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Review

Cellular therapy and tissue engineering for cartilage repair

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SUMMARY

Articular cartilage (AC) has limited capacity for repair. The first attempt to repair cartilage using tissue engineering was reported in 1977. Since then, cell-based interventions have entered clinical practice in orthopaedics, and several tissue engineering approaches to repair cartilage are in the translational pipeline towards clinical application. Classically, these involve a scaffold, substrate or matrix to provide structure, and cells such as chondrocytes or mesenchymal stromal cells to generate the tissue. We discuss the advantages and drawbacks of the use of various cell types, natural and synthetic scaffolds, multiphasic or gradient-based scaffolds, and self-organizing or self-assembling scaffold-free systems, for the engineering of cartilage constructs. Several challenges persist including achieving zonal tissue organization and integration with the surrounding tissue upon implantation. Approaches to improve cartilage thickness, organization and mechanical properties include mechanical stimulation, culture under hypoxic conditions, and stimulation with growth factors or other macromolecules. In addition, advanced technologies such as bioreactors, biosensors and 3D bioprinting are actively being explored. Understanding the underlying mechanisms of action of cell therapy and tissue engineering approaches will help improve and refine therapy development. Finally, we discuss recent studies of the intrinsic cellular and molecular mechanisms of cartilage repair that have identified novel signals and targets and are inspiring the development of molecular therapies to enhance the recruitment and cartilage reparative activity of joint-resident stem and progenitor cells. A one-fits-all solution is unrealistic, and identifying patients who will respond to a specific targeted treatment will be critical.

Introduction

Articular cartilage (AC) has limited capacity for repair, in part due to its intrinsic properties. It is a hypocellular tissue[1,2] with few progenitor cells[3]. Due to its pressurized proteoglycan- and collagen-rich matrix, cell mobility is low[4]. The tissue is avascular, aneural, and alymphatic[5], and cartilage nutrition relies on diffusion from synovial fluid[6] and subchondral bone[7,8]. When repair does occur, the tissue formed is often fibrocartilage, which is compositionally different to AC and thus biomechanically inferior.

Since 1977, when to our knowledge the first tissue engineering approach was described for AC repair[9], cellular therapies and tissue engineering strategies have been extensively pursued as treatment options for cartilage repair. The goal is to repair or regenerate damaged AC by restoring structure, zonal architecture, and function of the damaged tissue[10]. A wide range of design approaches, cell sources, biomaterials, and fabrication methods have been evaluated[11], and although there have been great advances, the optimal approach has yet to be delineated.

Cartilage tissue engineering is advantageous over current surgical practices which use auto/allografts. Osteochondral autograft transfer is not optimal, because osteoarthritis (OA) can develop at the harvest sites, and the size of the defect that can be repaired is limited. Procedures involving transplant of fresh osteochondral allografts (FOCAs) are limited by availability of donor tissue[12], often result in inadequate integration with surrounding cartilage, and can transmit disease. Importantly, tissue engineered cartilage constructs can be personalized to fit individual joint shapes and defect sizes.

To be successful, cartilage produced by cellular therapy or tissue engineering must have the characteristics of native AC. That is, regenerated cartilage must contain appropriate mechanical,

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compositional, and structural anisotropies[13] to be able to withstand compressive forces and enable tribological movement of the joint, as well as integrate with bone and surrounding cartilage. Crosstalk at the osteochondral junction[14] is also important for construct success. To be able to generate these tissues, it is necessary to understand mechanisms which regulate the formation of cartilage, including spatiotemporal cues which can be used to pattern cells, scaffolds, and the environment[14].

Tissue engineering for OA treatment introduces different considerations as compared to repair of focal defects. OA often involves larger and more diffuse involvement of articular surfaces and greater alteration of joint homoeostasis[15]. These changes include an inflammatory and catabolic microenvironment, bony changes such as osteophyte formation[16], joint space narrowing, and altered biomechanics[17,18], which may favour implant degradation. OA can also be associated with obesity and increased age[19], and both of those factors may alter the behaviour and success of cell therapies and tissue-engineered implants[20].

In this review, we discuss the regenerative medicine and tissue engineering approaches to cartilage repair (Fig. 1). The review is not exhaustive, and we apologize to those whose work was not cited because of space constraints.

Cell therapy for the repair of joint surface defects

Autologous chondrocyte implantation (ACI) has pioneered cell therapy for the repair of symptomatic, full-thickness AC defects[21]. In this treatment, chondrocytes are enzymatically released from a biopsy of cartilage taken from a healthy area of the joint, expanded in monolayer culture, and then implanted in the defect under a periosteal flap, or more recently a synthetic membrane. Compared with microfracture, a clinically used marrow stimulation technique for the treatment of AC defects[22], ACI has shown comparable clinical outcome at 12 and 18 months, but superior structural repair[23]. Results from up to 20 years follow-up have demonstrated that ACI is an effective and durable solution for the treatment of large cartilage defects in the knee[24,25], and ACI has entered routine clinical practice in some countries. Positive predictors of good outcome include age, location of defect, early intervention (<3 years), and no radiographical signs of OA[26]. However, chondrocytes dedifferentiate in culture[27,28], limiting their expandability and number of cells available for transplantation. In addition, tissue overgrowth especially when using a periosteal flap is not uncommon and may necessitate another surgery[29].

