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Surgical and tissue engineering strategies for articular cartilage and meniscus repair

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Abstract | Injuries to articular cartilage and menisci can lead to cartilage degeneration that ultimately results in arthritis. Different forms of arthritis affect ~50 million people in the USA alone, and it is therefore crucial to identify methods that will halt or slow the progression to arthritis, starting with the initiating events of cartilage and meniscus defects. The surgical approaches in current use have a limited capacity for tissue regeneration and yield only short-term relief of symptoms. Tissue engineering approaches are emerging as alternatives to current surgical methods for cartilage and meniscus repair. Several cell-based and tissue-engineered products are currently in clinical trials for cartilage lesions and meniscal tears, opening new avenues for cartilage and meniscus regeneration. This Review provides a summary of surgical techniques, including tissue-engineered products, that are currently in clinical use, as well as a discussion of state-of-the-art tissue engineering strategies and technologies that are being developed for use in articular cartilage and meniscus repair and regeneration. The obstacles to clinical translation of these strategies are also included to inform the development of innovative tissue engineering approaches.

Arthritis is a debilitating condition that affects ~50 million adults in the USA, a prevalence that is projected to rise by ~60% in the next two decades[1]. Osteoarthritis (OA), the most common type of arthritis[2], is associated with pain and loss of joint function. Although the aetiology of OA can be idiopathic, the disease is often characterized by cartilage degeneration in articulating joints as a result of 'wear and tear' or injury, including sports-related injuries. For example, in one study, individuals who sustained knee injuries were 7.4 times more likely to develop OA than those who had not sustained knee injuries[3]. Meniscus and anterior cruciate ligament (ACL) tears can also contribute to the development of OA because damage to these structures alters joint loading[4,5]; OA occurs 10–20 years after injury in ~50% of patients who sustain meniscal or ACL tears[5]. Globally, knee and hip cartilage degeneration is one of the leading contributors to disability[6]. Rheumatoid arthritis (RA), the second most common type of arthritis, is a chronic autoimmune disease characterized by inflammation and deterioration of joints that results in loss of function, and affects 1.3 million adults in the USA[7]. Worldwide, arthritides such as OA and RA represent a substantial burden to health-care systems[8,9].

Despite the pervasiveness of OA, most current treatments are palliative and do not prevent further joint degeneration[10]. Likewise, treatments for RA often reduce joint inflammation without treating cartilage damage[11]. Ultimately, many patients with arthritis will require total joint arthroplasty, an invasive end-stage treatment that uses implants that wear out over time. Current surgical strategies for cartilage repair are designed to treat small defects in cartilage and are not directly indicated for use in inflamed joints, such as those that occur in RA. However, using tissue engineering strategies, which focus on the complete regeneration of articular cartilage[12,13] and menisci[14,15], researchers can potentially create neotissue that has been modified to withstand immune-mediated degeneration. Thus, in the future, tissue engineering strategies could offer new therapeutic avenues for patients with RA before total joint arthroplasty is indicated.

In this Review, we begin by discussing current surgical techniques, including tissue-engineered treatments, defined here as cell-based (scaffold-free and scaffold-based) therapies, for the repair of articular cartilage and meniscus lesions. We then discuss advances in tissue engineering research for articular cartilage and meniscus regeneration, including novel scaffold-based and scaffold-free approaches, promising sources of cells for cell-based therapies and emerging data on biochemical and biomechanical stimuli. We also present data

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Key points

- Current cartilage repair techniques include surgery and cell-based therapies for articular cartilage, and surgery for meniscus repair; however, such treatments have limited capacity to induce regeneration.
- Tissue engineering strategies to create cartilage using a variety of cell sources and exogenous stimuli have made advances towards replicating the native architecture and functional properties of cartilage.
- Most cell-based tissue engineering products currently in clinical trials are indicated for knee articular cartilage, with very few indicated for hip cartilage or the meniscus.
- Allogeneic and non-articulating cartilage might serve as additional cell sources for engineered articular cartilage and meniscus products.
- The pro-inflammatory environment of arthritic joints and issues surrounding neotissue integration need to be addressed to maximize the clinical translation of new tissue-engineered products.

on cell-based tissue-engineered products for cartilage regeneration currently in development. Finally, we discuss scientific and regulatory obstacles to the clinical translation of tissue-engineered technologies, as well as future directions to encourage researchers in the field to overcome these challenges.

