles

egy, integrating scaffold-based and scaffold-free approaches, represents a new, genuinely convergent research direction with strong potential for enabling disruptive solutions and advancing the fields of TERM.

The focal point of scaffold-based approach is on the use of appropriate transient 3D template, skeleton or framework to support cellular attachment, proliferation and formation of new tissue and organ. The essence of the vital functions of the scaffold should be adequately designed to match the degradation profile of the scaffold to the formation of new ECM by the cells. This aspect must be balanced and is always necessary to maintain the compliance of the TEMPs, particularly for weight-bearing tissues such as cartilage [9]. Durable 3D scaffolds can protect cells from possible damage by external factors. Another aspect of design that must be taken into consideration is that the scaffolds should be able to equip a biomimetic microenvironment for cells as well as the delivery and controlled release of signalling molecules to facilitate new tissue formation [54].

With 89% coverage of research worldwide, the scaffold-based approach is seen as a popular and advantageous method, especially in addressing the mechanical properties and degradation profile of TEMPs. The choices of biomaterial scaffolds are many, and they can be tailored to suit the TERM applications (Appendix). There is also an option to deliver signalling molecules either by controlled release from the materials or by immobilizing them on the surface [55, 56]. In addition, rapidly progressing 3D printing technologies offer a wide range of possibilities from using bioinspired composites to the realisation of multiphasic TEMPs and shape-morphing systems [22–24].

The scaffold-free approach is a bottom-up strategy using cell sheet engineering [57, 58], spheroids [10, 11, 59] or tissue strands [60, 61] as building blocks. This approach depends on the intrinsic ability of these cellular materials to assemble and fuse to form larger tissue constructs or TEMPs. Unlike the scaffold-based approach, scaffold-free TEMPs need a high initial cell density. In this case, the proliferation and migration of cells are not absolute factors, so the time needed for new tissue formation can be reduced significantly. A notable advantage of this scaffold-free approach is its ability to address the structure or architecture of the multifaceted tissues or organs by the controlled assembly of various cellular sources [53].

However, one critical disadvantage of this scaffold-free approach is the inferior mechanical properties of the cellular sources in that the materials of the cell may break during the manipulation in vitro. In addition, the holding time needed to obtain a reliable TEMP may be longer than the scaffold-based approach because the scaffold-free cellular materials sometimes need to fuse themselves and prompt the ECM to deposit and thus develop the tissue. Despite lingering uncertainties concerning the above facts, "cell sheet engineering" perhaps is the most successful scaffold-free approach, developed using temperature-responsive culture dishes by a Japanese research team. This method is explored to overcome the limitations of tissue reconstruction using biodegradable scaffolds or single-cell suspension injection. Popularised by Yamato and Okano [62], the resulted cell sheets have been applied clinically for various tissue reconstructions, including ocular surfaces, periodontal ligaments, cardiac patches and bladder augmentation.

7.6 Conclusion

Basic research and scientific development reveal the potential of TERM applications. However, a significant number of unanswered questions about the actual requirements for tissue regeneration, the mechanisms associated with its pathophysiology and the unresolved ethical issues remain as challenges to the field. While an ideal formulation for cartilage regeneration has yet to be resolved, it is felt that the scaffold-based approach is still needed for cartilage TERM for years to come.

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Appendix

List of biomaterial scaffolds used as an individual or in combination in cartilage tissue engineering experimentation based on 1645 studies starting 1994 to 2017. Note Table (A) natural biomaterials and (B) synthetic biomaterials.