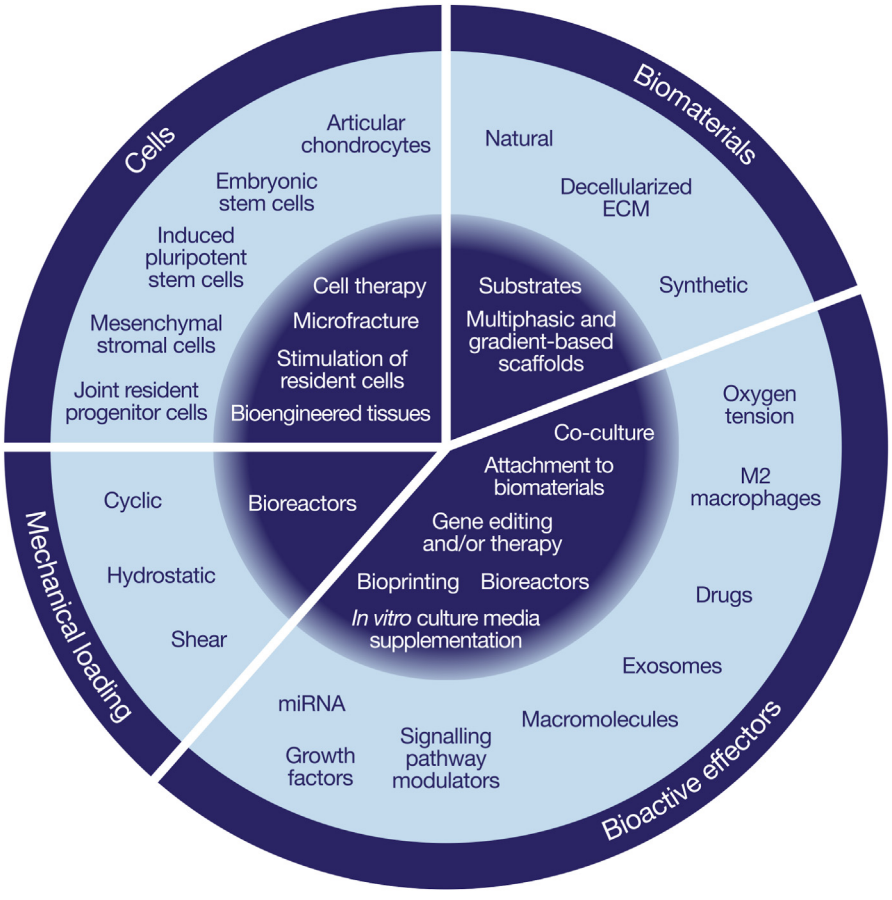

Fig. 1

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Components involved in cellular therapy and tissue engineering for cartilage repair. Therapies for cartilage repair require any combination of cells, biomaterials, mechanical loading, and/or bioactive effectors. The light blue ring shows examples of these four major components whereas the dark blue innermost ring represents the ways in which they can be utilized for cartilage tissue engineering as discussed in this review.

Mesenchymal stromal cells (MSCs) from various tissues are an alternative cell source as they are easy to expand in culture. Preclinical studies have shown promising results when adopting MSCs for osteochondral repair[30], although advancement of the bone front at the expense of the overlaying AC is not uncommon[31]. Studies in humans have reported variable structural outcome ranging from hyaline-like cartilage to fibrous tissue[32]. Autologous bone marrow MSCs were non-inferior to chondrocytes in clinical outcomes at 24 months in an ACI-like procedure[33], but longer-term follow-up will be essential to support their use in routine clinical practice. Allogeneic MSCs have shown an acceptable safety profile[34], and their production could be upscaled to generate large batches of cells ready for use, which would increase consistency and decrease cost of cell therapy.

MSCs for cell therapy can be derived from various tissues, including bone marrow[35,36], periosteum[37,38], synovium[39,40], or adipose tissue[41]. Bone marrow MSCs are most used but may not be ideal for the repair of AC due to their propensity to undergo chondrocyte hypertrophy, perhaps as an integral part of their endochondral bone formation programme[42,43]. Adipose-derived MSCs, while attractive due to their ease of harvesting, tend to be poorly chondrogenic[44–46], possibly due to their lack of expression of TGF-β type I receptor and low expression of BMPs[47]. MSCs from synovium displayed superior cartilage-forming potency compared to MSCs from bone marrow, subcutaneous adipose tissue, and periosteum[44,45], and have shown promise in preclinical and clinical studies[48,49]. AC and synovium have a common developmental origin from the embryonic joint interzone[50,51]. It is therefore fascinating to contemplate how potency including morphogenetic tissue repair ability may be imprinted in the MSCs based on their ontogeny.

Cell therapy for osteoarthritis

Intra-articular MSC therapy was pioneered with a study that showed regeneration of the medial meniscus and reduced secondary OA in goats in response to intra-articular injection of bone marrow MSCs after medial meniscectomy and anterior cruciate ligament resection[52], paving the way to clinical studies in patients with knee OA[30]. Recent systematic reviews of phase I/II clinical trials (not always controlled or blinded) concluded that intra-articular injection of MSCs, typically from bone marrow or adipose tissue, into the knee is overall safe and well tolerated. Furthermore, MSCs can decrease pain and improve function of the knee, with histological data indicating that hyaline-like cartilage repair can be achieved[30,53,54]. A meta-analysis of 11 trials of MSC therapy for knee OA, including a total of 582 patients, reported improvements across a range of clinical outcome measures[55]. While most studies have used autologous cells, allogeneic MSCs appear to have an acceptable safety profile[56,57]. However, large, controlled trials, as well as standardisation of cell product manufacturing, optimal delivery, and definition of target patient populations through stratification are needed to ascertain efficacy and allow comparisons of clinical study outcomes.

The mechanisms of action of MSC therapy in OA remain unclear, and there is limited evidence to support direct contribution of the injected MSCs to repair tissue. MSC-derived extracellular vesicles (EVs) can promote cartilage repair and protect against OA-induced cartilage degeneration[58–61], supporting the notion that MSCs could mediate tissue repair via release of EVs and other paracrine signals.

