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Review article

Progress and prospect of technical and regulatory challenges on tissue-engineered cartilage as therapeutic combination product

Xiaolei Guo [a,*], Yuan Ma [b], Yue Min [a], Jiayi Sun [a], Xinli Shi [a,**], Guobiao Gao [a], Lei Sun [a], Jiadao Wang [b,***]

[a] Center for Medical Device Evaluation, National Medical Products Administration, Beijing, PR China
[b] State Key Laboratory of Tribology, Tsinghua University, Beijing, PR China

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ABSTRACT

Hyaline cartilage plays a critical role in maintaining joint function and pain. However, the lack of blood supply, nerves, and lymphatic vessels greatly limited the self-repair and regeneration of damaged cartilage, giving rise to various tricky issues in medicine. In the past 30 years, numerous treatment techniques and commercial products have been developed and practiced in the clinic for promoting defected cartilage repair and regeneration. Here, the current therapies and their relevant advantages and disadvantages will be summarized, particularly the tissue engineering strategies. Furthermore, the fabrication of tissue-engineered cartilage under research or in the clinic was discussed based on the traid of tissue engineering, that is the materials, seed cells, and bioactive factors. Finally, the commercialized cartilage repair products were listed and the regulatory issues and challenges of tissue-engineered cartilage repair products and clinical application would be reviewed.

1. Introduction

Osteoarthritis (OA) is a chronic and debilitating joint disease that causes damage to the articular cartilage and underlying bone [1], which is associated with pain and loss of joint function. The latest survey result shows that there were around 654.1 million individuals (40 years and older) with knee OA in 2020 worldwide [2]. Cartilage tissue has no blood supply, lymphatic drainage, and nerve distribution, which makes it very difficult to repair itself after injury. The repair of damaged cartilage is a tricky issue as well as a focus in the field of sports medicine. The palliative treatments such as non-steroidal anti-inflammatory drugs, analgesics therapy, corticosteroid injection as well as injection lubricant (such as hyaluronic acid) just relieve the symptoms without preventing further articular cartilage degeneration, leading to a compelling choice of total joint arthroplasty ultimately. Surgeries, which include microfracture (MF), autologous chondrocyte implantation (ACI), matrix-assisted autologous chondrocyte implantation (MACI), and osteochondral transplantation (OCT) are applied for OA treatment [3]. Although successful in some aspects, each of these methods has limitations (Table 1), such as the formation of fibrocartilage in the repaired region, ineffective in repairing large cartilage defects, and the regenerated cartilage is difficult to closely integrate with the surrounding normal cartilage and donor-site morbidity [4–6].

With the development of biological and engineering technology, it has become possible to realize the regeneration of cartilage according to the principle of tissue engineering. In 1997, Cao and coworkers seed cow chondrocytes into a biodegradable ear-shaped scaffold and then implanted them under the skin of mouse to generate new cartilage [7]. This study demonstrated the regenerative of cartilage is realizable, which brings new hope to patients with cartilage defects. In the following decades, tissue engineering cartilage is being advanced to create biologically compatible cartilage constructs. Appropriate cell types, scaffolds with good biocompatibility, and appropriate microenvironments (including biological, chemical, and physical environments) are selected and fabricated for tissue-engineered cartilage. Various forms of cartilage repair products have also been developed and applied in clinical therapy. Meanwhile, with the emergence of new tissue cartilage implant products, the regulatory is also facing new technical and ethical challenges.

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* Corresponding author.
** Corresponding author. Center for Medical Device Evaluation, National Medical Products Administration, Beijing, 100081, PR China.
*** Corresponding author. State Key Laboratory of Tribology, Tsinghua University, Beijing 100084, China.
E-mail addresses: guoxl@cmde.org.cn (X. Guo), jdwang@mail.tsinghua.edu.cn (J. Wang).

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Abbreviated table

Abbreviations	*Full name*
OA	osteoarthritis
MF	microfracture
ACI	Chondrocyte implantation
MACI	Matrix-assisted autologous chondrocyte implantation
OCT	Osteochondral transplantation
BMSC	Bone marrow stem cell
OCA	Osteochondral allograft transplantation
PACI	Particulated articular cartilage implantation
AMIC	Autologous matrix-induced chondrogenesis
HA	Hyaluronic acid
PLA	Polylatide
PGA	Polyglycolide
PLGA	Polylatide/polyglycolide
PCL	Polycaprolactone
iPSC	Induced pluripotent stem cell
MSC	Mesenchymal stem cell
GMP	Good Manufacture Practice
GTP	Good Tissue Practice
QS	Quality System
HCT/Ps	Human Cells, Tissues, and Cellular and Tissue-based Products
RWE	Real World Evidence
QRM	Quality Risk Management
RM	Risk Management
ICH	International Council for Harmonisation
ISO	International Standardization Organization

Table 1
Limitations and advantages of the surgery therapies.

Techniques		Limitations	Advantages
MF		1. Repaired defect size is limited (1–2.5 cm^2) 2. Non-good long-term repair effect 3. Formation of fibrocartilage rather than hyaline cartilage	1. Easy to operate 2. Low cost
OCT	OAT	1. The transplant cartilage tissue is difficult to integrate with the surrounding cartilage tissue 2. Only suitable for Small defect size	1. Rapid healing 2. No immune rejection
	OCA	1. Insufficient transplant donors 2. Preservation of transplant tissue 3. Risk of disease transmission 4. High costs	1. Suitable for large-size cartilage repair 2. Less surgical trauma is caused 3. Both fresh and frozen allografts can be used
	PACI	Difficult to prepare	1. Less donor cartilage is required 2. Less damage to the donor
ACI	P-ACI C-ACI MACI	1. Two operations are needed 2. A long recovery time (6–12 months) is required to ensure neotissue maturation 3. Chondrocytes dedifferentiate into fibrochondrocytes in long-term cultivation *in vitro* and autologous chondrocytes of aged patients have reduced proliferation and differentiation potential 4. High costs	1. Less trauma formation 2. More simplicity to perform 3. No risk of disease transmission
	Chondrosphere	1. A long cultural time 2. A complex culturing procedure 3. High costs	1. Better maintain the phenotype of chondrocytes 2. No risk of interrupting the signal transmission among cells

In this article, the issues related to the defected articular cartilage repair will be discussed. First, the techniques for OA treatment in the clinic will be discussed. Subsequently, we will review the recent developments and the current state of tissue-engineered cartilage. Finally, the commercial products, especially the tissue engineering products for the therapy of OA in the clinic were reviewed. Moreover, regarding the development and application of tissue-engineered cartilage implants, the problems, and challenges encountered in the supervision of therapeutic combination products will also be discussed, as well as future directions to encourage researchers in the field to overcome these challenges.

2. The techniques for defect cartilage repair in the clinic

OA is a chronic musculoskeletal degenerative disease, the therapy of OA mainly focused on the repair and regeneration of articular cartilage through surgical therapy (Fig. 1). Many conservative treatments for OA have also been processed in the clinical trials, however, the effect of treatments was very poor. For example, hyaluronic acid injections, analgesics, and Non-steroidal anti-inflammatory drugs (NSAIDs) are recommended by at least 75% of the knee OA treatment guidelines [8], but the effect size of analgesics (acetaminophen) on knee OA pain is just 0.13, which implies a trivial clinical effect, and the effectiveness of NSAIDs in pain amelioration is similarly poor with no statistical difference detected between subjects taking NSAIDs vs. placebo [9]. A clinical research result showed that the viscosupplementation (hyaluronic acid injection) for knee OA just moderate effect on pain, function, and stiffness in the short-term (1–4 weeks), and clinical effectiveness diminished over time and, by 5–13 weeks, there was no evidence of benefit [10].

In 1959, Pridie used the method of drilling holes in the articular cartilage of osteoarthritis, accessing the bone marrow led to a clot formation which had the potential to form cartilage, the method of cartilage repair was achieved breakthrough [11,12]. And in the 1980s this method was refined by Steadman and coworkers, who coined the term MF, which is a typical representative of bone marrow stimulation procedures [13,14]. In this method, the primary goal of making small holes on subchondral bone is to release the bone marrow and form a marrow clot, and the secondary goal is to make a rough surface to hold the clot. The formed marrow blood clots contain bone marrow mesenchymal stem cells (BMSCs) and growth factors, the BMSCs can differentiate into chondrocytes and form fibrous cartilage tissue to repair the defected cartilage [15,16]. Low cost, short-term good outcomes, and easy operation make MF being considered the gold standard and the first-line treatment for the cartilage repair by some [17,18]. Although the MF method has made a breakthrough in the treatment of cartilage defects, there are still many shortcomings: (1) the best repair defect size of 1–2.5 cm^2 [19]; (2) the repair effect of elderly patients is worse than that of

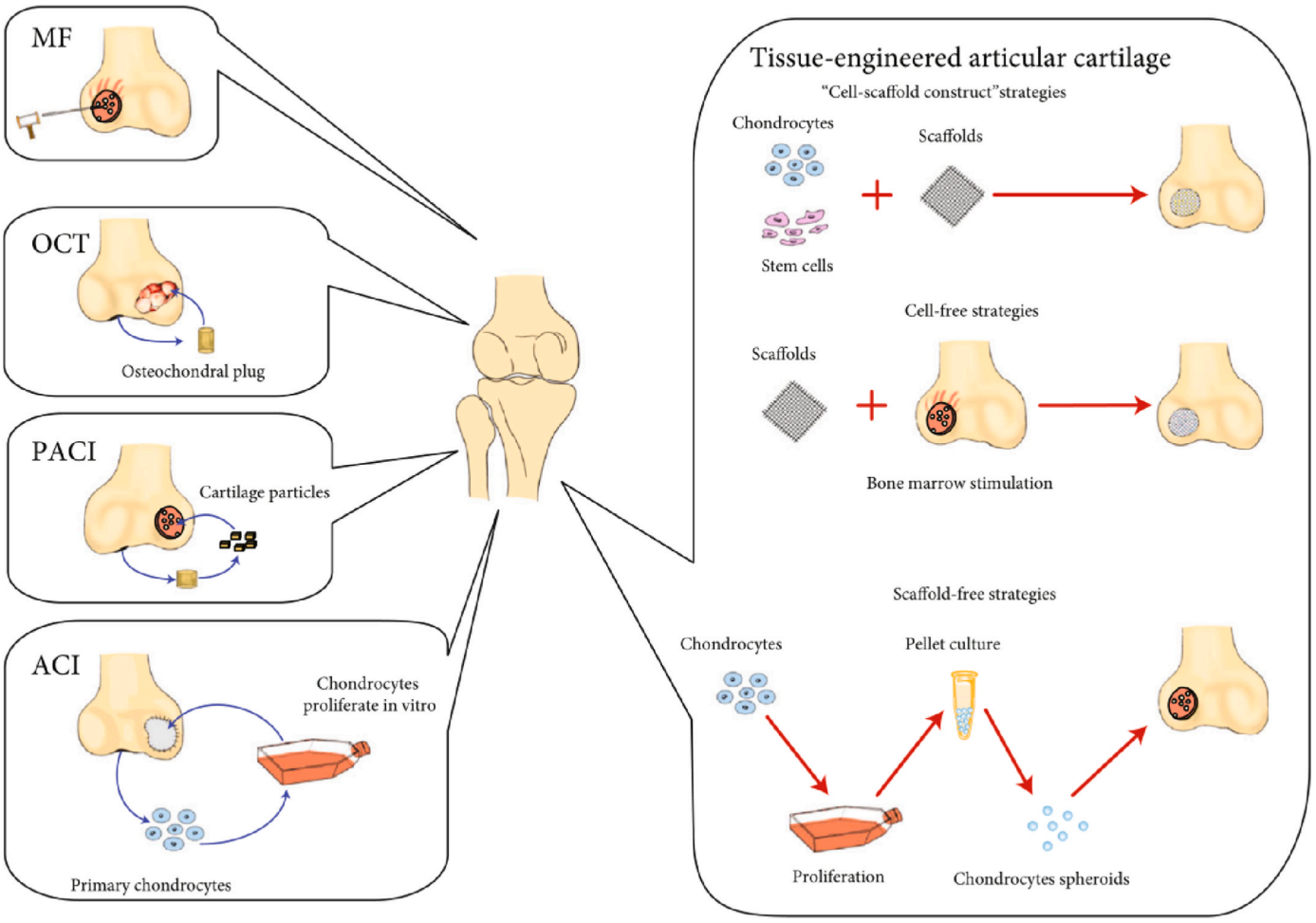


Fig. 1. Articular cartilage repair and regeneration techniques. Cell, scaffold, or cell-scaffold construct strategies were applied for cartilage regeneration. The cells can be stem cells or chondrocytes with or without *in vitro* expansion in various morphology, such as particles and spheroids [3].