Current surgical strategies

Repairing articular cartilage defects

Articular cartilage is predominantly composed of type II collagen and glycosaminoglycans and is avascular with low cellularity (FIG. 1a) and, therefore, has a low healing capacity. Clinicians encounter articular cartilage damage in more than half of knee arthroscopies performed as a result of injury or symptoms of cartilage damage[16,17]. Specifically, chondral lesions (defects that do not penetrate into the subchondral bone) and osteochondral lesions (defects that penetrate into the subchondral bone) were found in 61% of patients surveyed[12,17]. Because cartilage defects are often asymptomatic[18], careful assessment is required to determine whether the lesion is the source of pain in an individual. Current surgical strategies aim to repair small (<4 cm^2) defects in cartilage to prevent further degeneration and progression towards OA (FIG. 1b). Cartilage repair strategies for the knee are well-established and produce improvements in clinical outcomes for patients[19,20]. However, repair of hip cartilage is less frequently performed than repair of knee cartilage. The use of bone marrow stimulation, grafting and cell-based techniques for articular cartilage repair are discussed in the following section.

Bone marrow stimulation and augmentation. Bone marrow stimulation techniques for small (<4 cm^2), contained, defects have evolved from open debridement of damaged cartilage and removal of subchondral bone to the Steadman microfracture technique[21], in which the calcified cartilage is removed and an awl is used to create perforations in the subchondral plate. Bone marrow released into the defect forms a blood clot, which might ultimately lead to the formation of fibrocartilage. Unlike hyaline cartilage, fibrocartilage is rich in type I collagen and is of limited durability. Individuals treated with microfracture show initial clinical improvement after surgery, but have an accelerated decline in clinical outcome scores and a higher failure rate during long-term follow-up than those treated with osteochondral autograft treatment[22,23]. To overcome the shortcomings of microfracture, augmented bone marrow stimulation techniques were subsequently developed, including the concomitant injection of molecules such as growth factors, the use of acellular scaffolds (such as collagen membranes) or liquid hydrogels, and the use of micronized acellular cartilage extracellular matrix from allografts[24]. However, more high-quality studies are needed to demonstrate the superiority of augmented bone marrow stimulation techniques over other established procedures, such as microfracture or autologous chondrocyte implantation (ACI)[25].

Autografts and allografts. Osteochondral autograft transfer delivers viable, mature hyaline cartilage–bone units into chondral defects. These osteochondral grafts can bear load in the early postoperative period, enabling faster rehabilitation than following other, currently available, cell-based cartilage repair strategies[26]. Osteochondral autograft transfer involves the harvesting of 'plugs' from regions of the distal femur that bear low loads (such as the intercondylar notch or medial or lateral trochlea) and, therefore, its use is reserved for small chondral defects (<2 cm^2) owing to limited graft availability[27].

The avascular nature of cartilage renders it immune privileged[28], thereby opening up the potential for allogeneic approaches. Osteochondral allograft transplantation does not have the donor site limitations of osteochondral autograft transfer and can be used in revision surgery for failed cartilage repairs, making osteochondral allografting an appealing technique, although the availability of allograft tissue limits its use. Matching allografts to the shape and contours of the native knee architecture can also be difficult to achieve, potentially creating biomechanical loading imbalances and resulting in degenerative joint changes[29,30]. Techniques to improve the viability of chondrocytes in fresh osteochondral allografts and to accelerate the remodelling of graft tissue into host tissue are continually being investigated because both factors seem to be important for the longevity of the transplanted allograft[31,32].