Bioengineering cartilage tissue implants

Tissue engineering techniques for cartilage repair aim to create tissues which effectively mimic native AC and restore joint function[62]. Tissue engineering requires the use of 1) a scaffold, substrate or matrix to provide structure, 2) cells to generate the tissue, and/or 3) signalling in the form of chemical or physical cues to promote a cartilage or bone phenotype[63]. Implanted constructs must be sufficiently porous to allow for nutrient transport and waste removal, contain or promote formation of a mature zonal organization with a biochemically appropriate composition, and must integrate with the surrounding tissue to enable smooth articulation and transfer and dissipation of joint loads[64]. Constructs must also be biocompatible, customizable in shape and size to fill defects or to replace an entire joint, and be easy to place and secure in the defect during surgery[65].

Scaffolds are composed of natural or synthetic materials and may be coupled with bioactive molecules such as growth factors, drugs, or deoxyribonucleic acid (DNA). They can be used either seeded with cells, or without cells to support cell ingrowth following implantation. Scaffolds can differ in charge, wettability, material, microstructure (porosity, pore size, pore shape), and stiffness, each of which influence cell phenotype, proliferation, differentiation, migration, and extracellular matrix (ECM) production[66–68]. Scaffolds can influence tissue formation by activating intracellular signalling pathways via interaction with cell adhesion molecules, such as integrin-mediated mechanotransduction, and/or via release of soluble factors[69]. Thus, determining the optimal scaffold characteristics that induce and maintain articular chondrocyte phenotypes that produce cartilage tissue with a zonal architecture is critical.

While cartilage engineering scaffolds have been extensively studied, consensus on the optimal material, fabrication technique, or structure has not yet been reached[70]. However, certain scaffold characteristics have been identified[63]. Scaffolds must be biocompatible and biomimetic (if not derived from natural substances) to support chondrogenesis by promoting cell adhesion, cell proliferation, and ECM production[71]. Scaffolds and their degradation products should not produce immunological reactions following implantation[72]. They must be processable into different shapes and sizes[73], and allow integration with native tissue. The scaffold-containing construct must be mechanically strong and resistant to an applied load[73]. As scaffolds biodegrade, degradation rate must match tissue formation rate[73] to ensure sufficient load bearing function, and not generate cytotoxic by-products nor induce a fibrotic response.

There are many different methods for making scaffolds, including 3D printing, hydrogels[74], supercritical fluid technology[75], electrospinning[76,77] and weaving. 3D printing allows precise cell and biomolecule positioning in scaffolds consisting of different materials, and predefined designs and geometries, and can be combined with microfluidics to enhance cell seeding[66,77].

Tissue engineering approaches using natural scaffolds

Natural scaffolds are highly biocompatible, biodegradable, and have multiple cell attachment sites due to their similarity with native ECM[66,70]. Degradation of this type of scaffold is usually enzymatic, and consequently, degradation products should not result in immunological reactions[66]. Natural scaffolds that have been evaluated include proteins (i.e., silk fibroin, collagen, gelatin, keratin, fibrinogen, elastin), polysaccharides (i.e., chitosan, chitin, alginate, gellan gum), and glycosaminoglycans (i.e., hyaluronic acid) [Fig. 2(A) and (B)]. Structural proteins (elastin, fibrin, silk) may have an added benefit as they are suitable as well for drug delivery[78,79]. Natural scaffold limitations include poor shape customizability, batch to batch differences in degradation rate, and difficulties in functionalization[73]. Most of these scaffolds have been evaluated in small animals pre-clinically, and there have been some clinical

trials, although most of these are single-arm trials. One clinical trial using nasal chondrocytes and collagen scaffold (Chondro-Guide) implanted in a post-traumatic cartilage defect in the knee after 2 weeks in culture resulted in improved symptomatology. While there was variable fill of the defect as visualized by magnetic resonance imaging (MRI), glycosaminoglycan content of the repair tissue significantly increased between six and 24 months after the procedure, as determined by delayed gadolinium-enhanced MRI[80].

Another type of natural scaffold is tissue that has been decellularized to generate cartilage-derived matrix that preserves tissue macromolecules and structure[81]. Decellularization procedures include physical, chemical, and enzymatic treatments[82–85], but optimal decellularization has been difficult to achieve as there is often a trade-off between DNA removal and glycosaminoglycan loss[86,87]. Advantages of decellularized scaffolds include preservation of zonal architecture and growth factor distribution[64,88], potential for successful interface integration, provision of a cartilage-mimetic environment, and facilitation of differentiation of cells seeded into the matrix[89]. Limitations include poor characterization of decellularized scaffold composition unless analysed using proteomic analysis[64], and poor mechanical properties[64]. The success of decellularized scaffolds in cartilage tissue engineering may be improved by recellularization of the scaffold prior to implantation[90]. Additionally, decellularized ECM can be used as bioink for 3D bioprinting[64,91,92], or reinforced with hydrogels[89,93,94] or synthetic polymers. A recent study in pigs in which decellularized allogenic cartilage was implanted into knee defects had promising results at 6-month follow-up[95].

Tissue engineering approaches using synthetic scaffolds

Synthetic scaffolds can be manufactured with highly predictable properties[70], allowing for precise manipulation of construct mechanical characteristics[90]. Their advantages include potential to specify composition, reproducibility, ease of processing and preservation of sterility, and control of degradation times. Drawbacks include lack of natural binding motifs for cell attachment, insufficient biological activity, variable hydration, hydrophobic nature depending on the material, potential for inflammation, mismatch between degradation rate and tissue formation leading to tissue collapse *in vivo*, and failure to recapitulate zonal architecture of cartilage[90,96]. Some polymers have limited use as they generate acidic degradation products[73]. Examples of synthetic scaffolds include poly(alpha-esters) such as polyglycolic acid, polylactic acid, and their copolymers, polycaprolactone[97], biodegradable polyurethanes, and polyethylene glycol [Fig. 2(B)][73].