younger patients [20]; (3) the main shortcoming, the long-term repair effect is poor, the regenerated cartilage tissue is fibrocartilage not hyaline cartilage and the phenotype of chondrocytes is difficult to maintain for a long-time [20]. These disadvantages limit the MF application in the clinic.

The osteochondral transplantation technique had been processed for articular cartilage repair in the clinic by Eric Lexer since 1908 [21]. Osteochondral transplantation can be divided into osteochondral autologous transplantation and osteochondral allograft transplantation according to the source of the transplanted cartilage. Although osteochondral autologous transplantation surgery healing is rapid and no immune rejection, the limitation of healthy cartilage tissue source, which leads to this treatment is just suitable for patients with a small size of defected cartilage. Another limitation of osteochondral autologous transplantation application is that the transplanted cartilage tissue is difficult to integrate with the surrounding cartilage tissue, especially the cartilage surface, which may cause stress concentration and long-term surgical failure [22]. A systematic review by Andrade et al. showed that the average secondary lesion incidences at osteochondral autologous transplantation donor sites in the knee and ankle were 5.9% and 19.6%, respectively [23]. Compare with osteochondral autologous transplantation, osteochondral allograft transplantation can repair large-size cartilage defects, causing less surgical trauma, moreover, both fresh and frozen allografts could be used for osteochondral allograft transplantation [24]. Clinical statistics show that osteochondral allograft transplantation treatment of knee cartilage and osteochondral defects can improve the recovery rate, and the 10-year survival rate was 82% after fresh osteochondral allograft transplantation of the femoral condyle, and the patients' symptoms and functions continued to improve [25,26]. Although allograft osteochondral transplantation has some advantages in cartilage damage repair, the risk of disease transmission, insufficient transplant donors, the preservation of transplant tissue, as well as high costs limit its application in the clinic. In addition, particulated articular cartilage implantation (PACI) is another osteochondral transplantation style, which refers to crushing autologous or allogeneic articular cartilage into small particles sized 1–2 mm and then implanting them into articular cartilage defects. Compared with osteochondral autologous transplantation and allograft transplantation, PACI theoretically requires less donor cartilage and thus causes less damage to the donor.

Autologous/Allogeneic Chondrocyte Implantation (ACI) is another effective method for cartilage repair, which refers to the harvesting of chondrocytes from the non-weight-bearing area of the autologous articular surface, culture, and amplification *in vitro*, and then implantation into cartilage defects. ACI has undergone 3 times technical updates since Brittberg et al. first reported the repair of articular cartilage defects in 1994. We believe that ACI is the beginning of the use of tissue engineering principles to treat cartilage defects. ACI adopts the treatment concept of combining cells (Chondrocyte) and materials (first generation: autologous periosteum; second generation: collagen membrane). However, the function of the material in the first and second-generation ACI is to cover the damaged site and prevent the loss of cells, not to provide a scaffold for cell adhesion, proliferation, migration, and differentiation. The third generation of ACI, Matrix-induced

autologous chondrocyte implantation (MACI) is a real tissue engineering strategy for the treatment of cartilage injury, which is the most common "cell-scaffold constructs" based cartilage repair technique in clinical practice. Similar to ACI, MACI requires two surgical procedures. The first surgery collects autologous tissue, from which the patient's chondrocytes are isolated and expanded *in vitro*, then the cells are seeded on a scaffold and cultured for several days (3–7days). The second surgery debrides the lesion, after which the cells-scaffold complex is implanted in the defected cartilage position. A separate study found that the outcomes to be better with MACI than micro-fracture for patients with large (>4 cm^2) defects 2 years after surgery [27]. Compared with OCT, the advantages of ACI and MACI include less trauma formation, more simplicity to perform, and no risk of disease transmission. However, the drawbacks of ACI and MACI are also obvious: two operations are needed, long recovery time (6–12 months), the dedifferentiation of chondrocytes *in vitro* cultivation, and autologous chondrocytes of aged patients have reduced proliferation and differentiation potential, and the high costs [28–30].

In the normal physiological microenvironment, chondrocytes are affected by cellular and exogenous stimuli. However, the scaffolds would interrupt cell-cell signaling and shield exogenous stress stimulus signals. Moreover, the degradation rate of the scaffold does not match the rate of cartilage regeneration, which leads to the shape and mechanical strength of the cell-scaffold structure cannot be maintained. Due to these reasons, scaffold-free technology for articular cartilage regeneration is based on generating spheroids of autologous chondrocytes (Chondrospheres) for implantation. Chondrocytes are cultured *in vitro* for 6–8 weeks to proliferate and concentrate into spheroids, then implanted into the defects. The scaffold-free strategy can be considered an ACI, where chondrocytes are no longer used in the form of cell suspensions but rather prepared into spheroids. This method can better maintain the phenotype of chondrocytes than ACI. However, the scaffold-free strategy also faces the problem of requiring a long culture time and having a complex culturing procedure, which leads to an increase in costs [3].

Although ACI, MACI and Scaffold-Free strategies offer much promise for articular cartilage repair, the methods for cell isolation, proliferation, and phenotypic maintenance are time-consuming, costly and at high risk of contamination. Furthermore, because these strategies are based on cellular techniques, the government regulatory agency (such as FDA, NMPA) considers the products both as medical devices and as biological medicine, resulting in long and expensive regulatory approval. The stricter regulations thereby enhance interest in developing cell-free material-based products for cartilage regeneration and repair. Cell-free strategies are not absolutely "cell-free" and usually use endogenous stem cells indirectly. Autologous matrix-induced chondrogenesis (AMIC) is a cell-free technique that can be performed in a single surgery. To perform AMIC, a mini-arthrotomy exposes and cleans the defect site, then microfracture releases both blood and bone marrow containing MSCs, and finally matrix is sutured or glued into the cartilage defect [31]. The implanted matrix and the released bone marrow, as well as the BMSCs, are combined to form a cell-matrix complex, where the matrix provides space and mechanical support for BMSCs adhesion, proliferation, and differentiate into chondrocytes, and further forms new cartilage tissue. AMIC technique is a "one-step" method for cartilage repair, which avoids the problems of iatrogenic trauma resulting from the harvesting of autologous chondrocytes or MSCs, as well as the time and expense of cell culture and expansion *in vitro*. To enhance the effect of AMIC in repairing cartilage, the growth factors are added to the matrix to promote the recruitment of BMSCs from the subchondral marrow into the implanted matrix. The implant matrix combined with autologous serum or platelet-rich plasma is then injected into the defect caused by initial microfracture, which has been proposed as a novel strategy for active in situ AMIC [32]. Currently, very few AMIC products are on the market, the number of application cases is small, and a greater number of clinical cases and long-term follow-up data are needed to prove effectiveness and safety.

Compared with cartilage transplantation (OCT) and MF, the methods (MACI, AMIC, and Chondrosphere) that are based on the concept of tissue engineering have certain advantages in the cartilage defect treatment, but there are still some problems that need to be solved in these methods. From the perspective of materials, preparing the scaffolds with physical and chemical properties mimic the natural cartilage tissue, which promotes the integrability of the scaffold with surrounding tissue, and improves the microenvironments for new cartilage tissue formation. From the cell perspective, the stable seed cells, that have a wide range of sources, can be mass-produced, and have high biological activity are another strick issue that tissue-engineered cartilage products must face. Appropriate biological and physical signal stimulation after implantation is also a problem that needs to be solved for tissue-engineered cartilage products. These issues will be discussed in the subsequent part of tissue-engineered cartilage research progress.

3. Tissue engineering cartilage

3.1. Materials of scaffolds

Among the traid of tissue engineering cartilage, the development of suitable scaffolds or matrix plays a pivotal role during the cartilage regeneration process. The overall properties of the scaffold may directly affect the incorporated cells or the migration of surrounding cells, which finally influence the regeneration of articular cartilage. For an ideal media, the following elements should be considered, including the chemical properties, mechanical properties, porosity, roughness, stability, biocompatibility, bioactivity, biodegradability, as well as fabrication technologies. The scaffolds used for articular cartilage regeneration are made primarily from natural and artificial polymers, as well as composite materials. Natural protein or polysaccharides such as collagen, chitosan, and hyaluronic acid are good candidates due to their good biocompatibility and biodegradability. Multiple types of research have demonstrated that the cultivation of chondrocytes on these 3D media tends to stabilize their phenotype and facilitate chondrogenic differentiation. Fabrication technologies of scaffolds can also be manifold, including conventional ones like solvent casting, gas forming, freeze-drying, electrospinning and novel 3D printing technology, which allows for independent control over macroscale and microscale features as well as achieve the development of customized tissue scaffolds. Following, the common materials and fabrication methods that are under research or in the clinic for tissue-engineered cartilage will be discussed, respectively.