Both osteochondral autograft transfer and osteochondral allograft transplantation have produced high rates of long-term graft survival, as well as high degrees of reported patient satisfaction and return-to-play among athletes[26,33–35]. For example, a 2016 systematic review found that ~90% of patients who underwent osteochondral autograft transfer had good or excellent outcomes at up to 10 years after surgery[19]. Another study showed that the survival of fresh osteochondral allografts was 82% at 10 years and 66% at 20 years after transplantation[33]. Cryopreserved osteochondral allografts (Cartiform), fresh osteochondral allografts (ProChondrix) and particulated juvenile allograft cartilage (DeNovo NT), which are processed by laser cutting or mincing, have also been used to treat articular cartilage defects[36]; however, short-term and long-term data are needed to determine the clinical success of these products.

Debridement
The removal of damaged tissue and/or torn fragments from a defect.

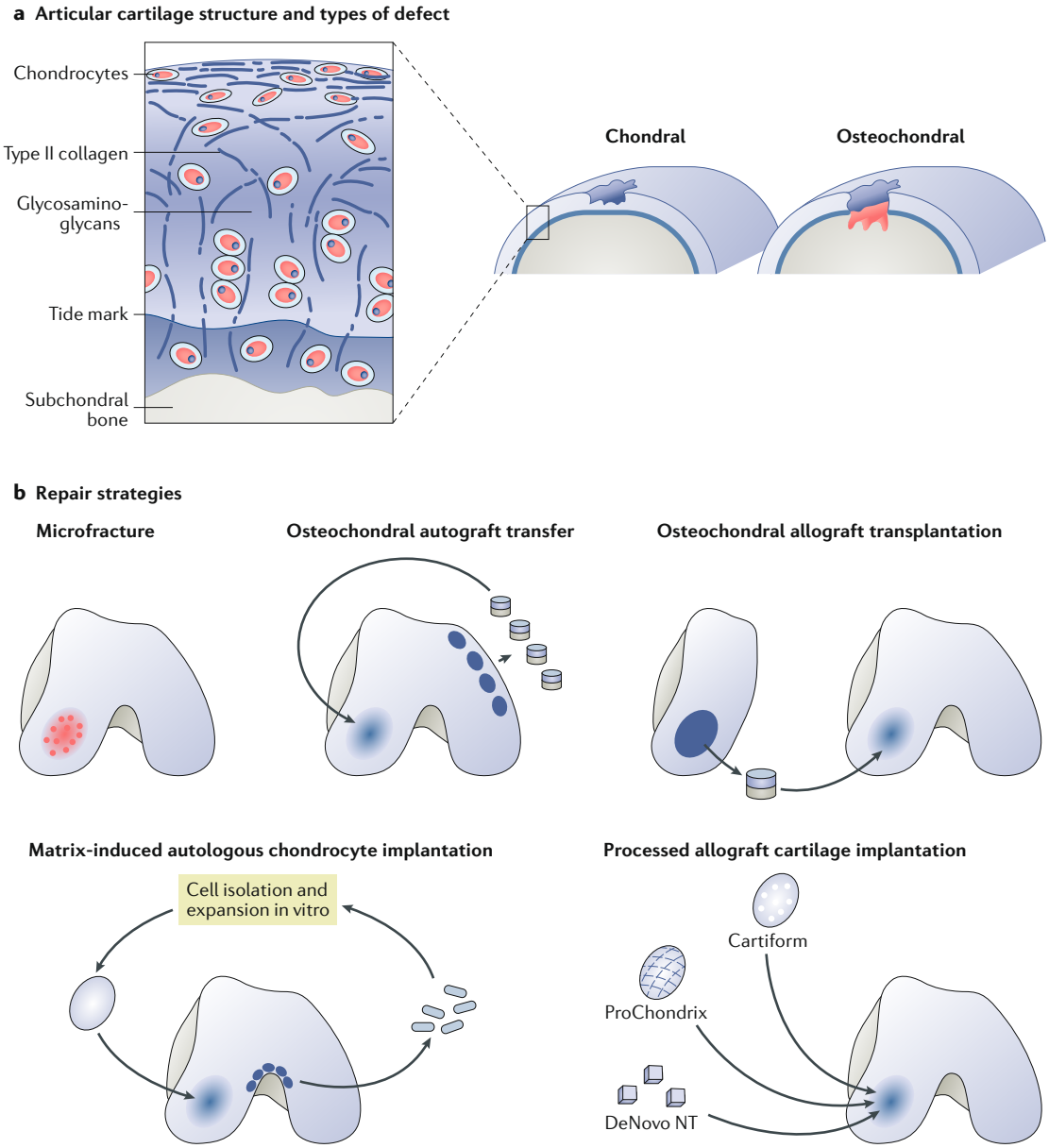

Fig. 1 | **Articular cartilage structure and treatment methods. a** | Articular cartilage consists of chondrocytes embedded in a defined structure of collagen fibres and glycosaminoglycans. Two main types of defect can occur: chondral defects, which only penetrate the cartilage, and osteochondral defects, which also penetrate the subchondral bone. **b** | Currently used repair strategies for cartilage defects include microfracture, osteochondral autograft transfer, osteochondral allograft transplantation, implantation of processed allograft cartilage such as DeNovo NT, ProChondrix and Cartiform, and matrix-induced autologous chondrocyte implantation. The choice of treatment method depends on the size and type of the defect, the expertise and preferences of the surgeon and patient-specific factors such as age and activity level.

Cell-based techniques. Current cell-based cartilage repair techniques enable the implant to be contoured to the recipient defect, making these techniques attractive for treating large (>3–4 cm^2) chondral lesions in areas with variable topographies, such as the patellofemoral joint or acetabulum. ACI requires two operations: chondrocytes are harvested from healthy articular cartilage in one operation and are then re-implanted into the chondral defect in a second operation after expansion in culture. A newer iteration of this technique, known as matrix-induced ACI (MACI), includes seeding of the chondrocytes onto a scaffold before implantation[37]. Patients treated with MACI have reported substantial long-term improvements in