There are numerous evaluation studies of scaffold implants in animal models, as demonstrated by the publication of 334 papers over the past 5 years in Pub Med which were identified by the search terms scaffold and cartilage repair. They describe variable outcomes, and many are short-term studies that do not address durability of repair[98]. For example, in one study using minipigs, polycaprolactone woven scaffold was anchored into a focal full thickness chondral defect (4 mm) in the knee joint. At two months, the scaffold was retained and there was fibrovascular ingrowth of the scaffold which suggested that this scaffold had promise to be effective for cartilage repair. However, the 12-month results were poor as repair was impaired, the tissue that developed had lower mechanical properties than AC and the implant had subsided into bone and induced extensive remodelling[98,99]. This emphasizes the need for longer term studies (6 months or greater depending on the species) to better assess the utility of an implant and the extent of remodelling over time. Of note, a 6-month study in dogs using a modification of this woven implant did not show bone resorption.

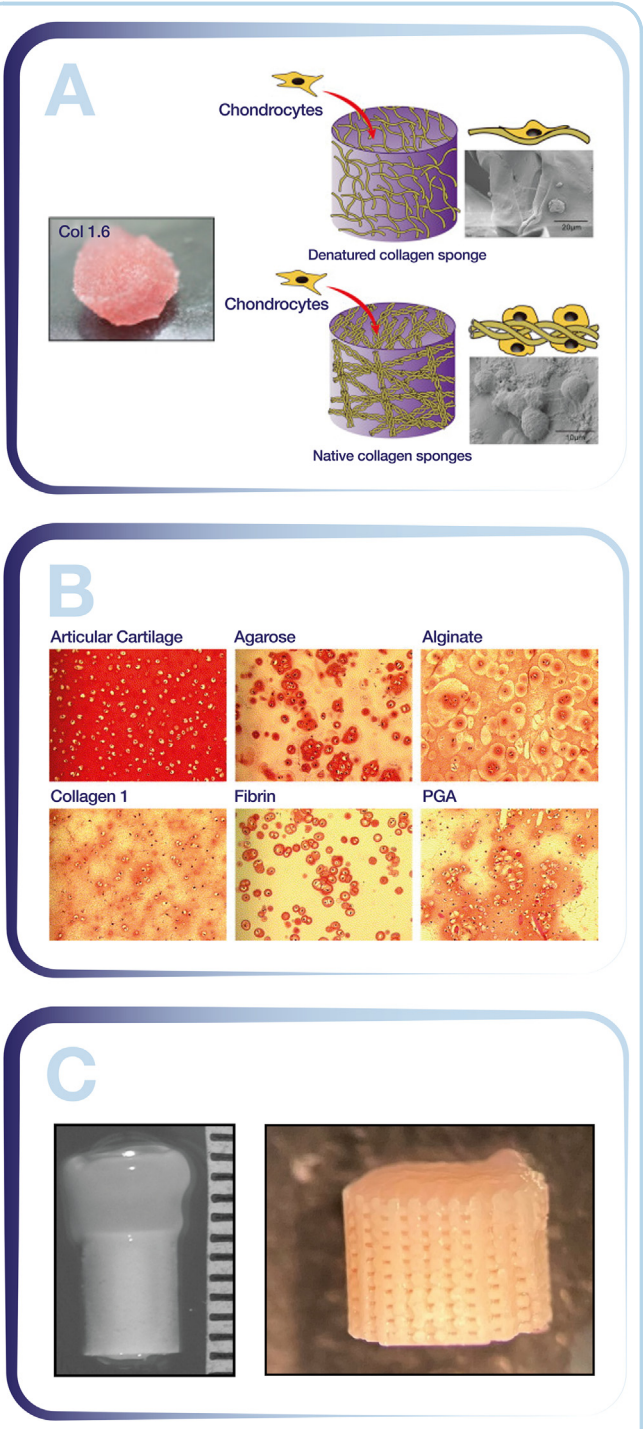

Fig. 2 Osteoarthritis and Cartilage

Examples of cartilage tissue engineering. (A) Cartilage tissue formed by chondrocytes within a scaffold of denatured or native collagen. Composite image is reproduced from Ref. 190. (B) Cartilage tissue formed by chondrocytes within an agarose, alginate, collagen, fibrin or PGA scaffold. Shown are interior sections stained with Safranin O after culture for 20 days, with articular cartilage for comparison. Image is reproduced from Ref. 191. (C) Cartilage tissue formed on the top surface of a substrate (porous polyphosphate, left) or scaffold (3D-printed polycaprolactone, right). PGA: polyglycolic acid.

This raises the question as to whether the animal model used to evaluate implants may itself influence outcome[100].

There have been very few clinical trials using synthetic scaffolds, and they have been used for focal defect repair. An example of one of these trials was placement of an acellular scaffold composed of photoreactive chondroitin-sulfate/polyethylene glycol hydrogel in a post-traumatic defect in the femoral condyle following microfracture[101]. Variable repair by MRI was observed at 24-month follow-up, with five out of 18 patients showing cartilage delamination and four showing cartilage overgrowth. These complications are not uncommonly seen in scaffold-based implants.