3.1.1. Hyaluronic acid

Hyaluronic acid (HA) is an important extracellular matrix molecule with multiple physical and biological functions in many tissues, including cartilage. The application of HA for tissue engineering is usually chemically modified and then crosslinked into 3D scaffolds with suitable crosslinking methods, including photo-crosslinking, chemically induced crosslinking, and enzyme-catalyzed crosslinking, among others. For example, Jin and coworkers formed an injectable hydrogel by enzyme-mediated crosslinking based on HA and gelatin. Briefly, a tyramine-conjugated HA (HA-t) was synthesized first. Then hybrid hydrogels were made by mixing HA-t solution with the gelation solution, and epigallocatechin-3-gallate (EGCG) was incorporated into the hydrogel to modulate inflammation and scavenge radical species. The composite hydrogel protected chondrocytes against the pro-inflammatory factor, IL-1β, and can lead to chondrogenic differentiation *in vitro* [33]. In another study, HA-based hydrogel was applied to reinforce and seal damaged cartilage, restoring native function and preventing further deterioration. HA was first modified with three consecutive steps to yield a final product RGD-MeHA-ALD and then formed into hydrogel by adding a photo initiator to allow for UV light-induced polymerization [34]. He et al. engineered HA-based

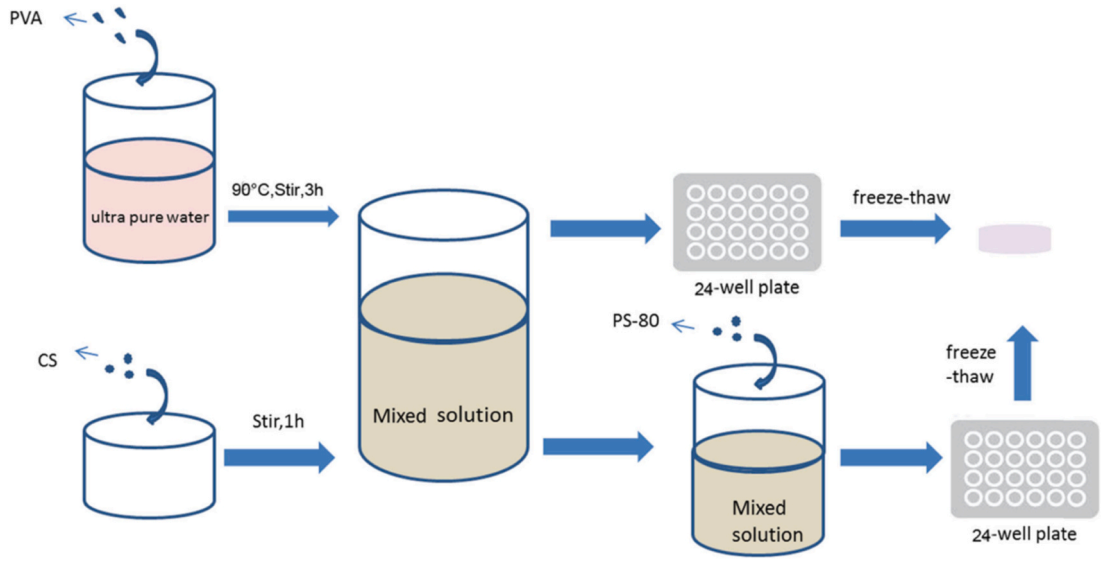


Fig. 2. Diagram of PVA/CS composite hydrogel preparation. PVA and CS were dissolved in relevant solvents and a mixed solution of various proportions was prepared. The mixture solution with or without PS-80 was added to a 24-well plate, sealed and frozen at −20 °C, and thawed at room temperature. The freeze-thaw cycle was repeated several times and the final solution was neutralized with sodium hydroxide to get the final hydrogel [38].

biomimetic shape-memory cryogel scaffolds with the light-induced process, which can contract and regain their shape following syringe injection to non-invasively fill cartilage defects [35].

Besides chemical crosslinking methods, others like electrospun can be applied to increase the recruitment and infiltration of surrounding MSC and nutrition. The nanofibrous hydrogel could deliver factors specifically designed to enhance cartilage repair. In another study, 3D bioprinting was manipulated to construct a HA-based bio-inspired hydrogel To produce cartilage constructs with optical mechanical properties, HA was co-printed with PLA. The hydrogel construed by 3D printing exhibited good chondrogenic supporting and cartilage tissue formation. The HA-based bio-ink could form cross-linked gels physically in the presence of calcium and alginate [36].

Though various HA-based scaffolds have been studied to promote cartilage repair, they are still in the process of research development, no one has transferred to clinical application, which may contribute to its complicated preparation process, improving the difficulties for market regulation and clinical transformation.

3.1.2. *Chitosan*

Chitosan is a promising polymer to design scaffolds for promoting cartilage lesion repair, numerous studies have suggested that chitosan could promote chondrogenic differentiation and reduce inflammatory of chondrocytes. For instance, chondrocytes implanted in a chitosan-based hydrogel scaffold could adhere, proliferate and secrete extracellular matrix after culture for 1 week *in vitro,* and further *in vivo* studies suggested that the hydrogel-chondrocytes could promote the repair of the defect in rabbits [37]. In another study, a porous hydrogel was fabricated with polyvinyl alcohol and chitosan by a method of freeze-thaw cycles. The hydrogel showed good cartilage healing ability and no cytotoxicity (Fig. 2) [38].

A photo-crosslinked layered gelatin-chitosan hydrogel with graded compositions was designed for osteochondral defect repair. The layered structure mimics the multi-layered gradient structure of the cartilage-

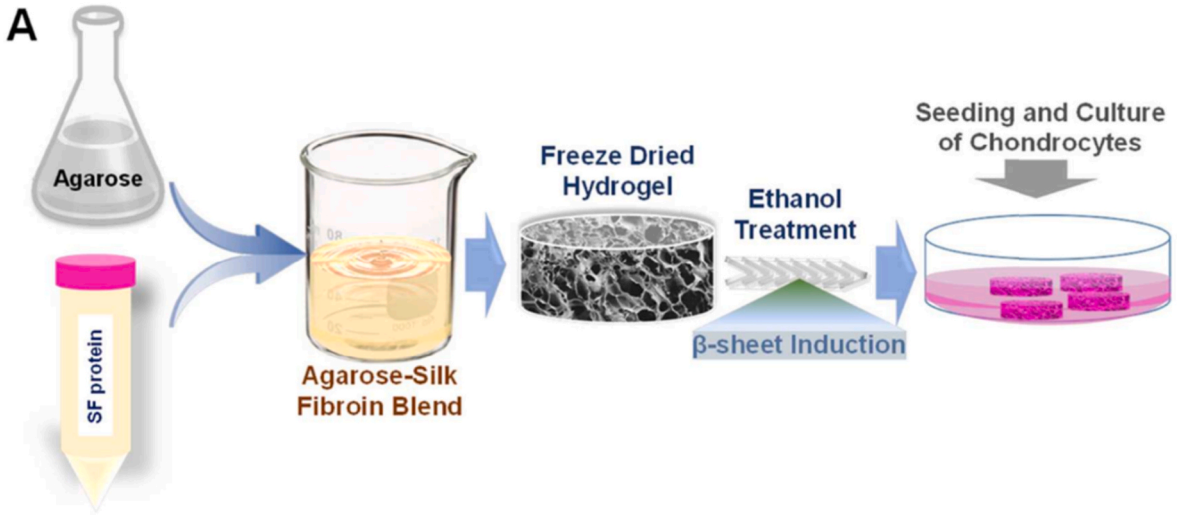


Fig. 3. Schematic representation of the fabrication of SF/Agarose hydrogel. SF protein solution was added to the dissolved agarose solution to get a blend and then freeze-dried to get the hydrogel. The lyophilized hydrogels were treated with 70% for 4–6 h to induce the β-sheet in SF protein and for further chondrocyte seeding [41]. Reproduced with permission. Copyright 2016, American Chemical Society.

Table 2
The techniques and products for cartilage repair in the clinic.

Techniques		Materials	Product (company)	Seed cells	Application status	Surgical steps	Number of clinical studies	References
MF		N/A	N/A	N/A	Applied in clinical therapy	One step	/	[19,20]
OCT		OAT	N/A	N/A	Applied in clinical therapy	One step	/	[22,23]
		OCA	N/A	N/A	Applied in clinical therapy	One step	/	[24,26]
ACI	**P-ACI**	Autologous periosteum	N/A	Autologous chondrocytes (Expand *in vitro*)	Applied in clinical therapy	Two steps	/	[119,120]
	C-ACI	Collagen membrane	Chondro-Gide® (Geistlich Biomaterials)	Autologous chondrocytes (Expand *in vitro*)	On the market	Two steps	67 (phase 3)	[104,121]
		Porcine collagen I/III membrane	MACI®(Vericel)	Autologous chondrocytes (Expand *in vitro*)	On the market	Two steps	144 (phase 3)	[105,122]
	PACI	N/A	CAIS (Depuy)	Autologous cartilage particles	Phase I clinical trial has been completed, but the project was terminated	One step	/	[106]
		N/A	DeNovo NT (Zimmer)	Allogenic juvenile cartilage particles	On the market	One step	25	[107,108]
TEAC	**MACI**	Fibrin, polyglycolic/ polylactic acid, polydioxanone	BioSeed® (BioTissue)	Autologous chondrocytes (Expand *in vitro*)	On the market in European	Two steps	/	[123,124]
		Hyaluronic acid scaffold	Hyalograft® C (Fidia Advanced Biopolymers)	Autologous chondrocytes (Expand *in vitro*)	Off the market, clinical applications exceeding 5000	Two steps	/	[125]
		Fibrin based gel	Chondron™ (Sewon Cellontech)	Autologous chondrocytes (Expand *in vitro*)	Available in Korea	Two steps	127	[113]
		Human fibrin and recombinant hyaluronic acid	BioCart II (Histogenics Corporation)	Autologous chondrocytes (Expand *in vitro*)	Available in Italy, Greece, and Israel; ongoing clinical trials in the United States;	Two steps	/	[126,127]
		Hydrogel of agarose and alginate	Cartipatch (TBF Tissue Engineering)	Autologous chondrocytes (Expand *in vitro*)	Ongoing phase III clinical trials;	Two steps	58 (phase 3)	[128,129]
	MACI	Atelocollagen (bovine) gel	AteloCell® (Koken)	Autologous chondrocytes (Expand *in vitro*)	Available in Japan	Two steps	27	[130]
		Collagen I hydrogels	CaReS® (Arthro Kinetics Biotechnology GmbH)	Autologous chondrocytes (Primary)	On the market in some European countries	Two steps	245	[131]
		Collagen I scaffold	NeoCart® (Histogenics)	Autologous chondrocytes (Expand *in vitro*)	Phase III clinical trial has been completed	Two steps	245 (phase 3)	[110]
		Collagen sponge	NOVOCART®3D (AESCULAP Biologics)	Autologous chondrocyte	Phase III clinical trial is in progress	Two steps	233 (phase 3)	[122]
	AMIC	Polylactide-co-glycolide copolymer, calcium sulfate, polyglycolide, and surfactant.	TruFit scaffold (Smith & Nephew)	Autologous BMSCs (Primary)	On the market	One step	/	[115,116]
		Collagen I and hydroxyapatite	MaioRegen (Fin-Ceramica Faenza SpA)	Autologous BMSCs (Primary)	On the market	One step	145 (phase 4)	[132]
CFS	N/A		Chondrosphere® (Co.Don AG)	Autologous chondrocytes (Expand *in vitro*)	On the market	Two steps	102 (phase 3)	[133,134]

MF: Microfracture; OCT: Osteochondral Transplantation; OAT: osteochondral autologous transplantation; OCA: osteochondral allograft transplantation; ACI: Autologous Chondrocyte Implantation; P-ACI: Periosteum- ACI; C-ACI: Collagen membrane-ACI; PACI: Particulated Articular Cartilage Implantation; TEAC: Tissue Engineered Articular Cartilage; MACI: Matrix-induced Autologous Chondrocyte Implantation; AMIC: Autologous matrix-induced chondrogenesis; CFS: Scaffold -free strategy.

bone interface tissue. The multilayered hydrogel showed good cytocompatibility and remarkable osteochondral defect recovery [39]. Ken et al. reported a chitosan-based hydrogel fabricated by the 3D printing method. Infrapatellar fat pad adipose stem cells were then incorporated into the scaffold to undergo chondrogenesis using TGF-β3 and BMP-6. The final results showed a cartilage-like tissue formation on the surface of the scaffold, proving its promising application as an osteochondral graft [40].