knee function and high rates of satisfaction[38,39]. In one study, at 5 years after surgery, 93% of patients expressed satisfaction with their postoperative pain relief, 90% had an improved ability to perform daily activities and 80% were able to participate more in sports compared with before the operation[38]. However, procedures that require only one operation are currently more appealing for clinicians than ACI or MACI.

Repairing meniscus defects

Two semicircular, wedge-shaped menisci are located between the distal femur and the tibial plateau and serve to distribute loads and protect articular cartilage. Each meniscus has two distinct regions (FIG. 2a): the outer, vascular, neural region (the red–red zone), which contains elongated fibroblast-like cells and predominantly type I collagen, and the inner, avascular, aneural zone (the white–white zone), which contains rounded chondrocyte-like cells (fibrochondrocytes) and predominantly type II collagen. These two zones are separated by the red–white zone, which has characteristics of both the red–red zone and the white–white zone. The meniscus functions by distributing load through its circumferentially aligned collagen fibres (FIG. 2a). Meniscus tears disrupt this function; however, only a small proportion of tears are considered repairable on the basis of tissue vascularity, tear pattern, anatomical location and tear acuity (FIG. 2b). For example, vertical longitudinal tears within the red–red or red–white zone of the meniscus are often amenable to repair[40]. Horizontal and radial tears are thought to rarely heal owing to incursion into the avascular white–white zone. Furthermore, radial tears disrupt the circumferential collagen fibres that are critical for maintaining hoop stresses, whereas circumferential vertical or horizontal tears can leave the meniscus with the potential for residual functionality because these tears follow the circumferential collagen fibres. The length, depth and size of tear, as well as joint stability and other patient-related factors such as age and symptoms also affect healing[41,42]. Despite our understanding of the crucial function of the meniscus in knee biomechanics, partial meniscectomy to remove unstable, damaged portions of the tear remains the gold standard for surgical treatment of meniscus tears, and accounts for half of the knee arthroscopic procedures performed in the USA[43]. However, both partial and total meniscectomy are linked to the development of knee OA[44], a fact that provides motivation for the development of novel interventions such as cell-based regenerative therapies.