Scaffold-free tissue engineering approaches

Another approach to cartilage repair is scaffold-free cartilage tissue engineering, whereby cells are induced to produce ECM and form a tissue *in vitro* prior to implantation [Fig. 2(C)]. This approach aims to mimic, in a short time period *in vitro*, developmental, mechanical, structural, and cellular changes which occur over several years during the development and maturation of native AC[73]. Scaffold-free tissue engineering includes self-organizing and self-assembling approaches. Self-assembly occurs in closed systems where cells undergo condensation, proliferation, differentiation, ECM production, and tissue maturation[102]. This is likely driven by differential cell adhesion and interfacial tension[103–105]. For example, deep zone articular chondrocytes can be grown scaffold-free *in vitro* and produce biphasic tissue rich in proteoglycans[106] with a localized calcified layer, similar to *in vivo* calcified cartilage[107]. Self-organizing culture systems require exogenous input of energy[62] and include pellet culture, aggregate culture, cell sheets, or high-density cell culture on the top surface of a substrate. To generate cell/tissue sheets, cells are expanded in monolayer to high confluency and released as a sheet from mechanically or temperature-responsive substrate systems[96], and released sheets are rolled, layered, or applied to molds[108] to generate thick tissues[109]. Aggregate culture involves subjecting cells to rotational culture in the presence of growth factors[62]. The cells that can be used in these approaches are chondrocytes, MSCs from various tissue sources, induced pluripotent stem (iPS) cells and embryonic stem cells.

Scaffold-free systems circumvent some of the limitations of scaffolds[110–112]. Scaffold-free systems may work by decreasing stress shielding[113], altering mechanotransduction[62], enhancing matrix deposition[62], promoting a rounded chondrocyte phenotype[62,110] and/or enhancing integration with native AC due to increased cell numbers at tissue edges[62,114]. Limitations of scaffold-free systems include the large number of cells required[62], limited tissue thickness and potential for necrosis in the core[96,115], need for longer culture times[115], and poor tissue mechanical properties[96]. Scaffold-free constructs have been used to successfully repair focal defects in pigs[116] and sheep[117]. One study using cartilage tissue sheets to treat focal defects in humans ($n = 5$) resulted in symptom relief at up to 2 years follow-up and showed repair tissue (biopsy) at 48 weeks that resembled hyaline cartilage, suggesting that this approach may have clinical utility[118].

Multiphasic or gradient-based tissue engineering constructs

Tissue-engineered constructs can be multiphasic or gradient-based[119], and this is being pursued so the construct better resembles the joint surface, with a zonal architecture consisting of non-mineralized cartilage, calcified cartilage, and subchondral bone. Incorporation of a zone of calcified cartilage would help to maintain construct integrity by regulating force transmission across the interface and preventing cell migration between layers[120]. This can be accomplished for example by using mechanical cues to direct cell differentiation, i.e., scaffold stiffness and topography can be modified to influence cell fate[121,122]. Soft matrices favour cartilage formation, while stiff matrices favour chondrocyte hypertrophy and osteogenesis, driven by nuclear transduction of mechanical cues involving Yes-associated protein (YAP) and WW-domain-containing transcription regulator protein 1 (WWTR1, also known as transcriptional co-activator with PDZ binding motif (TAZ))[123–127].

Biphasic scaffolds, consisting of a soft zone and a hard zone that may or may not include calcium, have been evaluated clinically for the repair of focal cartilage defects. An example of this is a BiCRI (polylactic-co-glycolic acid (PLGA) and PLGA plus β-tricalcium phosphate) construct which is currently in clinical trial[128]. While biphasic scaffolds with an apatite-containing inferior layer have been created, the presence of a calcified cartilage interface was not confirmed[129]. To our knowledge, this has only been shown when cartilage was formed on the top surface of a substrate[130]. Layers and gradients may differ in terms of composition (cellular and scaffold), fabrication technique, and structural characteristics, which can create transitional or stepwise depth-dependent differences in composition, arrangement, distribution, dimensions, orientations, and interfaces of the tissues[119]. These gradient-type constructs have not been tested clinically as yet.

Cyclic loading to improve the mechanical properties of engineered cartilage

Tissue engineering approaches for cartilage repair commonly result in tissue that is less mechanically robust than native cartilage. Application of mechanical loading, either cyclic, hydrostatic and/or shear, under the appropriate conditions, during tissue formation *in vitro* has been successful in increasing matrix content. However, it is important to identify the optimal parameters for a specific tissue engineering methodology as these applied forces, if excessive, can induce tissue degradation[131]. Factors to consider in the determination of the load include type and amount of load, and frequency, duration and timing of application. Identification of optimal conditions from the literature is hampered by the use of different methods to apply load and the variability in metrics that are assessed in different studies, making comparisons difficult. However, there are a series of experiments using one type of scaffold-free self-assembly tissue engineering approach and one type of instrument to apply the load that demonstrate the importance of selecting the right parameters. For example, one application of cyclic compression for 30 min, 1 day after cell seeding in 3D culture, resulted in an increase in dry weight of the tissue, higher collagen and proteoglycan content, and just over double the maximum equilibrium stress and equilibrium modulus of the tissue 4 weeks later. In contrast, the same force applied 8 or 14 days later had either no or a negative effect on matrix synthesis[132]. In another study, cyclic compression after 4 weeks of culture could increase tissue formation, but a larger force was required[133]. Interestingly, cyclic compression applied after cartilage had formed, for as little as 6 min every other day for 4 weeks, was sufficient to induce a stimulatory effect[133]. This series of studies highlights the need for further rigorous standardized studies to investigate the use of mechanical stimulation to improve cartilage tissue formation. It should be noted that there are very few *in-vitro*-formed cartilage tissues that attain mechanical properties approaching those of native cartilage even in the presence of mechanical loading. However, this goal may not be necessary, as the loading that occurs with use post-implantation could lead to improved mechanical properties, as was shown to occur in a biphasic implant (cartilage integrated with a porous biomaterial) in a sheep model[117]. At present, it is not known what mechanical properties are required of

bioengineered cartilage to be able to withstand the complex forces experienced by the human joint during daily acts of living (ranging from 7 to 23 MPa of compressive strength and 5–15 MPa tensile modulus[69]).