3.1.3. Elastin, gelatin, silk

Another kind of polymer often selected for tissue engineering is natural proteins and their derivatives, such as silk, elastin, and gelatin. For example, a hydrogel of mulberry and non-mulberry silk fibroin blended with agarose were processed with the free-drying method for cartilage tissue formation. The formed hydrogel showed a porous structure with water retention and degradation capability. Besides, the stable and stiffer hydrogel supports chondrocytes survival, proliferation, and chondrogenic differentiation of stem cells (Fig. 3) [41]. Nazarov et al. fabricated a cartilaginous scaffold with the gas foaming technology

with silk fibroin, taking the ammonium bicarbonate as a porogen. The scaffold showed higher compressive strength and interconnectivity compared to the salt leaching method, resulting in higher connections among cells [42].

3.1.4. Collagen

Collagen is the most abundant mammalian protein in the connective tissue, cartilage, and skin. The biodegradability and low antigenicity made collagen the primary resource in medical applications, including drug delivery systems and tissue engineering. For example, a collagen-based scaffold was fabricated with the pentaerythritol PEG ether tetrasuccinimidyl glutarate (PTE-PEG) as the crosslinker, in which atelocollagen was selected to suppress immunogenicity. Chondrocytes were embedded into the collagen-based hydrogel and injected into the damaged rabbit cartilage without a periosteal graft. At 8 weeks after the injection, favorable hyaline cartilage regeneration with good chondrocyte morphology was observed [43]. In another study, a physically crosslinked composite hydrogel was prepared with type I and type II collagens. The hydrogel was formed by self-assembly of collagen at 37 °C for 30 min and the compressive modulus can be regulated by changing the type I collagen content in the hydrogel. Chondrocytes encapsulated in the hydrogel could maintain their natural morphology and secrete cartilage-specific ECM [44]. Similarly, collagen type I and II were blended to form a hybrid hydrogel to examine its function on cartilage repair. The results showed that adding collagen type II could promote GAG production and support cartilage repair verified by the cartilage defects models of rabbits [45]. Lim et al. applied the clinical-grade soluble Col I that was provided by Ubiosis of Korea to evaluate the collagen gel-associated human nasal septum-derived chondrocytes as an injectable hydrogel for cartilage repair. Implantation of the cell-laden collagen increased the repair of osteochondral defects in rats compared with collagen only [46]. Apart from the conventional crosslinkers or physical crosslinking, the 3D printing method is often used with collagen-based materials for hard tissue engineering. For example, A 3D printed cartilage tissue was constructed by the extrusion printing method, taking the collagen type I or agarose mixed with sodium alginate as the 3D bioprinting bio-inks. Chondrocytes were incorporated in the bio-inks to function as seed cells. Results showed that collagen mixed with alginate could provide favorable mechanical strength and biological functionality supported by distinctly facilitated cell adhesion, accelerated cell proliferation, and enhanced chondrogenic differentiation [47].

To enhance the mechanical strength or scaffold formation ability, the collagen was always hybrid with other biomaterials to achieve a better function. For example, a collagen-poly (vinyl alcohol) nanofiber scaffold was fabricated by the electrospinning process and sterilized by ultraviolet irradiation. MSCs were incorporated into the scaffold and implanted into the full-thickness osteochondral defects of the stifle joint in the rabbit. After 12 weeks implantation, the MSC incorporated in the collagen-PVA group had better chondrocyte morphology, continuous subchondral bone, and much thicker newly formed cartilage compared with the control group, supporting its induction of MSC for cartilage repair [48].

Besides the scaffold fabrication methods difference, the treatment process of collagen including the preparation technology, and sterilization process all influence the final properties of the collagen-based scaffold. Jiang et al. fabricated a shape memory scaffold with the native collagen or denatured collagen and further examined their function on chondrocytes, tissue compatibility in a rat model, and repair ability in rabbits. The results showed that the native collagen scaffold showed better cell promotion *in vitro* and better cartilage regeneration *in vivo* due to the maintained triple-helical structure compared with the denatured collagen [49].

Besides nature collagen derived from various tissues, recombinant collagen has also been broadly studied for cartilage repair in recent years. A recombinant human type II collagen/polylactide scaffold (rhCo-PLA) was designed to examine its function on cartilage repair in porcine. 4 months after transplantation, hyaline cartilage formed most frequently in the rhCo-PLA group, and a less adverse subchondral bone reaction was developed compared with the collagen membrane group [50]. In another study, recombinant collagen type II was used as a scaffold for chondrocyte incorporation. Results demonstrated that recombinant human type II collagen is a promising biomaterial for cartilage repair, allowing homogeneous distribution in the gel and biosynthesis of ECM components [51]. Being free of animal products, recombinant collagen can eliminate the risk of undesirable immunological responses and transmission of animal-derived pathogens. What's more, the recombinant technology enables batch consistency and manufacture of high purity collagen. Therefore, recombinant collagen may be a good candidate for cartilage tissue engineering in the near future.

Due to the good biodegradability and low antigenicity, lots of collagen-based products have got into the market or in clinical research for cartilage repair, such asNeocart, CaReS, and Novocart 3D (Table 2). NeoCart: produced by seeding a type I collagen matrix scaffold with autogenous chondrocytes and bioreactor treatment, the scaffold was extracted from bovine collagen type I matrix. CaReS: utilize rat-tail tendons derived collagen I gel which were extracted in a standardized controlled process to avoid contamination, cells are mixed with the collagen gel and allowed to polymerize at 37 °C, cultured for 2 weeks before transplantation. Novocart 3D: a collagen-chondroitin sulfate sponge, cells are cultured in a monolayer and then seeded onto the scaffold for further culture 2 days before implantation.

Currently, almost most of the collagen-based scaffolds on market or in the clinic are in a solid state before transplantation, which increases the difficulty of operation. To reduce the trauma and improve the filling of materials to the defect, the injectable hydrogels may be a good candidate in the near future for cartilage repair, which is injectable before transplantation but solid after implantation to the defect.

3.1.5. Synthetic polymers (PLA, PLGA, PCL)

Due to their significant mechanical strength, immunologically neutrality, and no risk of transmitting pathogens, synthetic polymers are another good candidate for tissue-engineered cartilage. The commonly used synthetic polymers for cartilage regeneration include polylactide (PLA), polyglycolide (PGA), polylactide/polyglycolide (PLGA), and polycaprolactone (PCL), among others [52]. A large-pore scaffold was constructed by the 3D-bioprinting technology with acrylonitrile butadiene styrene (ABS) and PLA. Cells cultured on the scaffolds witnessed good cell proliferation, high viability, ample amounts of proteoglycan and collagen type II, which are primary components of the ECM of cartilage [53]. A PCL-based scaffold was constructed by the salt leaching method. The function of the scaffold on chondral defects was examined by the rabbit model. After 3 months with PCL scaffolds, defects were filled with cartilaginous tissue and better integration with the surrounding native cartilage [54].

In another study, PLGA and icariin based composite scaffolds were prepared by electrospinning to explore their function on OA. The spinning scaffold showed higher hydrophilicity and good biocompatibility, which was further verified by the mouse OA model results, promoting the synthesis of ECM [55].

3.2. Cells

The development of therapeutic strategies for cartilage repair should not only consider the scaffolds that can mimic the extracellular matrix, but also that of the lost cartilage cells. Various cell types can be used for promoting cartilage repair, including chondrocytes, mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs) as well.

3.2.1. Chondrocytes

As the exclusive cells residing in cartilage and maintain the

functionality of cartilage tissue, chondrocytes are the first considered cell type that could be used in promoting cartilage repair [48]. Chondrocytes are an attractive choice for the seed cells to be used in tissue engineering due to their ability to produce both ECM and type II collagen. However, there are some drawbacks to chondrocytes-based therapy. First, chondrocytes can lose their chondrogenic phenotype during cultivation *in vitro*. Second, the instability of chondrocyte source, the cells always have a great difference between different donors, such as the chondrocyte of the older patient become smaller and less uniform aggrecans, which greatly limited their further regeneration function.

3.2.2. Mesenchymal stem cells

Compared to chondrocytes, stem cells are much easier to obtain and get a large demand of cell quantity for application. MSCs are the important cell sources in tissue engineering approaches due to their higher proliferation rate, chondrogenic differentiation capacity, easy to obtain from the specific tissue, and more importantly low immune response. The commonly used MSCs included adipose-derived stem cells (ADSCs), bone-marrow mesenchymal stem cells (BMSCs), umbilical cord mesenchymal stem cells (UCMSCs), placenta-derived stem cells (PDSCs), among others.

Numerous studies have reported the function of ADSCs incorporated in scaffolds for promoting cartilage repair. For example, Imam et al. developed the cartilage tissue through seeding human ADSCs on a *Bombyx mori* silk fibroin scaffold and culturing in the medium was supplemented with 10% platelet-rich plasma. After seeding in the scaffold for 21 days, the GAG synthesis, growth factor-β1 secretion, and increased surface marker proteins were observed [56]. Besides, many other different types of biomaterials such as hydrogels, and fibers derived from biodegradable synthetic and natural polymers have been investigated to support the regeneration process of incorporated ADSCs. The scaffolds incorporated with ADSCs showed reparative hyaline cartilage and a significant amount of type II collagen, while the non-ADSC incorporated scaffolds observed lesions filled with fibrosis on follow-up, proving the benefit of ADSCs to the cartilage regeneration process in tissue engineering. Zhang et al. demonstrated the near normal cartilage regeneration by 12 weeks after the addition of a water-soluble polypeptide scaffold with ADSCs to a full thickness cartilage defect in rabbits [57].

BMSCs are one of the important stem cell options in tissue engineering. Lots of studies have examined the potential of these cells in tissue engineering for cartilage repair. Bernd et al. reported a PLGA-fleece combined with autologous BMSCs as a resorbable implant for cartilage repair in sheep. Cells incorporated in the implant differentiated into chondrocytes [58]. The predifferentiated autologous MSCs embedded in a collagen I hydrogel showed significantly better histological outcomes compared with the undifferentiated MSCs gels, chondrocyte transplantation group, and the untreated controls. Besides, the repair of chronic osteochondral defects with collagen hydrogel composed of chondrogenic predifferentiated MSCs showed no signs of degradation after 1 year *in vivo*, suggesting its advantages compared to the autologous chondrocyte transplantation [59]. In another study, the functions of a freeze-dried PGA-HA implant preloaded with human BMSCs were examined *in vitro* and a rabbit articular cartilage defect model. The results showed that MSC-laden PGA-HA scaffolds formed new tissue, rich in chondrocytes and a hyaline-like cartilage appearance after transplantation of 30 and 45 days. While the control group showed no articular resurfacing, tissue repair in the subchondral zone and fibrin formation [60].