Reduction of meniscal tears. Lesions in the meniscus that are mechanically unstable, complex or of a degenerative nature are conventionally treated with partial meniscectomy; however, attempts to reduce meniscal tears instead of performing partial meniscectomy have become more common during the past 15 years[45] (FIG. 2c). Meniscus defect reduction (often described by clinicians as meniscus repair) is usually accomplished by closure of the tear with sutures and/or anchors. For example, suturing of defects in the red–red and red–white zones led to satisfactory clinical healing in 76% of patients with meniscal tears[46]. Tear reduction also resulted in meniscus preservation without degeneration in younger patients (aged between 16 and 52 years)[47,48]. Meniscal tear reductions performed concurrently with ACL reconstruction have superior healing rates than meniscal tear reductions alone[49], potentially owing to the intra-articular release of cells and growth factors from the bone marrow that occurs when drilling a bone tunnel during ACL reconstruction[50]. Parameters affecting meniscus repair are probably multifactorial, but biological augmentation techniques, such as mechanical stimulation of the adjacent synovium or meniscus by rasping or radial trephination[51,52], the addition of an exogenous fibrin clot[53] or the introduction of bone marrow stem cells by marrow venting[54], are thought to promote healing.

Allografts. Meniscus allograft transplantation is the only option for total meniscus replacement, and is widely performed following total or near total meniscectomy (FIG. 2d). Allograft transplantation is indicated in patients who have a stable, correctly aligned joint and, at most, early knee OA[55]. Meniscus allografts can be inserted with several forms of attached bone, such as bone plugs, a common bone bridge or a hemi-plateau, or without attached bone[56]. In particular, meniscus fixation using bone plugs leads to better load transmission than fixation without using bone plugs[56]. Appropriate allograft sizing to the recipient knee[56] is also an important factor for tissue healing[57] and for the preservation of knee biomechanics[58]. Allograft recipients have good rates of clinical improvement. In a long-term follow-up study (mean 152 months) in 30 patients who received meniscal allografts, all patients had improved function (as measured by Lysholm score, short form-36 (SF-36) score and Knee Injury and Osteoarthritis Outcome Score (KOOS)), and 90% were satisfied with the outcome of the surgery[59]. However, meniscus replacement does not prevent joint space narrowing[60].

Synthetic implants. Partial meniscus replacements, such as collagen meniscus implants (CMI, available in the USA) and polyurethane polymeric implants (Actifit, available in Europe), can be used in patients with segmental meniscus defects, an intact peripheral rim and limited articular cartilage damage[61]. CMI provided substantial pain relief and functional improvement and had a low rate of implant failure at follow-up (mean 9.6 years) in patients receiving implants following partial meniscectomy[62]. Similarly, polyurethane polymeric implants improved clinical outcomes in patients following partial meniscectomy up to 4 years after implantation[63]. For replacement of the entire meniscus, a polyethylene-reinforced polycarbonate urethane prosthetic (NuSurface) is currently in FDA clinical trials[64]. Although synthetic meniscus implants can improve clinical outcomes, their use is limited by several shortcomings and technical difficulties: synthetic implants do not result in meniscus regeneration; the ability of synthetic implants to stop progression of OA is unproven; synthetic implants are difficult to place properly within the defect using an arthroscopic approach; and synthetic implants are challenging to handle and suture[65]. Therefore, a great need exists for cell-based approaches that can regenerate damaged meniscus.