(A) Natural biomaterials
1. Agarose
2. Collagen type I (Integra®) commercial
3. Hyaluronic acid (HYAFF®-11)
4. Fibrin
5. Alginate
6. Collagen I
7. Collagen I/GAG
8. Gelatin
9. Calcium phosphate tribasic
10. Collagen type I
11. Collagen II
12. Hyaluronic acid, alginate (NS) [NS]
13. Silicone rubber membranes coated with type I collagen; ~agarose
14. Atelocollagen I
15. Silk fibroin
16. Calcium polyphosphate
17. Chitosan
18. HYAFF-11; HYAFF-11-S
19. Sodium alginate
20. Methacrylated form of hyaluronan (HA-MA) (hydrogel, cylindrical) [Photocross-linking]
21. Collagen type I (Cellagen™) commercial
22. Self-assembling collagen type I
23. Alginate; agarose; gelatin; fibrin
24. Agarose; alginate; gelatin
25. B-TCP
26. Cartilage ECM
27. Hyaluronic acid methacrylated
28. Bacterial cellulose; collagen type II; alginate
29. Hyaluronan
30. Self-assembled (collagen type II-coated; aggrecan-coated); ~agarose
31. Collagen I; II; III
32. Pellet; ~atelocollagen
33. Fibrinogen
34. Hyaluronic acid;{atelocollagen}
35. Hyaluronic acid (HA) hydrogels (2 wt% 1100 kDa, 2 wt% 350 kDa, 5 wt% 350 kDa, 2 wt% 50 kDa, 5 wt% 50 kDa, 10 wt% 50 kDa; 20 wt% 50 kDa)
36. Pellet; ~collagen
37. Macroporous gelatin-coated microcarrier beads CultiSpher
38. Gelatin (photopolymerisable styrenated gelatin)
39. Alginate beads; agarose
40. CaReS (rat-tail collagen type I); atelocollagen (bovine collagen type I); dermal regeneration template (bovine collagen type I); Chondro-Gide (bovine collagen type I/III); atelocollagen honeycomb small (bovine collagen type I); atelocollagen honeycomb large (bovine collagen type I)
41. Human amniotic membrane (epithelial side of intact HAM (IHE), basement side of denuded HAM (DHB) and stromal side of denuded HAM (DHS))
42. Collagen type I (Resorba®) commercial
43. Collagen
44. ECM (cell-derived)
45. Pellets vitro and vivo; ~alginate gel vivo
46. Micromass; collagen honeycomb
47. Osteochondral cores (cylindrical) [NS]
48. Collagen I (Antema®) commercial
49. Pellet → engineered ECM
50. Collagen type II
51. Alginate hydrogel; agarose hydrogel
52. Hyaluronan biomaterial (HYAFF-11, Fidia) {cylinder} [NS]
53. Alginate bead → coralline hydroxyapatite
54. Hyaluronic acid {hydrogels}
55. Decellularised (cartilage ECM)
56. Collagen I (Helistat®) commercial
57. Chitosan + Arg-Gly-Asp (RGD); chitosan + epidermal growth factor (EGF)
58. Coral
59. Whole blood, agarose
60. Cell sheet → cell plate in culture insert; ~atelocollagen honeycomb-shaped
61. Chitin (di-butyryl-chitin)
62. Alginate beads → calcium phosphate Calcibon®
63. Cellulose
64. Gellan gum
65. Hyaluronic acid HA (0.5, 1 and 2 g)
66. Aragonite matrix
67. Layered agarose hydrogel

(continued)

(A) Natural biomaterials
68. Chondron ECM
69. Cross-linked methacrylated hyaluronic acid hydrogels (MeHA);{agarose}
70. HA; agarose {gel}
71. Collagen II (recombinant human)
72. Calcium alginate
73. Gelatin, chitosan (cylindrical) [NS]
74. Gellan um;{agarose}
75. Alginate (hydrogel) [NS]; demineralised bone matrix (NS) [3D printing]
76. Atelocollagen
77. Hyaluronic acid (nonwoven mesh) [NS]
78. Decellularised (osteochondral graft)
79. Demineralised joint condyle
80. Alginate beads; ~hydroxyapatite (HA) carrier
81. Collagen type I (CaRes®)
82. Collagen I (CaReS®)
83. Hydroxyapatite, chitin, chitosan (NS) [NS]
84. Collagen type I (Arthro Kinetics Biotechnology)
85. Collagen (Chondro-Gide®)
86. Pellet; cross-linkable hyaluronan hydrogel
87. Decellularised osteochondral explant
88. Gelatin; chitosan
89. Silk fibroin;{hyaluronic acid (HYAFF®-11)}
90. Fibrin glue hydrogel; platelet-rich fibrin glue hydrogel; fibrin glue hydrogel containing heparin-binding delivery system; platelet-rich fibrin glue hydrogel containing heparin-binding delivery system
91. Nonbiomedical and biomedical grade alginates
92. Collagen I (Porcogen™)
93. Sodium alginate (Sea Matrix®)
94. Methacrylated glycol chitosan
95. Collagen type I, collagen type III (disc) [NS]
96. Self-assembled; fibrin
97. Collagen I (Ultrafoam®) commercial
98. Pellet culture; agarose
99. Hyaluronic acid (HA) hydrogel; agarose hydrogel
100. Hyaluronic acid methacrylated; agarose
101. κ-Carrageenan
102. "Hydrogel: (1) soluble rat-tail type I collagen (0.2% w/v) (BD Biosciences, San Jose, CA, USA); (2) type I collagen (0.2%) incorporating transglutaminase (TG)-2 (100 lgml–1) (Sigma); (3) type I collagen (0.2%) incorporating microbial transglutaminase (mTG; 100 lg ml–1) (Ajinomoto Food Ingredients LLC, Chicago, IL); (4) type I collagen (0.2%) incorporating genipin (GP, 0.25 mM) (Wako, Richmond, VA, USA); (5) type I collagen (0.2%) incorporating GP (0.25 mM) and control agarose beads (without heparin) (Sigma); and (6) type I collagen (0.2%) incorporating GP (0.25 mM) and heparin-agarose type I beads (10% weight of heparin/weight of collagen) (Sigma)