Based on the functions of animals, the function of MSCs in cartilage repair has also been verified in the clinic, with some MSCs-based products on the market for cartilage repair. For instance, CARTISTEM®, a combination of human UCMSCs and sodium hyaluronate for cartilage repair, got into the market in Korea in 2012. Short-term and long-term studies have verified its functions and advantages compared to the traditional methods (MF). In a randomized controlled phase 3 clinical trial, 89 participants with large, full-thickness cartilage defects were treated with CARTISTEM® and conducted for 48 weeks, followed by an underwent extended 5-year follow-up. At 48 weeks, ICRS grade, and overall histologic assessment score were all significantly superior in the UCB-MSC-HA group versus the MF group. And the clinical results were also better at 3–5 years to follow up [61]. In another study, the safety and efficacy of CARTISTEM® on cartilage regeneration were proved in seven osteoarthritic patients with 7 years of extended follow-up, suggested by maturing repair tissue at 12-week and stable clinical outcomes over 7 years of follow-up. MRI at 3 years showed persistence of the regenerated cartilage [62].

3.2.3. Induced pluripotent stem cells

The iPSCs, which were predifferentiation towards chondrogenic lineage, have been shown to successfully repair cartilage defects in a variety of rat models. In a study, human iPSCs induced MSCs were plated onto a PLGA scaffold and transplanted into the cartilage defects of the New Zealand rabbits. Cartilage-like tissue was observed in the experimental group but not in the control group without cells after 6-week transplantation [63]. In another study, the undifferentiated iPSCs were cultured on the nanofiber-based polyethersulfone scaffold and the chondrogenesis function was examined. Their results showed that the nanofiber-scaffold iPSCs group enhanced the chondrogenesis of iPSCs and the highest capacity for differentiation into chondrocyte-like cells [64]. Similarly, iPSCs incorporated in a 3D nanofibrous PCL/gelatin scaffold showed higher levels of chondrogenic markers than the control group. The animal model further confirmed the scaffold-cell complex can enhance cartilage repair as well as subchondral bone regeneration. The iPSC-containing scaffolds enhanced the restoration of cartilage defects to a greater degree than that of the scaffolds alone [65]. However, a problem should be taken into consideration that the undifferentiated iPSCs could form teratoma *in vivo*, which is the main drawback of iPSCs for the application in tissue engineering.

3.2.4. Embryonic stem cells

ESCs, another type of pluripotent cell, have the potential to develop into a wide range of tissues including the chondrogenic lineage. Compared to MSCs, ESCs could potentially provide an unlimited supply of differentiated chondrocytes and chondroprogenitor cells. ESCs are always applied in the differentiated phenotype, including chondrogenic cells, MSCs, chondroprogenitors and so on, instead of its immature phenotype considering the risk of forming teratomas by ESCs upon transplantation [66–68]. ESCs are always incorporated in the 3D microenvironment, such as polymeric scaffolds, sponges, and hydrogels. For example, mouse ESCs were induced into chondrogenic cells *in vitro* and then transplanted into the PCL scaffolds to generate cartilage *in vivo*. Postimplant analysis of the retrieved tissues demonstrated cartilage-like tissue formation in 3–4 weeks [69]. In another research, the ES-like colonies from *in vitro*-produced vitrified embryos were pooled in groups of two or three, followed by embedded into fibrin glue and transplanted into osteochondral defects in the medial femoral condyles of sheep. The final result demonstrated that the ES-like cells transplanted into cartilage defects could stimulate the repair process to promote better organization and tissue bulk [70]. Another human ESCs-derived MSCs incorporated collagen bilayer scaffold was designed to examine whether neonatal desensitization could support relative long-term survival of the seeded cells and promote cartilage regeneration. *In vivo* studies in rats showed that abundant collagen type II was detected in the research group after transplantation of 8 weeks [67]. Toh et al. designed a HA-based hydrogel seeded with human ESCs-derived chondrogenic cells and further explored its potential to form an ECM-enriched cartilaginous tissue construct. By 12 weeks, an orderly spatial-temporal remodeling of cells into osteochondral tissue including a hyaline-like neocartilage layer with good surface regularity and complete integration was observed [71].

3.3. Bioactive factors and biophysical stimuli

Numerous studies have already identified that bioactive factors are essential for chondrogenesis both *in vitro* and *in vivo* [72–74]. The bioactive factors include growth factors, mineral ions, intracellular signaling molecules, kinases, transcription factors, exosomes and signaling mimetics derived from synthetic and natural compounds, which could promote cellular proliferation, migration, secretion, differentiation and maturation during the process of cartilage repair and regeneration.

A previous study has demonstrated that the relatively high concentration of Mg ions (2–10 μM) facilitated chondrogenesis and osteogenesis instead of adipogenesis of BMSCs and tendon-derived mesenchymal stem cells (TDSCs) under induction conditions respectively *ex vivo* [46]. The transforming growth factor β1 or 2 (TGF-β1 or TGF-β2), insulin-like growth factor (IGF-1), growth and factor differentiation 5 (GDF-5), Interleukin-1β (IL-1β) and bone morphogenic proteins (BMP-2, BMP-4 and BMP-7), as well as kartogenin (KNG), have been demonstrated to maintain chondrocytes phenotype and chondrogenic differentiation of stem cells *in vitro*. TGF-β1 is the most common growth factor used in chondrogenic induction medium, which promotes the synthesis of DNA and glycosaminoglycans as well as collagen II expression [75]. BMP 2 and BMP 4 stimulate the formation of cartilaginous tissue and GDF-5 triggers increased production of pre-chondrogenic precursors and the transcription factor SOX-9 [76]. Moreover, BMP4 enhances MSCs chondrogenic differentiation and forms mature chondrocytes [77]. Appropriate concentration of IL-1β promotes synovial MSCs proliferation and chondrogenic differentiation ability [78]. BMSCs are treated with IL-6R, which could enhance the effect of defected articular cartilage repair *in vivo* [79]. IGF-1 has been demonstrated to promote the production of collagen II and proteoglycans, and prevent the release and degradation of proteoglycans, moreover, IGF-1 treated mature chondrocytes could well maintain their characteristic rounded phenotype [80].

In addition, stem cell secretome is also used to repair damaged cartilage tissue. Secretome is a general term for bioactive factors and extracellular vehicles (EVs) secreted from the cell to the extracellular space. EVs are a heterogeneous complex of membrane-bound carriers including proteins, lipids, and nucleic acids. EVs play an important role in intercellular and even interorganismal communication [81]. Several EV subtypes have been characterized. Among them, exosomes are a kind of small EVs with nano-sized diameter capable of transferring the DNAs, microRNAs, non-coding RNAs and lipids with or without direct cell contact, a novel way of intracellular communication. Stem cell-derived EVs promote cartilage regeneration and prevent cartilage degeneration induced by OA, *in vitro* studies showed that MSC-EVs mediate cartilage repair by promoting chondrocytes proliferation, reducing cell apoptosis, and regulating the immune response [82,83]. The function of BMSCs extracted exosomes was verified *in vitro* and in a rat OA model *in vivo*. In the animal model, at 2,4,6 weeks, the paw withdarway latency (PWL) was significantly improved in the exosome-treat group compared with the non-group [84]. In another study, osteochondral defects were created in the knees of micropigs and then treated with intra-articular injection of 2 ml MSC exosome with HA. Results showed that the exosome + HA group showed better MRI scores and functional cartilage and subchondral bone repair than HA-treated defects at 2 and 4 months. The underlying mechanisms of MSC-EVs may attribute to the delivered cargos, as well as the triggering of relevant signaling pathways via cell surface interactions. However, there are two distinct views on the mode of action. One mode of action is the miRNA-mediated group and the other is the protein-based group. In 2007, Valadi et al. reported that mRNA and microRNA can be sent to other cells by exosomes, suggesting its potential function in mediating intercellular communication by delivering nucleic acids. Moreover, more and more studies have shown that the therapeutic effect of EVs is due to their nucleic acid composition, and the miRNA could influence OA [85]. For example, exosome miR-23b induced human MSCs to differentiate into chondrocytes by inhibiting the protein kinase A (PKA) signaling pathway, miR-92a regulates the PI3K/AKT/mTOR signaling pathway by targeting noggin3, thus upregulating chondrocyte proliferation and matrix synthesis [86,87].. MSC-sEVs shuttled miR-92a-3p increase chondrocyte proliferation and the expression of aggrecan and col 9a2. In another study, they found five highly enriched miRNAs in the hUCMSC-EVs, including has-miR-122–5p, and has-miR-148a-3p by high-throughput sequencing of miRNAs, with targeted genes mainly focus on the PI3K-Akt signaling pathway [88]. All these results proved that the main functional components of MSC-sEVs are miRNAs, which can regulate the expression of multiple target genes and participate in various cell signaling processes [89].However, the extent to which miRNAs are exposed via the EV route and whether they contribute to cell-cell communication is controversial. Some researchers independently indicated that EV-derived miRNAs are rarely contained but predominantly associated with RNA binding proteins [90].Wei et al. reported that the MSC exosome works most through a protein-based mechanism of action rather than the RNA, based on the biologically relevant concentration, biochemical functionality and the potential to elicit an appropriate timely biochemical response [91]. John reported that exosomes contained a small minority of miRNA by a quantitative analysis using a stoichiometric approach, which argues with the miRNA-based intercellular communication hypothesis [92].

Although both miRNA and protein in MSC exosomes have the potential to biologically influence disease or injury-associated processes, their role in mediating MSC exosome-mediated therapeutic activity has to be considered beyond a presence in the exosomes, and which component plays the main mechanism of action needs further studied and clarified, including their concentration, structure and the accessory components.

Besides, it was reported that only a fraction of the miRNAs identified in MSCs were secreted in MSC exosomes, suggesting that miRNA secretion by MSCs is a regulated process, the isolation, purity of EVs concerning other factors in the MSC secretome, including the potential co-isolation of contaminating proteins and nucleic acids, may result in invalid conclusions of EV content and function.

The small molecular compounds protect stem cells and improve the effectiveness of stem cells on cartilage regeneration. KNG is identified as the most promising small molecule for inducing BMSC chondrogenesis [93]. In a mouse OA model, the addition of KNG induced MSCs chondrogenic differentiation and reduced collagen II degradation [93]. Vitamin E can make MSCs resistant to H_2O_2-induced oxidative stress, upregulate the expression of proliferation markers and transforming growth factor-β (TGF-β), and downregulate the expression of apoptosis-related genes [94]. Enhancing stem cells homing to the injured cartilage site is another method for promoting cartilage repair. The stromal cell-derived factor (SDF-1)/C-X-C chemokine receptor type 4 (CXCR4) signaling pathway has been shown to play a key role in endogenous stem cell homing, and mediated cartilage regeneration [95,96].