Other approaches to improve engineered cartilage constructs

To improve cartilage thickness, organization and mechanical properties, constructs can be grown in a bioreactor, such as a perfusion, spinner or rotating vessel, to enhance nutrient diffusion and/or to apply loading[134]. Culture under hypoxic conditions to more closely mimic *in vivo* conditions where the O_2 can go as low as 1% could improve cartilage tissue development[135]. Culture media supplementation with naturally occurring macromolecules, such as polyphosphate[136], link N[137], and platelet-rich plasma[138,139], have also been shown to enhance cartilage tissue formation. Additionally, there have been many studies exploring the use of proteins, particularly growth factors[140]. The major signalling molecules and pathways controlling the process of joint repair are similar to those involved in joint morphogenesis during embryonic development, including transforming growth factor (TGF)-β superfamily, Wnt fibroblast growth factor (FGF), hedgehog, parathyroid hormone (PTH)/PTH-related protein (PTHrP), Wnt, and NOTCH signalling[32]. Targeting these signalling pathways can offer opportunities to enhance cartilage formation, but fine-tuning of intensity, duration, and downstream signalling cascades will be essential. Indeed, excessive or sustained activation of TGF-β signalling can lead to cartilage degradation and OA[141,142], while inhibition of TGF-β signalling protects cartilage integrity in models of OA[143–145]. Similarly, excessive or sustained activation of Wnt/β-catenin signalling can be detrimental[146–149]. Optimal growth factor stimulation may also require sequential exposure to multiple growth factors. Current investigations are focussed on the effects of spatial and temporal release of growth factors from scaffolds on cartilage and bone formation[150].

Other approaches to improve tissue formation in engineered cartilage include use of exosomes[151], microRNA[152], anti-inflammatory M2 macrophages[153], and modified cells using clustered regularly interspaced short palindromic repeats-based gene editing[154], but these are still in the experimental stage.

Interestingly, a recent study suggested that increased temperature, as occurs with mechanical loading (thermomechanical stimulation), can enhance chondrogenic gene expression in chondroprogenitor cells. It was postulated that this could lead to better cartilage formation by these cells[155]. Finally, identifying ways to establish and maintain the superficial zone chondrocyte phenotype, and their expression of joint lubricating factors such as *Prg4*/lubricin that protect against the development of OA[156,157], is an important goal. A recent study showed that the transcription factor Creb5 is selectively expressed in the superficial zone and augments TGF-β and epidermal growth factor receptor-induced expression of *Prg4*/lubricin in superficial zone chondrocytes[158]. In addition, YAP and TAZ have been shown to regulate expression of *Prg4* and tenascin C in superficial zone chondrocytes[159], linking mechanosensing to joint lubrication.

Clinical studies employing tissue engineering approaches for osteoarthritic cartilage repair

There have been very few clinical studies evaluating the efficacy of tissue-engineered cartilage in the treatment of OA. A review of studies registered on clinicaltrials.gov (February 2022) did not identify any. There are papers describing clinical evaluation but they are usually small studies and not always controlled. One study described the use of ChonDux hydrogel (chondroitin sulfate (CS)/polyethylene glycol (PEG)), with or without microfracture, to repair full-thickness cartilage defects in individuals with no or early OA. At 2 years, there was significantly increased defect fill and less pain compared to microfracture alone[101], although 30% of treated individuals had dropped out of the study. In another study, adipose-derived MSCs were loaded into a fibrin scaffold and used to treat knee OA. Outcome was compared to adipose-derived MSCs alone at an average follow-up of 28.6 months (minimal followup – 24 months). There was an increase in activity scores in both groups and the fibrin scaffold group had better International Cartilage Repair Society macroscopic scores at second-look arthroscopy[160]. A cell-free aragonite-based scaffold was evaluated in individuals with mild to moderate knee OA who had at most three discrete cartilage lesions[161]. Two-year follow-up showed symptom improvement and variable fill as determined by MRI, but there was no control group. Hollander *et al.* showed that implantation of an esterified hyaluronic acid scaffold (Hyalograft[131]) seeded with passaged chondrocytes in nine patients with OA resulted in formation of hyaline-like cartilage in some of these individuals at 14 months follow-up[162]. These studies raise the possibility that biological repair of cartilage using tissue engineering approaches in knee OA is possible.

Enhancing endogenous repair by joint-resident stem and progenitor cells

An exciting prospect would be to promote endogenous repair using pharmaceuticals. Investigations of joint-resident stem and progenitor cells, and their molecular regulation, will generate the knowledge base that is essential for targeted molecular interventions aiming to activate and modulate intrinsic repair mechanisms. In recent years, genetic cell-lineage tracing and cell transplant studies have provided insight into the stem and progenitor cells that form, maintain and repair skeletal tissues. The most-well studied are skeletal stem cells (SSCs) in bone, which are heterogeneous and enriched in the perivascular bone marrow niche[163–167] and growth plate region[168–170]. SSCs in mice can contribute to repair of osteochondral lesions that extend into the underlying marrow[167,171], and activation of SSCs in subchondral bone marrow is considered to be at the basis of microfracture therapy. However, microfracture typically results in fibrocartilage repair tissue in both mice[171] and humans[23]. Stem and progenitor cells are also present in the superficial zone of the AC[172–175], synovium[39,51,176] and periosteum[37,177,178], and these could all potentially contribute to the repair and remodelling of joints throughout life.