(A) Natural biomaterials
103. Sponge-like scaffolds were prepared: (1) porcine type I/III collagen (CI) (0.5% w/v) (Geistlich Biomaterials, Wolhusen, Switzerland); (2) CI (0.5%) additionally supplemented with CS (7% w/w relative to CI) (Sigma Chemical Co., St Louis, MO, USA); and (3) CI (0.5%) additionally supplemented with HS (7% w/w relative to CI) (Sigma)"
104. Cell pellet – collagen type II nanoarchitectured molecules; collagen fibrils (CNFs); collagen spheres (CNPs)
105. Gelatin; chitosan; agarose
106. Decellularised (dermal ECM)
107. Hyaluronic acid (HYAFF®-11); collagen (Bio-Gide®) commercial
108. Self-assembled (agarose mould) → collagen cross-linking via lysyl oxidase (timing)
109. Collagen type I (PureCol®) commercial
110. Bacterial cellulose
111. Pellet culture (aggregate);~micromass (self-assembled) in plate; ~collagen II
112. Devitalised cartilage explant
113. Alginate (beads) [NS]; cell pellet (NS) [NS]; collagen, chitosan (NS) [NS]
114. Alginate bead → scaffold free on b-tricalcium phosphate carriers []
115. Fibrin hydrogel in agarose well; agarose well only
116. Osteochondral cores, agarose (disc) [NS]
117. Extracellular matrix (ECM) by ASCs; ECM by synovium-derived stem cells (SDSCs). The cell in pellet condition
118. TCP
119. Glycerol phosphate
120. Decalcified bone matrix
121. Hyaluronic acid hydrogel
122. Recombinant human collagen type II (Fibrinogen Europe, Helsinki, Finland)
123. PRP
124. Micromass; pellet culture model; vivo~fibrin gel
125. Injectable hydroxypropylmethylcellulose (HPMC) hydrogel
126. Hyaluronic acid methacrylate (HA-MA), chondroitin sulphate methacrylate (CS-MA); (hydrogel) [NS]
127. Collagen type I, collagen type III (NS) [NS]
128. Sulphated alginate
129. Agarose; plasma; whole blood
130. Photocross-linkable gelatin-methacrylamide (Gel-MA); varying concentrations (0–2%) of hyaluronic acid methacrylate (HA-MA)
131. Human acellular cartilage matrix powders
132. Self-assembled (polyethylene terephthalate (PET)-coated); agarose hydrogel encapsulation
133. Methacrylated gelatin

(continued)

(A) Natural biomaterials
134. ECM (MSC-derived)
135. Demineralised bone matrix
136. Hybrid organic-inorganic (HOI) material photopolymer ORMOSIL SZ2080; ∗collagen type I membrane
137. Decellularised (meniscus ECM)
138. Alginate; chitosan; fibrin
139. Heparin-conjugated fibrin (gel) [NS]
140. Microcavitary alginate hydrogel (microsphere)
141. Chondroitin sulphate methacrylate
142. Micromass cell pellets; alginate hydrogels
143. 45S5 Bioglass®
144. Graphene oxide (NS) [NS]
145. Amniotic membrane
146. Hyaluronic acid (NS) [NS]
147. Collagen type I, collagen type II, hydroxyapatite (cylindrical) [NS]
148. Porcine articular cartilage extracellular matrix (ACECM) (disc) [directional crystallisation and freeze-drying]
149. Cartilage ECM powder
150. Self-assembled; ~alginate
151. Pellet; ECM hydrogel
152. RGD-immobilised microcavitary alginate hydrogels; microcavitary alginate hydrogel
153. Gelatin methacryloyl
154. Gelatin methacrylamide (GelMA), hyaluronic acid methacrylate (HAMA), alginate (ALG), hydroxyapatite paste (HAP) (hydrogel) [3D printing]
155. Chitosan; alginate; collagen I
156. Demineralised cancellous bone
157. Human dermal fibroblast-derived ECM (hECM)
158. Decellularised (cartilage ECM) and methacrylated; methacrylated gelatin
159. Calcium-cobalt alginate
160. Devitalised cartilage
161. Transglutaminase-cross-linked hyaluronan hydrogels (HA-TG); alginate
162. Pellet; alginate bead; {monolayer}
163. ECM
164. Alginate; agarose
165. Decellularised (bone matrix) and demineralised
166. Gelatin methacrylamide; polyacrylamide
167. Pellets; agarose
168. Monomeric type I and type II collagen
169. Sodium alginate, collagen type I, collagen type II, chondroitin sulphate (hydrogel) [NS]