Articular cartilage is a load-bearing tissue, the biophysical stimuli play an important role in defected cartilage tissue repair. In designing a system for defective articular cartilage repair, mechanical properties such as compression, fluid-promoted shear stress, and hydrostatic pressure must be considered. Previous research found that cell scaffold pretreated by hydrostatic pressure significantly increased the formation rate and matrix content of new cartilage and enhanced its mechanical properties [97]. Cell-scaffold complex subjected to hydrostatic pressure (10,000 Pa) at 1 Hz for 4 h per day, 5 days per week, for 8 weeks, the cells exhibited higher levels of collage production and more rounded phenotype, as well as lower levels of GAGs, compared with the cells that did not subject to pressure [98]. In another research, continuous low-intensity ultrasound pretreatment upregulated SOX9 gene expression and enhanced the nuclear localization of SOX9 protein in MSCs, which further promotes collagen II production [99]. Hypoxia is another biophysical stimulus that influences cartilage regeneration. In natural

cartilage, cells are exposed to low oxygen pressure (surface area 7% and deep area 1%), researches had demonstrated the hypoxia environment enhanced stem cells' survival, migration, proliferation, and differentiation after implantation [100,101]. Moreover, in the hypoxia environment, matrix deposition of MSCs is promoted, but the hypertrophy markers such as collagen X are reduced [102]. In the rabbit OA model, hypoxia-pretreated MSCs + HA hydrogel showed significant improvement in the cartilage repair score [103]. And the mechanism of hypoxia regulates cells behavior is mainly through regulation of HIF-1, HIF-1α enhance cartilage matrix gene expression and HIF-3α increase the cartilage phenotype stability. In contrast, HIF-2α upregulates hypertrophy gene and matrix-degrading enzymes expression [102].

4. Commercial products and their regulatory challenges

4.1. Currently commercialized cartilage repair products

With the continuous advancement of clinical treatment technology and material processing technology, a variety of corresponding cartilage repair materials have been researched and developed. The commercial cartilage repair products are summarized in Table 2.

In OCT surgery, due to the constraints of the source of transplanted cartilage and the timeliness of transplantation, the transplanted products are difficult to commercialize. Based on the ACI repair method, the cover membranes have been commercialized. The collagen membranes from Geistlich Biomaterials company (Chondro-Gide®) and Vericel company (MACI®) have been applied for several years in the clinic, and show positive repair effects [104,105]. The commercial products for PACI also have been developed. The autologous cartilage particles product CAIS from Depuy company has finished the phase I clinical trial. Although the follow-up clinical study of CAIS was terminated in 2014 due to the extremely low enrollment rate, its phase 1 study still showed positive repair results [106]. Another commercial product derived from allogeneic juvenile cartilage particles DeNovo NT has sale on the market. The repair effect is reportedly good within 2 years after implantation, and most of the repaired tissues are a mixture of hyaline cartilage and fibrocartilage [107]. However, both the CAIS and DeNovo NT are just suitable for the repair of small size cartilage defect (<3.5 cm^2), and the repair effectiveness lack long-term follow-up data [108]. The safety and efficacy of these products need more clinical data to be verified.

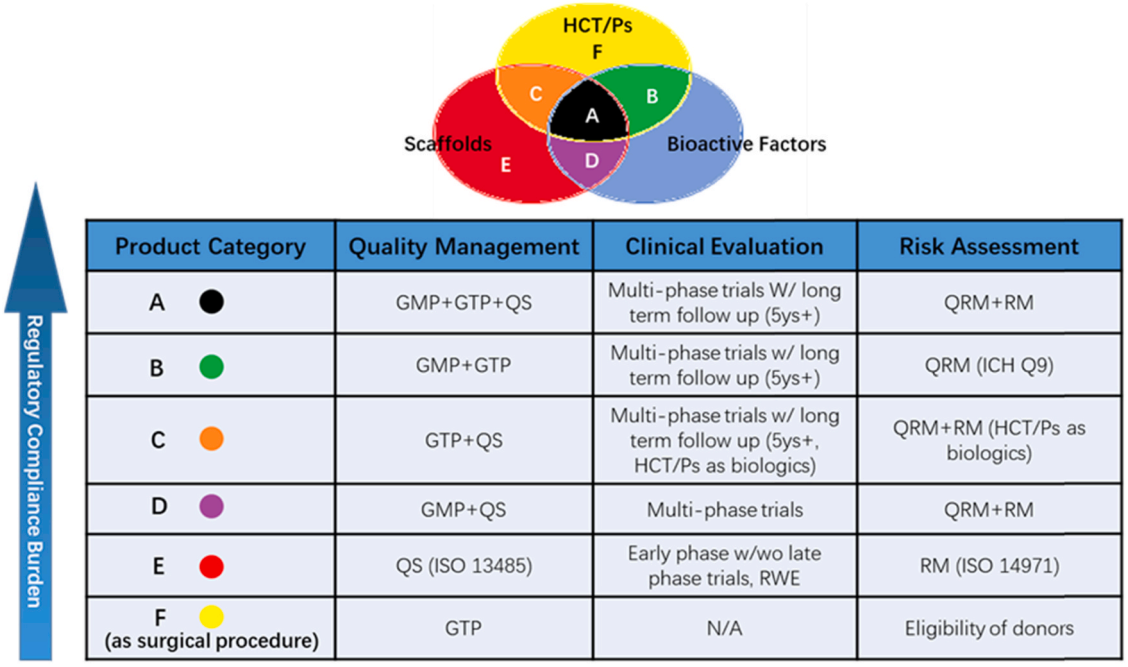
Tissue engineering is recognized as the most promising cartilage repair strategy. MACI is a typical tissue-engineered articular cartilage technology based on the "cell-scaffold construct" strategy. Until now, several commercial products (Table .1) have been developed based on the MACI concept, and show good clinical efficacy. For example, NeoCart is a chondrocyte-based implant which consists of a 3D bovine collagen I scaffold. The chondrocytes were seeded into the scaffold and then proliferate in a low-oxygen bioreactor, which mimics the native intra-articular environment to preserve the chondrocyte phenotype. After implantation, the graft is fixed to the defect with a bioadhesive [109,110]. Another typical product is the CaReS. In this system, the rat-derived collagen I gel was used to support the adhesion and proliferation of chondrocytes. The chondrocytes were seeded into the collagen gel to preserve the cartilage phenotype and then transplanted into the lesion [111]. Besides collagen-based scaffolds for chondrocyte incorporation, hyaluronic acid-based scaffolds, fibrin-based scaffolds, alginate-based scaffolds et al. are also widely used for MACI. For example, the Hyalograft C scaffold is formed based on the benzylic ester of hyaluronic acid. Chondrocytes were cultured directly on the scaffold and the implant was input without additional adhesive [112]. The fibrin-based gel scaffold named Chondro™ is another material combined with chondrocytes for cartilage repair. Chondrocytes are mixed with fibrin in a 1:1 ratio and then directly injected onto the defect. Clinical results showed no graft-related complications and nearly normal cartilage formed in patients after 12 months of transplantation [113,114]. Another option is a self-assembling process that requires no scaffold. The scaffold-free product (Chondrospheres®) is already on the market, according to the clinical feedback of National Institute for Health and Clinical Excellence (NICE) recommends the use of Chondrospheres® for the treatment of femoral condyle and patellar cartilage defects only when (1) no previous repair of articular cartilage has been performed, (2) osteoarthritic damage is minimal; and (3) the area of cartilage defects exceeds 2 cm^2 [3].Using these products for cartilage repair requires twice surgeries and chondrocytes *in vitro* expansion, which increases the complexity of surgeries and prolongs the recovery time. To overcome these drawbacks, repair materials based on the AMIC concept have been developed. These kinds of materials are usually called cell-free scaffolds, in which endogenous stem cells are indirectly loaded. TruFit scaffold and MaioRegen are two such kinds of scaffolds that are available on the market. TruFit scaffold is composed of a polylactide-co-glycolide copolymer, calcium sulfate, polyglycolide fibers, and surfactant. Clinical studies have shown that the TruFit scaffold repair of articular cartilage defects have a good long-term effect [115–117]. And MaioRegen scaffold is a three-layer biomimetic scaffold consisting of collagen I and hydroxyapatite. A multicenter prospective study showed that MaioRegen scaffold implantation significantly improved knee symptoms of the patients with full layer cartilage defects of knee [118]. However, these stents are solid, inconvenient implantation during surgical treatment.

Lots of research needs to be done before launching on the market to ensure the safety and efficacy of the products, including materials, biocompatibility, and clinical evaluation with medical devices, for instance. For different kinds of materials, the test standard is quite different. But generally speaking, there are similarities among different products. For materials property, the physical, chemical, biological, packing properties, and stability should be considered. ISO 13485, and ISO 14971 should be followed to make sure the quality of certain products. For biocompatibility, ISO 10993 is a generally used standard to ensure the biosafety of the products. As for clinical trials, ISO 14155 is a good standard during the process of clinical evaluation. Of course, different countries may have different local regulatory standards. Certain standards should be followed based on the products that are approved.

4.2. Regulatory identification of therapeutic products

Both technologies and commercialized medical products should comply with specific regulatory environments, which ensure their regular and ordered implementation. Regulatory compliance refers to the supervision of particular drugs, biological products, medical devices, and related manufacturers. It also includes the guidelines and ethical requirements from the clinical institutions, who are the final operator of the technologies or products, especially in autologous and allogeneic cartilage tissue transplantation [135]. Therefore, the primary challenge for product transformation is to define a clear regulatory environment. Following we will discuss the related regulations from the authorities' point of view for the above-mentioned aspects.

According to the classification of the statute, tissue-engineered cartilage is a typical combination product, which may include medical devices, biological products and drugs. There are quite different definitions in different jurisprudence. Based on the Code of Federal Regulations, the combination products are classified into four types in the US [136]. Tissue-engineered cartilage mostly belongs to the first type, which is physically, chemically, or otherwise combined or mixed and produced as a single entity. Sometimes it is classified into the other three types, according to the packaged form of the components. In Japan, combination products include three categories [137], they are classified mostly based on the components and their properties, not on the package form. The combination products will be finally marketed as a single drug, medical device, or cellular and tissue-based product. Similarly, in China, the combination products will be regulated as a single entity, either drug or medical devices based on certain products. Besides, the



Product Category	Quality Management	Clinical Evaluation	Risk Assessment
A ●	GMP+GTP+QS	Multi-phase trials W/ long term follow up (5ys+)	QRM+RM
B ●	GMP+GTP	Multi-phase trials w/ long term follow up (5ys+)	QRM (ICH Q9)
C ●	GTP+QS	Multi-phase trials w/ long term follow up (5ys+, HCT/Ps as biologics)	QRM+RM (HCT/Ps as biologics)
D ●	GMP+QS	Multi-phase trials	QRM+RM
E ●	QS (ISO 13485)	Early phase w/wo late phase trials, RWE	RM (ISO 14971)
F ● (as surgical procedure)	GTP	N/A	Eligibility of donors

Fig. 4. Regulatory compliance outline tissue-engineered cartilage as combination products.
GMP: Good Manufacture Practice; GTP: Good Tissue Practice; QS: Quality System; HCT/Ps: Human Cells, Tissues, and Cellular and Tissue-based Products; RWE: Real World Evidence; QRM: Quality Risk Management; RM: Risk Management; ICH: International Council for Harmonisation; ISO: International Standardization Organization.

biological products or cell and tissue-based components are not isolated from the drug category, which is different from Japan or US.