Age-related differences in outcomes

Parameters that affect the outcomes of articular cartilage and meniscus repair are multifactorial, but generally, increased patient age has a negative correlation with good outcomes, in particular after bone marrow stimulation techniques. Treatments that are acceptable for use in paediatric and adolescent patients might not be suitable for use in adults, who tend to have degenerative,

Hoop stresses
Compressive forces experienced by the meniscus in the circumferential direction.

Rasping
Mechanical scraping to expose fresh and/or bleeding tissue.

Radial trephination
Puncturing small holes into the joint lining and/or synovium and into the tissue to stimulate healing.

Bone plugs
Created or fashioned bone cylinders containing the enthesis of the meniscal roots.

Common bone bridge
Excised bone containing and preserving the anatomic relationship between the anterior and posterior meniscal horns (also known as 'slot').

Hemi-plateau
Half of the tibial plateau, containing the articular surface, subchondral bone and meniscus with root attachments.

Lysholm score
A scoring system used to measure changes in limping, support, locking, instability, pain, swelling, stair climbing and squatting (originally developed to evaluate outcomes of knee ligament surgery).

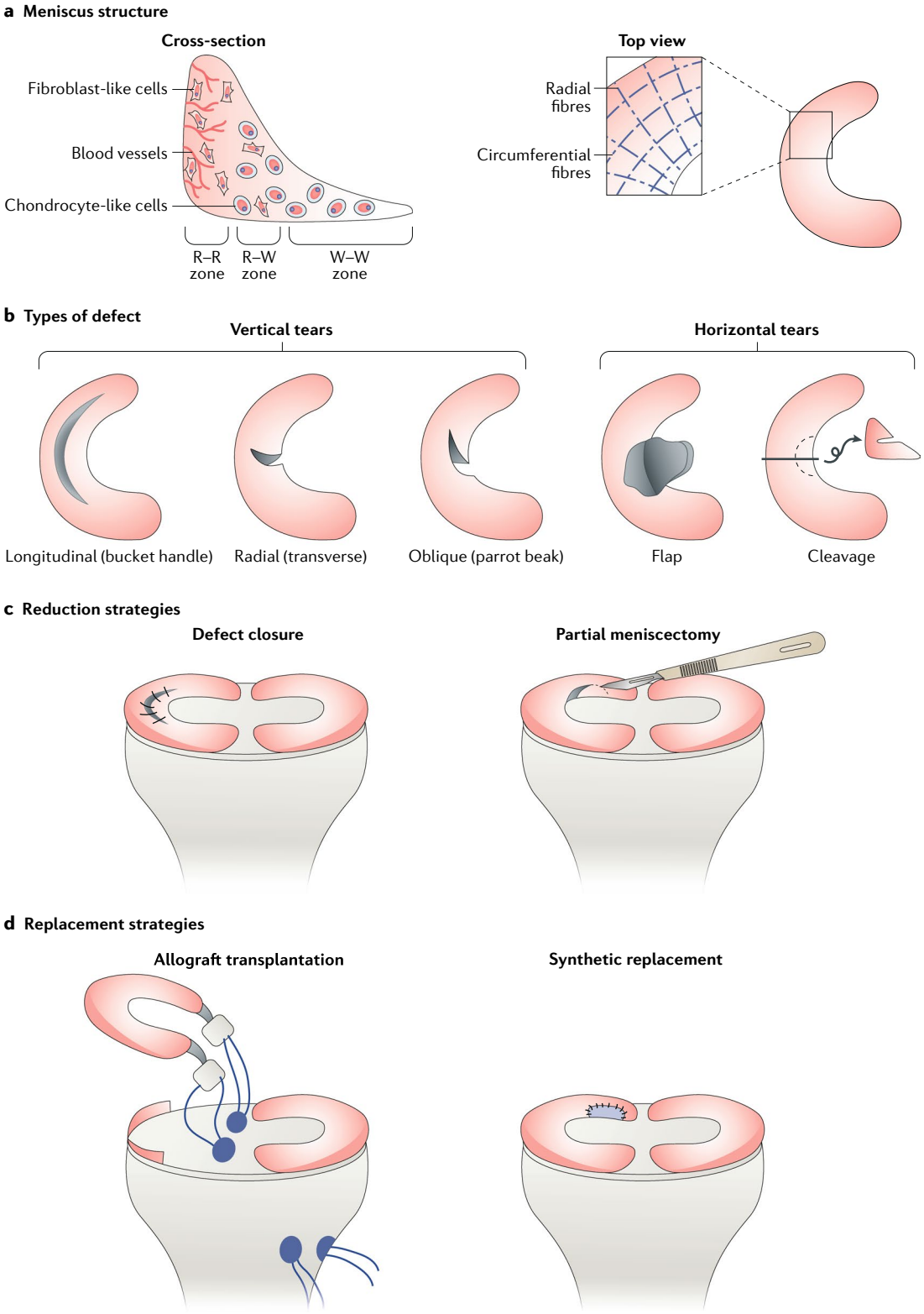

Fig. 2 | **Meniscus structure and treatment methods. a** | The meniscus consists of three main zones: red–red (R–R), red–white (R–W) and white–white (W–W). The R–R zone is fully vascularized and the W–W zone is avascular. **b** | A variety of different types of defect can occur in the meniscus, some of which are easier to repair than others owing to their intrusion into vascular or avascular zones. **c** | Reduction strategies in current use include defect closure with sutures or anchors and the trimming of torn pieces (partial or total meniscectomy). **d** | Replacement strategies in current use include allograft transplantation and the use of synthetic implants. As with articular cartilage, the size and type of defect, the expertise and preferences of the surgeon and patient-specific factors such as age and activity level affect the choice of treatment method.