Traditionally, stem cells are identified by the tissue they reside in. However, stem and progenitor cells within the same tissue are ontogenetically and functionally diverse, while stem and progenitor cells that reside in different tissues can share a common ontogeny. Perivascular SSCs in bone marrow derive, at least in part, from the neural crest[179,180], and play an important role in the regulation of haematopoietic stem cells[164,166]. Perivascular cells expressing SSC markers are also present in synovium and periosteum, but their functions are less clear. They do not appear to directly contribute to cartilage repair after injury[51] or osteophyte formation in OA[181]. Instead, these processes are largely mediated by *Gdf5*-lineage cells, mesodermally derived cells that are progeny of

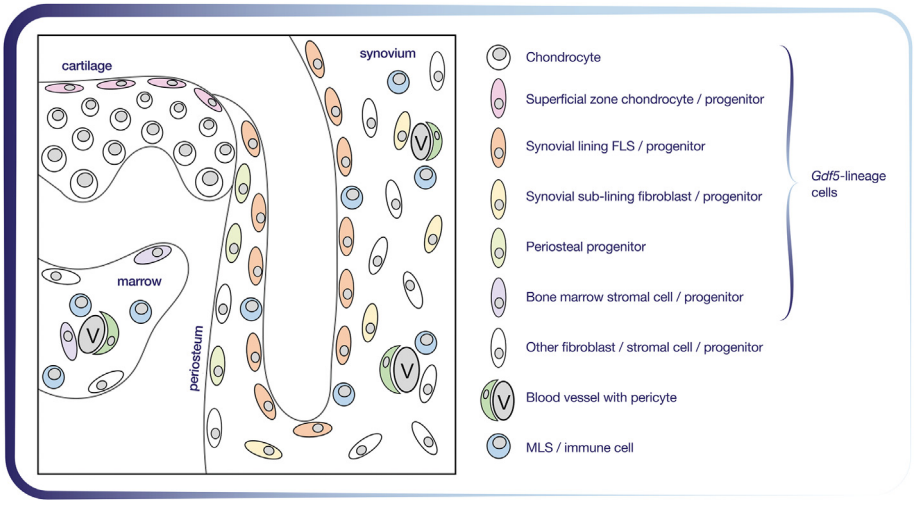

Fig. 3

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Joint-resident stem and progenitor cells. Cells with progenitor activity are present in multiple joint tissues, including synovium, periosteum, cartilage, and subchondral bone marrow. The main joint-reparative cells are found in the *Gdf5*-lineage cell population that descends from the joint interzone, the embryonic tissue that gives rise to the synovial joint during development. *Gdf5*-lineage progenitor cells include *Prg4*-expressing cells in the superficial zone of articular cartilage, *Prg4*-expressing cells in synovial lining, and *Sox9*-expressing cells at the periosteal surface. *Gdf5*-lineage cells are also present in synovial sub-lining and perivascular and endosteal niches in subchondral bone marrow. Other stromal cells in synovium, periosteum and subchondral bone marrow may contribute to repair. Pericytes, including cells expressing SSC markers such as Nestin or Leptin receptor, as well as macrophage-like synoviocytes in synovial lining and other immune cells, may contribute to regulating the reparative response. However, there is little evidence of a direct contribution of pericytes to joint surface repair. FLS: fibroblast-like synoviocyte; MLS: macrophage-like synoviocyte.

the *Gdf5*-expressing joint interzone cells in the embryo that form the synovial joints during development[50,182]. *Gdf5*-lineage cells in the adult mouse knee respond to acute cartilage injury by proliferation, homing to the site of injury, and chondrogenic differentiation to repair the defect[51], while they respond to chronic injury resulting from joint destabilisation by forming osteophytes[181].

The adult *Gdf5*-lineage cell population is not specific to any one tissue in the joint and contains several progenitor populations that could contribute to repair of the AC after injury (Fig. 3). There may be cooperation of different progenitor populations, as observed during osteophyte formation in experimental OA in mice, which is mediated by *Sox9*-expressing progenitors in periosteum and *Prg4*-expressing progenitors in synovial lining[181]. *Prg4*-expressing synovial lining cells may also be involved in AC repair[175], which could involve direct synovial attachment to the defect[175], or migration of synovial cells along the cartilage surface[183] or via synovial fluid[184,185]. Adverse environmental conditions could make the cells ineffective and unable to repair damaged cartilage, highlighting the need to understand the context-specific regulation of stem and progenitor cells in their own environment.

Recent studies have focussed on the identification and manipulation of molecular signals that can promote endogenous stem cell recruitment and their differentiation into a stable chondrocyte phenotype. Suppression of canonical β-catenin signalling and activation of the CaMKII/CREB pathway by the proteoglycan Agrin was shown to enhance recruitment of endogenous *Gdf5*-lineage progenitor cells to an osteochondral defect, and to improve osteochondral repair in mice and sheep[186]. Other studies have investigated molecular signals related to the avascular nature of cartilage. In mice, physically preventing vascular invasion during femoral fracture healing, or blocking vascular endothelial growth factor (VEGF) signalling in a renal capsule implant model of bone marrow SSCs, favoured chondrogenic over osteogenic differentiation[169,187]. Delivery of PEG hydrogels loaded with BMP2 together with a VEGF inhibitor in osteochondral defects that were created in OA mouse knees induced the formation of a cartilage repair tissue with biomechanical properties similar to native cartilage[171]. The promotion of chondrogenesis in an avascular environment may be driven by hypoxia-induced upregulation of hypoxia-inducible factor (HIF)-1α and HIF-2α, which bind to the *Sox9* promoter[188,189]. In addition, limited nutrient supply, and specifically lipid scarcity, regulates chondrogenesis in skeletal progenitors via activation of FoxO transcription factors that bind to and activate the *SOX9* promoter[187]. Thus, the avascular nature of cartilage is intricately linked to the molecular signals that regulate its formation and maintenance, and manipulation of these signals could induce formation of more stable cartilage by SSCs. Whether this will be sufficient to induce durable repair in synovial joints by stem or progenitor cells not ontogenetically derived from the joint interzone, or whether *Gdf5*-lineage cells remain the best candidate