(B) Synthetic biomaterials
1. 2-Hydroxyethyl methacrylate-L-lactate–dextran (HEMA–LLA–D)
2. B-TCP
3. Calcium carbonate (Calcibon®)
4. Calcium polyphosphate
5. Cell pellet; ~PLGA
6. Collagen-like proteins
7. Compact polyelectrolyte complexes (CoPECs)
8. Elastin-like polypeptide (ELP)
9. Hyaluronan benzyl ester (disc) [NS]
10. Injectable PLGA microsphere
11. Macromers of PEG-caprolactone (PEG-CAP) endcapped with norbornene (PEG-CAP-NOR)
12. Nonporous microcarriers poly(lactic-co-glycolic acid) (PLGA); porous PLGA; amine-functionalised PLGA-NH2
13. Nonwoven PGA fibres
14. Nonwoven polyethylene terephthalate fibre
15. NS polycarbonate membrane
16. Oligo(trimethylene carbonate)-poly(ethylene glycol)-oligo (trimethylene carbonate) diacrylate (TPT-DA)
17. OPF
18. PBT
19. PCL
20. PEG
21. PEG hydrogel; PLGA microfibers
22. PEGDA
23. PEGDM
24. PEG–oligo(lactic acid) dimethacrylate PEG–LA-DM
25. Peptide-modified PEGDA (hydrogel) [NS]
26. PGA
27. PGA; PLGA (disc) [NS]
28. PGA; PLLA; PDLLA; PLGA; PCL
29. PGA-PLA (Ethisorb 210); poly-L-lactic acid
30. PGLA (polyglycollic-co-lactic acid)
31. PHBV (3-hydroxybutrate-co-3-hydroxyvalerate)
32. PLA
33. PLA (OPLA®)
34. PLA; PGA; PLGA
35. PLAG
36. PLCL
37. PLG
38. PLGA
39. PLGA, poly(ethy1ene oxide)-dimethacrylate, poly(ethy-1-ene glycol) (NS) [double emulsion]

(continued)

(B) Synthetic biomaterials
40. PLGA; polydioxanone (PDO)
41. PLGA-fleece (darts) [NS]
42. PLLA
43. PLLA (NS) [electrospinning]
44. PLLA (RESOMERL207S)
45. PLLA; PLGA(L); PLGA(H); PLA/CL; PDLA
46. PLLA; PGA; PLGA; PLAO3
47. Poly(1,8-octanediol citrate)
48. Poly(2-acrylamido-2-methyl-1-propanesulfonic acid (NaAMPS)-co-N,N-dimethylacrylamide(DMAAm))
49. Poly(2-hydroxyethyl methacrylate)
50. Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx)
51. Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB)
52. Poly(ethyl acrylate-co-hydroxyethyl acrylate) [P(EA-co-HEA)]
53. Poly(ethylene oxide) dimethacrylate (PEODM)
54. Poly(ethylene terephthalate) (PET)
55. Poly(glycerol sebacate) (PGS)
56. Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV)
57. Poly(lactic-glycolic acid) (PLGA)
58. Poly(L-lactide-co-e-caprolactone) (PLCL) (NS) [supercritical fluid foaming; solvent-casting and salt leaching method]
59. Poly(L-lactide-co-e-caprolactone) (PLCL) {sponge} [supercritical fluid foaming; solvent-casting and salt leaching method]
60. Poly(L-lactide-co-e-caprolactone) (PLCL); articular cartilage explant (control)
61. Poly(N-isopropylacrylamide)-g-methylcellulose (PNIPAAm-g-MC) thermoreversible hydrogel
62. Poly(N-isopropylacrylamide-co-acrylic acid) (p(NiPAAm-co-AAc)) (hydrogel) [NS]
63. Poly(N-isopropylacryl-amide-co-acrylic acid) thermoreversible gel
64. Poly(propylene fumarate-co-ethylene glycol) [P(PF-co-EG)]; {agarose}; {alginate}
65. Poly(urethane urea) Artelon®
66. Poly(γ-benzyl-L-glutamate) (PBLG)
67. Poly(ε-caprolactone) (PCL) nanofibrous electrospinning
68. Poly3-hydroxybutyrate4-hydroxybutyrate (P34HB)
69. Polycaprolactone; poly(L-lactide); poly(lactic-co-glycolic acid); polyurethane
70. Polydimethylsiloxane (PDMS)