rather than acute traumatic, lesions. Two main principles exist for treating paediatric articular cartilage or meniscus defects: techniques must be effective to help prevent the risk of developing OA at a young age; and joint anatomy and functionality must be restored to ensure symptomatic relief and resumption of pre-injury levels of physical activity[66]. Given the increase in paediatric joint injuries[67,68], potentially as a result of increased participation in sports, the development of therapies that will withstand the test of time is greatly needed.

Treatment of articular cartilage defects in young patients. Although many of the same techniques are used to treat cartilage lesions in children and adolescents as in adults, outcomes can differ. For microfracture, patients older than 40 years had worse outcomes than younger patients (<30 years of age) in many studies[69–72], potentially because older patients have fewer bone marrow progenitor cells and diminished regenerative capacity compared with younger patients. A similar trend occurs with osteochondral autograft transfer, for which better outcomes have been reported in young patients (<30 years of age)[73]. By contrast, 88% of paediatric and adolescent patients had successful outcomes following osteochondral allograft transplantation after a median of 2.7 years[74], similar to success rates reported in adults[75]. ACI in young patients (≤18 years of age) produced an improvement in postoperative outcomes in 84–96% of patients at 2–4 years of follow-up[76,77], which was higher than the rate of improvement in adults for the same follow-up period (78–83%)[78,79]. Overall, in younger patients (≤40 years of age), many of whom are athletes, osteochondral autograft transfer[22,80] and ACI or MACI[81] might result in better long-term outcomes and higher rates of return-to-play than microfracture.