Before the transformation of the combination products, it is critical to figure out the product attributes and corresponding regulatory classification, which directly influence the registration designation and premarket review process. Definition of Primary Mode of Action (PMOA) of a Combination Product contributes enormously [138].PMOA is the single mode of action that provides the most important therapeutic action of the combination product, which is expected to make the greatest contribution to the overall intended therapeutic effects. The action modes include physical, biological, and chemical functions, among others. If the combination product does not achieve its primary intended purposes through that chemical action within or on the body, it may still be classified into the medical device category, even if it is metabolized for the achievement of the primary intended purposes, e.g. the implant cell-free scaffolds made of tissue engineering materials [139].Correspondingly, the cells, active factors, and physiological stimulating substances are achieved through chemical action, so they are identified as biological products in the US, which are belonging to drugs in China, rather than medical devices.

Therefore, it is noteworthy that tissue-engineered cartilage of cell-based and cell-free strategies may follow entirely different regulations due to the different PMOA, i.e. the cell-based as biological products or drugs, while the cell-free are regarded as medical devices. The products used in clinical applications are often judged as combined products with drug/biological substance as the mainstay and the device as the supplement [140].

In the US, although they are all Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps), different cell sources may comply with different regulatory modes. For instance, allogenic cell sources need to determine donor eligibility, while autologous cells do not [137,141]. According to FDA 21 CFR Part 1271 and the Public Health Service Act (PHSA), there is no need for premarket approval for autologous or allogenic cell productss, as long as they are minimally manipulated and for homologous use [142].

For structural cartilage, processing that alters the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement, is not considered minimal manipulation. Both *in vitro* expansion and particle/spheroid generation from allograft are processes altering the original relevant characteristics of cartilage to absorb shock and reduce friction. Thus, OCT products in Table 2 are explicitly excluded from biological products or medical devices, while ACI, CFS and MACI are identified as biologic/device combined products. As cells or nonstructural cartilage, the autologous BMSCs released by MF surgery in AMIC do not alter the relevant biological characteristics of cells or tissues, so AMIC is minimal manipulation without regulatory concern on the cell aspect, and the scaffold or matrix is a medical device unless mixed with biological stimuli [143]. Homologous use means the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with a HCT/P that performs the same basic function in the recipient as in the donor. Therefore, other stem cells discussed in 3.2 except BMSCs are non-homologous use and are identified as cellular therapy products, which belong to biological products.

In EMA, the cell-based products are classified as special Tissue-Engineered Products (TEP) or Somatic Cell Therapy Medicinal Products (SCTMPs), that is Advanced Therapy Medicinal Products (ATMPs) with regulatory priority to foster the development. In China, cell products implement a dual-track development model based on the executive body. For medical institutions, cell-based products were taken as medical technology and needed to be out of the negative list established by National Health Commission (NHS) before application [144]. For manufacturers, it should be approved by the NMPA before on the market. Therefore, different regulations should be considered depending on the jurisdictional areas.

4.3. Three key regulatory compliance pillars on tissue-engineered cartilage products

Tissue-engineered products as combination products usually contain at least one of three components: scaffold material, cells, and growth factors. At present, there is no regulatory specifically for tissue

Table 3
CMC for tisue-engineered cartilage components.

Components	Chemistry	Manufacturing	Control
Scaffolds	● Materials Source ● Preparation method ● Physical, chemical and biological property ● Degradability ● Metabolism ● Reagent quality ● Residual reagents ● Toxicity ● Analytical methods ● …	● Flow chart ● Process verification ● Formulation ● Storage ● Package ● …	● Sterility ● Pyrogenicity/ Endotoxin ● Residual reagents ● Container/ closure ● Stability ● Labeling ● …
● Cells or HCT/Ps	● Cell source, mobilization ● Cell bank system ● Cell identity, purity and activity ● Pathogen and viral agent ● Reagent quality ● Adventitious agents ● Residual reagent toxicity ● Excipient identification ● Analytical methods ● …	● Cell collection, processing and culture ● Cell characteristics ● Process timing and storage ● Formulation ● …	● Sterility ● Mycoplasma ● Virus ● Cell identity ● Purity ● Potency ● Cell number and viability ● Environment impact ● Stability ● Labeling ● …
● Biological factors	● Drug substance source and identification ● Raw material identification ● Other ingredients ● Analytical methods ● Quality ● Purity ● Toxicity ● Drug delivery device ● …	● Raw material and reagents specification ● Flow chart ● Animal source and model ● Cellular source and banking ● Expression vector system ● Purification and downstream ● In-process testing ● aseptic processing ● …	● Sterility ● Microbial contamination ● pH ● Residual moisture ● Viable cell determination ● Stability including potency ● …

engineering products, and it is mainly regulated by a combination of medical device and drug (biological products) regulatory regulations. Depending on the composition of the product, the applicable regulations are also different. Like medical devices and drugs, the quality control of tissue engineering products is carried out through three aspects: quality management, clinical evaluation and risk assessment management. The regulatory compliance outline of tissue-engineered cartilage products is shown in Fig. 4.

4.3.1. Quality management

To achieve regulatory compliance products of tissue-engineered cartilage products, the first pillar is the Quality System for manufacturing and tracking through the product life cycle, which plays a pivotal role in maintaining the stability of the product safety and efficiency. Good quality management is the common goal for drugs, biological products and medical devices. However, drugs and medical devices follow different requirements. The principles of ISO 13485 are always used and transformed into a relevant Quality Management System (QMS) globally for medical devices. While for drugs and biologics, the R&D and manufacturing process always follow the Good Manufacturing Practice (GMP). For combination products, the manufacturing quality system is a common issue for the global regulatory agencies. In China and Japan, the regulatory agencies have not yet formed a unified quality management system for such products, only requiring the components of each attribute to meet the requirements of their respective systems. In US and Europe, although relevant guidance has been issued to promote the compliance manufacturing of combination products, the overall principle is still to emphasize that each part should meet the corresponding system requirements [145,146], including Good Tissue Practice (GTP) for the HCT/Ps component. Both Japan and the United States have full-time regulatory agencies for human cells and tissues, which separate biological products and other regenerative medicine products from drugs and medical devices and have issued corresponding guidelines and specifications [147,148]. This supervision model may be more favorable for combined products.

The chemistry, manufacturing and control of each component of the tissue-engineered cartilage are summarized in Table 3. CMC of active factors and physiological stimulating substances must comply with the corresponding regulations on biological products and drugs [149,150].

4.3.2. Clinical evaluation

The second issue is the clinical evaluation. The term of clinical evaluation is often used in medical devices, while for drug or biological products, clinical studies are always applied instead. The clinical evaluation of medical devices is a set of ongoing activities that use scientifically sound methods for the assessment and analysis of clinical data to validate the safety, clinical performance and/or effectiveness of the medical device when used as intended [151]. Clinical data refers to safety, clinical performance, and/or effectiveness information that is generated from the clinical use of the medical device.

Clinical data from clinical use rather than sole clinical studies is essential content for clinical evaluation of medical devices, which lays the foundation for the assessment using real-world and even non-random clinical application data (Fig. 5). Correspondingly, for drugs and biological products, the data generated by multi-phased premarket clinical trials is the main content of the clinical evaluation, although it has recently been proposed to take the clinical experience data in the real world as a supplement for the prospective clinical trials [152]. But generally speaking, given the limitations of non-human models in terms of pharmacokinetics e.g. absorption, distribution, metabolism, and excretion (ADME), the pre-market technical review of drugs and biological products depend more on direct evidence from human clinical studies. In contrast, medical devices are largely based on pre-clinical and non-clinical engineering experimental evidence to prove that they are substantially equivalent to on market products, and then apply the clinical evidence of equivalent products to estimate the safety and effectiveness of products applying for regulatory clearance [153–155]. That is why a large number of collagen scaffold materials used as medical devices have been approved for marketing through laboratory evidence [153].

When clinical trials are conducted, drugs, medical devices and biological products follow different rules. For instance, many innovative medical devices can accumulate sufficient clinical evidence after early feasibility studies, and it is not required to carry out pivotal clinical studies [156], e.g. for customized medical devices. While in the clinical trials of biological products, cell therapy, gene therapy or active factors, it is still essential to follow the phased and progressive clinical trials. Even if there is the concept of early clinical trials, it generally exists as the beginning of multi-phase clinical trials, and the investigators and sponsors need to consider the overall planning of all phases of clinical trials to design early trials [157]. Therefore, to a large extent, the attributes of the combination products affect the cost of pre-market clinical evaluation, including the time cost, due to the different clinical

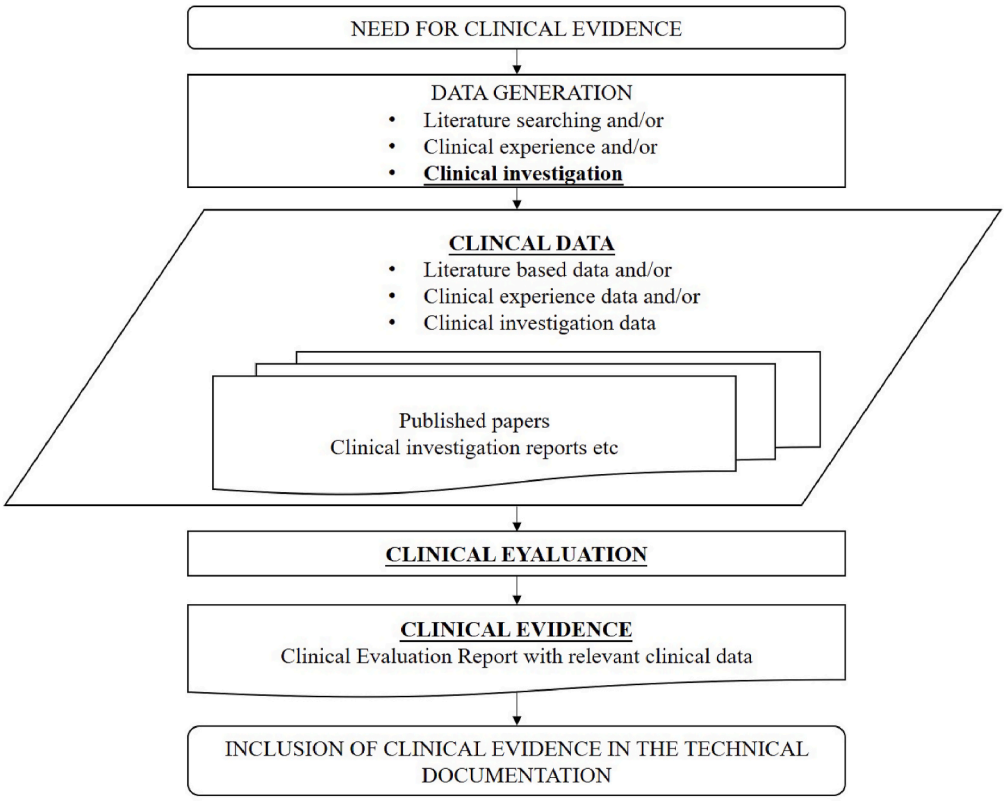


Fig. 5. Overview of process for data generation and clinical evaluation [151].

evaluation requirements of drugs, medical devices and biological products.