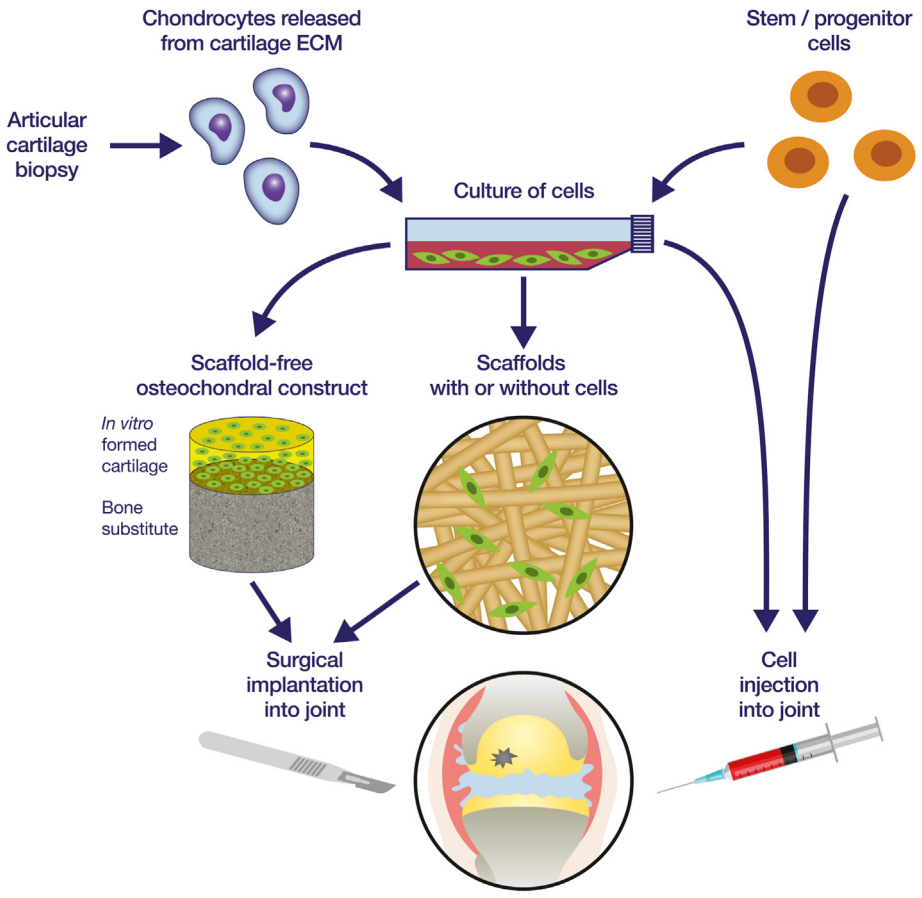

Fig. 4 Osteoarthritis and Cartilage

Schematic summary of methods for cartilage repair. All approaches shown can be enhanced with, for example, bioactive effectors or mechanical loading.

cells to target for the enhancement of endogenous repair, remains to be clarified.

Conclusions

Regenerative interventions have entered clinical practice in orthopaedics, with potential for long-term and possibly life-long benefit to patients, and a multitude of tissue engineering approaches to cartilage repair are in the translational pipeline towards clinical application (Fig. 4). While cellular products for cartilage repair have pioneered the field of tissue engineering, a common challenge is the standardization of processing and manufacture to obtain a consistent product of defined identity and known potency to patient benefit. The use of cell-free biomaterials and/or bioactive molecules that activate endogenous reparative processes might render the regulatory pathway more straightforward, but their utility has yet to be shown in clinical trials. The use of biomaterials and bioactive molecules, not only in combination with seeded exogenous cells but also as acellular functionalised scaffolds to promote intrinsic repair mechanisms, is an active area of investigation. Identifying the ideal scaffold and the ideal spatio-temporal delivery of bioactive molecules remain extremely challenging tasks. As our understanding of the intrinsic cellular and molecular mechanisms of tissue repair advances, new signals and targets will be identified that will inspire the development of molecular therapies that are more in line with classical pharmacological interventions. While these will target small cartilage lesions and will possibly lead to the long-awaited disease-modifying OA drugs (DMOADs), more comprehensive approaches relying on exogenous cells and/or combination tissue engineering products will still be needed for the repair of larger defects. The engineering of biological spare parts or even custom-made prostheses could be achieved through the coordinated design of consistent, fully controlled, and upscalable manufacturing processes using advanced technologies such as bioreactors, biosensors and 3D bioprinting. Efforts should be devoted to understanding the underlying mechanisms of action of cell therapy and tissue engineering approaches, not only to enhance our scientific knowledge and fulfil the regulatory requirements, but also, and most importantly, to help improve and refine therapy development over the years. Finally, properly designed, randomised, controlled clinical studies are required to define an evidence-based

treatment algorithm for selection of patients with cartilage defects and/or OA who will respond to the treatment. Additionally, appropriate rehabilitation programs will need to be developed. A one-fits-all solution is unrealistic, and stratification of patients will be necessary for targeted treatments to be successfully delivered to the right patient group at the right time.

Contributors

All authors contributed to drafting, editing and approving the manuscript.

Conflict of interest

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