(B) Synthetic biomaterials
71. Polydimethylsiloxane (PDMS) concave microwells
72. Polyester poly(3-hydroxybutyrate) (PHB) film
73. Polyethylene glycol diacrylate
74. Polyglycolic acid (PGA)
75. Polyglycolic acid (PGA); cartilage explant
76. Polyglycolic acid (PGA); poly(glycolic acid-e-caprolactone) (PGCL); poly(l-lactic acid-glycolic acid) (PLGA), poly(l-lactic acid-e-caprolactone;75:25 (w/w)) [P(LA-CL)25]; poly-e-caprolactone (tetrabutoxy titanium) [PCL(Ti)]; fullerene C-60 dimalonic acid (DMA)
77. PolyHIPE polymer (PHP)
78. Polyhydroxyalkanoate (PHA) = poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxy-10-38 undecenoate] (PHBU)
79. Poly-L,D-lactic acid (PLDLA)
80. Polylactic acid (PLA); Acrylonitrile butadiene styrene (ABS) (NS) [3D printing]
81. Polylactic acid poly-e-caprolactone (PLCL)
82. Polylactic acid-polyglycolic acid (PLGA)
83. Polylactic glycolic acid (PLGA)
84. Polylactic glycolic acid (PLGA)
85. "Polylactic glycolic
86. acid (3D-PLGA) (NS) [NS]"
87. Polylactide-polyglycolic acid (PLGA)
88. Polylactide-co-glycolide (PLGA) 85:15 microspheres/biodegradable hydrogel
89. Poly-L-lactic acid (PLLA)
90. Poly-L-lactic acid (PLLA) microsphere; poly-L-lactic acid (PLLA) microsphere + tripeptide Arg-Gly-Asp
91. Polymer solutions of poly(ethylene) oxide diacrylate
92. Polyurethane
93. Polyurethane (PU); poly(L/DL-lactide) (PLA)-control
94. Polyurethane/poly(L-lactide-co-D, llactide) (PU/PLDL) [6:4; 5:5; 8:2]
95. Poly-ε-caprolactone (NS) [electrospinning]
96. PuraMatrix (hydrogel) [NS]
97. PVA
98. Recombinant streptococcal collagen-like 2 (Scl2) protein with heparin-binding, integrin-binding and hyaluronic acid-binding peptide sequences (HIHA) [nonviral bacteria]. ScrMMP7-HIHA-Scl2, MMP7-HIHAScl2, MMP7:ACAN(75:25)-HIHA-Scl2, MMP7:ACAN(50:50)-HIHAScl2, MMP7:ACAN(25:75)-HIHA-Scl2 and ACAN-HIHA-Scl2 hydrogels.

(continued)

(B) Synthetic biomaterials
99. Self-assembling peptide (KLD) AcN-(KLDL)3-CNH2
100. Self-assembling peptide (KLD); cartilage explants
101. Self-assembling peptide (KLDL)
102. Self-assembling peptide (RADA)4
103. Self-assembling peptide AcN–(KLDL)3–CNH2 hydrogels;{agarose}
104. Self-assembly aggrecan (0.6% w/w), aggrecan–HA (0.6% w/w) and HA (1% w/w) solutions; ~type II collagen/aggrecan; ~PVA hydrogel
105. Silanised hydroxypropyl methylcellulose (Si-HPMC) hydrogel [E4M®]
106. Silated hydroxypropyl methylcellulose (hydrogel) [NS]
107. Silk; collagen; gelatin
108. Silk-elastin-like-protein polymer SELP-47 K
109. Sodium cellulose sulphate; polydiallyl dimethyl ammonium chloride (NS) [NS]
110. Tantalum
111. Tetramethacrylate prepolymer
112. Thermoreversible gelation polymer [poly(N-isopropylacrylamide-co-n-butyl methacrylate) (poly(NIPAAm-co-BMA))]
113. Titanium

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