4.3.3. Risk Assessment

The third pillar is the product risk assessment, an essential element of pre-market decision-making. Although the approval of any medical product is based on an analysis of the balance of risk and benefit, the focus of risk analysis and residual risk control is slightly different. Furthermore, since there is no clear boundary between original products and imitation products, medical devices are always in the process of mutual simulation, continuous improvement and iteration of similar products among companies. Therefore, the pre-market review of medical devices emphasizes the bottom line of safety first (such as biocompatibility, biosafety, etc.) while using substantial comparisons with similar products in terms of efficiency. The second issue that should be considered is the Risk Assessment. Although the marketing of any medical product is based on an analysis of the balance of risk and benefit, the methods of risk analysis and residual risk control are different. Since there is no clear boundary between original products and imitation products, medical devices are always in the process of mutual simulation, continuous improvement and iteration of similar products among companies. Therefore, the pre-market review of medical devices tends to emphasize the bottom line of safety first (such as biocompatibility, biosafety, etc.), while use using substantial comparisons with similar products in terms of efficiency, that is, substantiality is the same [153]. The Quality Risk Management (QRM) for innovative drugs or biological products, which used to be part of the Quality Management System, focused more on the manufacturing quality and relevant safety control. Drugs, especially original innovative drugs or biological products, used to be part of the quality management system for product risk management, but more focused on the quality risk management of the manufacturing process.

The Hazard Analysis and Critical Control Points (HACCP) is a well-known risk management tool for drugs (Fig. 6)The Hazard Analysis and Critical Control Points (HACCP) is a well-known risk management tool for drug risk assessment [158], while the ISO 14971 is always for medical devices. While ISO 14971 is always used for the risk assessment of medical devices [159]. There are Other recognized risk management tools are also commonly used by the pharmaceutical and medical device industry, e.g. Fault Tree Analysis (FTA) and Failure Mode Effects Analysis (FMEA). At present, medical devices have a more straightforward classification method (Fig. 7& Fig. 8) in the conclusion of risk assessment on overall residual risks, while drugs and biological products have a more complex calculation on Risk Prioritization Number (RPN) and risk ranking [160]. For the pre-market technical review, the U.S. FDA has proposed the risk-benefit evaluation guidelines for the pre-market technical review process of drugs and biological products until recently, certain differences between the two in the risk analysis process (Fig. 6). At present, medical devices have a clearer classification method (Figs. 7 and 8) in the conclusion of risk assessment, while drugs and biological products have not been classified for residual risk management. Returning to the pre-marketing technical review, the U.S. FDA has just recently proposed the risk-benefit evaluation guidelines for the pre-marketing technical review process of drugs and biological products [154], while the device field has already implemented this for decades. Due to discrepancies of uncertainties about benefits and risk, risk-benefit balance assessments are different between medical devices and drugs/biologics. In the regulatory decision-making stage, medical devices are much more pre-clinical laboratory evidence-driven rather than clinical evidence-driven. While the device field is already in the process of technical review. The concept of risk-benefit balance is implemented, which highlights that the technical review of medical devices emphasizes process evidence, while traditional medicines and biological products emphasize result evidence [155]. Like other cell therapy products, regulators usually assess the risk and corresponding mitigation approach of cell-based tissue-engineered cartilage on adventitious unintended immunogenicity, tumorigenicity, cell homeostasis, ectopic expression/differentiation, contaminants from the administration

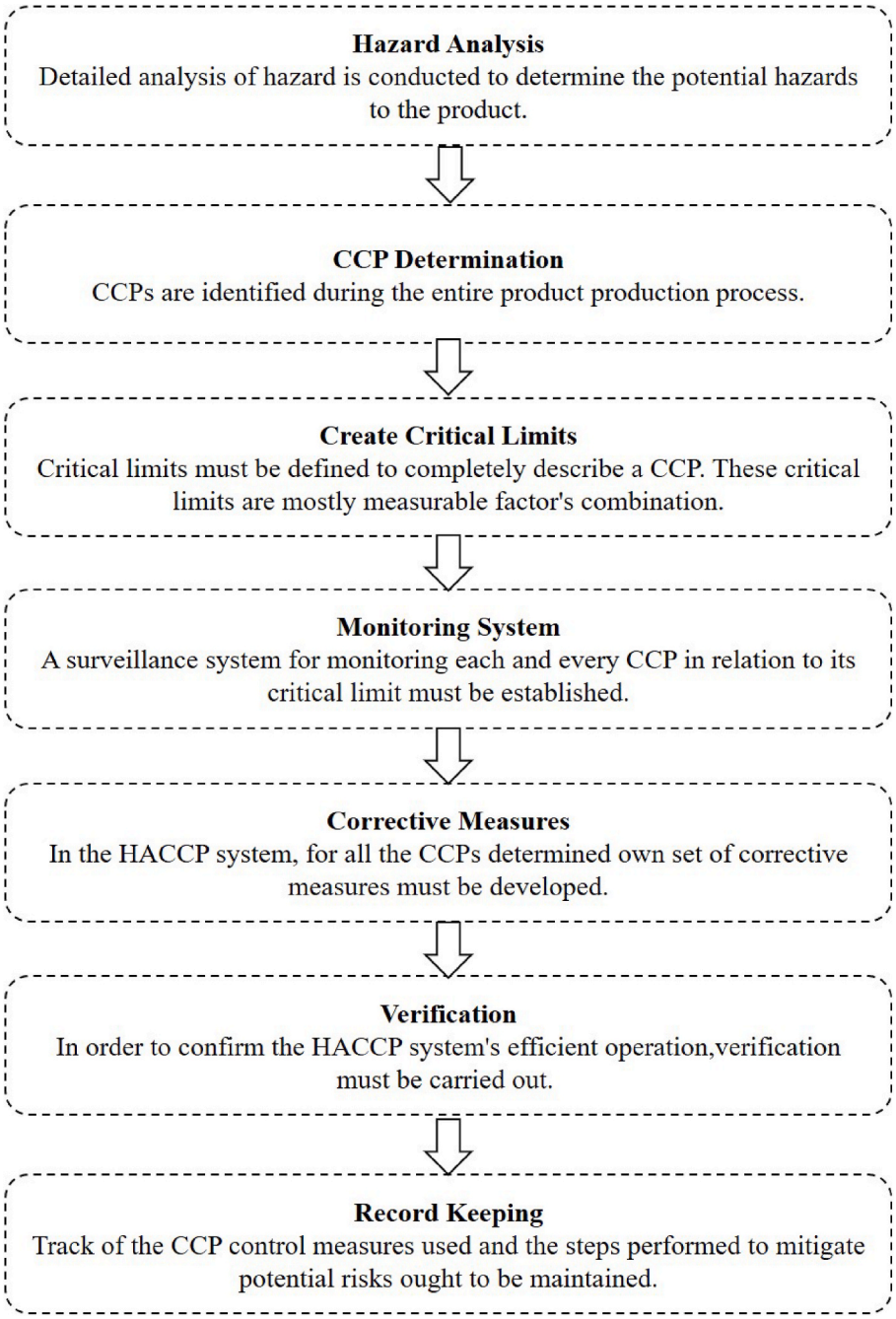


Fig. 6. HACCP principles for pharmaceutical industries [158].

process, as well as exposed toxicity and premature mechanical failure from scaffold component degradation [161].

Besides the above three pillars, there is much specialized technical thinking during the technical review of non-clinical research data before marketing, often named regulatory science. For example, the "Guidelines for the Registration and Evaluation of Drug Qualitative, Quantitative, and In Vitro Release of Drugs in Medical Device-Led Combination Products" was published by China NMPA recently, which shows that the medical device industry has begun to explore the mechanism of the pharmaceutical constituent of combination products those are different from traditional drugs and biologics [162]. With the evolution of products, the regulatory issues are also increasing.

5. Conclusion

With the rapid development of engineering technology and the deepening understanding of life sciences, cartilage engineering has achieved some notable progress. Nevertheless, the process of translating proof-of-concept research into preclinical or clinical investigation gives rise to escalated challenges. For clinical applications, the tissue-engineered cartilage products need to consider convenience, effectiveness and safety in the treatment process. The injectable product is a suitable option, which can reduce the trauma, improve the filling of materials to the defect and enhance the operability in the clinic. Natural polymers, especially those can physically assemble into scaffolds, such as collagen may be good candidates for clinical transformation, because of the simple preparation process, low residue of additional chemicals, controllable mechanical properties, etc. As for the incorporated seed

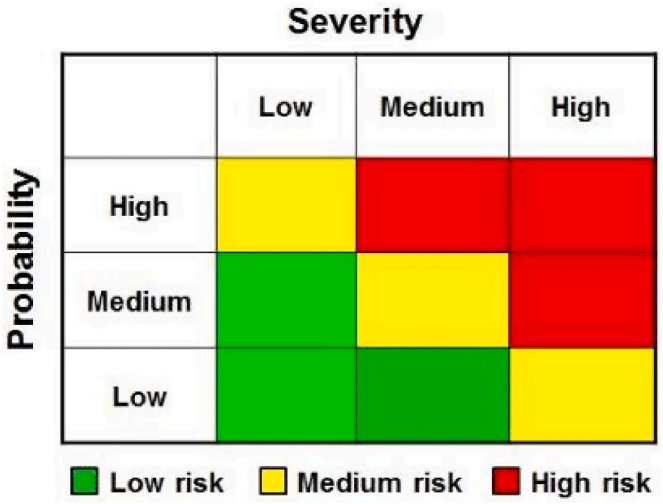


Fig. 7. Graphical determination of medical device risk [109].

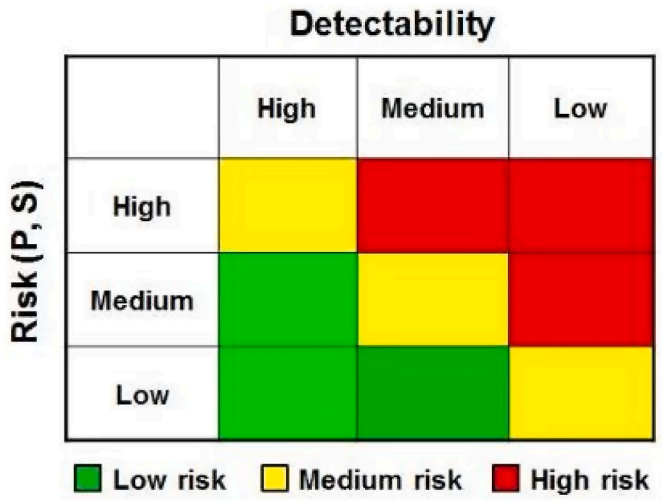


Fig. 8. Graphical determination of medical device Risk including Detectability. Risk Prioritization Number (RPN) = Severity (S) x Probability (P) [109].

cells, MSCs derived from bone marrow, adipose, umbilical cord or placenta may be good choices, due to their low immunogenicity, high proliferation and abundant tissue source, either autologous or allogeneic. An adequate microenvironment, including the trauma site and the surrounding tissues, should facilitate cell proliferation, differentiation and cartilage regeneration. Therefore, debridement, anti-inflammation and edema reduction are necessary before transplantation.

Finally, tissue engineering cartilage repair products involve combinations of scaffold materials with cytokines or cells, or even both, which are typically combined medical products. Different regulatory environments and compliance processes that match R&D costs must be paid attention to the transformation of tissue-engineered cartilage technology into products as well as product applications. In this process, R&D personnel must first determine the main mode of action (PMOA) of the product, which determines the attributes of the product is drug-led, biologic-led or medical device-led. Products with different attributes have many different requirements in terms of production quality system, risk assessment and clinical evaluation, which profoundly affect the progress of product development and marketing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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