Treatment of meniscus defects in young patients. As with articular cartilage, outcomes associated with treating meniscus pathologies differ as a result of multiple factors, including age and tear type. In general, meniscus allograft transplantation is indicated in young patients (<50 years of age) with meniscal deficiency, and is contraindicated in patients with evidence of advanced OA[82]. In patients aged 16 years or younger, an improved Lysholm score and a revision rate of 22% have been reported after a mean follow-up of 7.2 years following meniscus allograft transplantation[83]. For meniscal tear reduction, most studies in a meta-analysis showed little difference in failure rates between patients under and over the age of 40 years[84,85]. Another meta-analysis on meniscus repair that included 13 studies in adults showed a healing rate of 62–79% and a pooled re-tear rate of 23% after >5 years[86]. Comparisons between surgical outcomes in paediatric and adolescent patients versus adult patients need to take into consideration the types of tear that are being reduced. In paediatric and adolescent patients, meniscus defect reduction can be attempted for most meniscal tears regardless of zone, size and patient-specific factors, as the priority is to preserve the knee. By contrast, in adults, meniscus defect reduction is usually only performed for tears that have a high potential to heal, such as peripheral tears. Thus, despite the beneficial healing environment in paediatric and adolescent patients that results from a high degree of vascularization and increased cellular metabolism[87,88], healing rates in paediatric and adolescent patients compared with adult patients can seem similar because of the types of tears that are treated.

Tissue engineering strategies

Current surgical approaches do not provide long-term solutions for articular cartilage and meniscus regeneration, but tissue engineering techniques could provide alternative treatment strategies. Scaffolds, cells and biochemical and biomechanical stimuli, the main tools used to create engineered tissues (FIG. 3), are discussed in this section, as well as advances in cartilage engineering and the results of preclinical and clinical studies using engineered articular cartilage and meniscus products.

Scaffold and scaffold-free approaches

A variety of synthetic or natural materials, including polylactides, polyglycolides and silk, have been investigated for use as scaffolds for engineered articular cartilage[89] and meniscus[88]. Decellularized cartilage-derived matrix has also been investigated for use as a scaffold in cartilage regeneration[90,91]. For example, decellularized cartilage-derived matrix scaffolds inhibit the hypertrophic differentiation of embedded mesenchymal stem cells (MSCs) and promote the synthesis of cartilage matrix by these cells[90]. Decellularized extracellular matrix scaffolds derived from inner and outer regions of the meniscus support the differentiation of MSCs towards fibrochondrocyte and elongated fibroblastic phenotypes, respectively[91]. Various other types of scaffolds, including hydrogels and porous polymeric structures, are also under investigation for use in articular cartilage and meniscus tissue engineering. For example, injectable hydrogels, which can form irregular shapes to better fill defects, enable the use of minimally invasive implantation methods[92]. In the past 20 years, both natural materials (for example, alginate and hyaluronan) and synthetic materials (for example, polycaprolactone and polylactic acid) have been used in 3D printers to create anatomically shaped scaffolds for articular cartilage and menisci[93,94]. The advantages of using scaffolds for cartilage engineering include the ability to incorporate growth factors into the scaffold and the initial mechanical stability that they provide[95].

Despite the advantages of scaffolds, scaffold use can also result in degradation-associated toxicity, stress shielding, altered cell phenotypes and hindrances to remodelling[95]. These difficulties have provided the motivation for investigations into scaffold-free techniques to engineer cartilage[96] and menisci[97]. In particular, the scaffold-free self-assembling process facilitates cell-to-cell interactions by minimizing free energy, and recapitulates the conditions of cartilage development, which result in changes in the ratios of chondroitin 6-sulfate to chondroitin 4-sulfate and type VI collagen to type II collagen within the engineered neocartilage as it develops[98]. Through the use of biochemical and biomechanical stimuli, cartilage engineered using a scaffold-free approach has attained functional properties on a par with native tissue[99]. For example, engineered articular

Stress shielding
Protection of tissue from normal mechanical stresses by the presence of a much stiffer implant, often resulting in tissue loss.

Self-assembling process
A scaffold-free technology that produces tissues that demonstrate spontaneous organization without external forces via the minimization of free energy through cell-to-cell interactions.

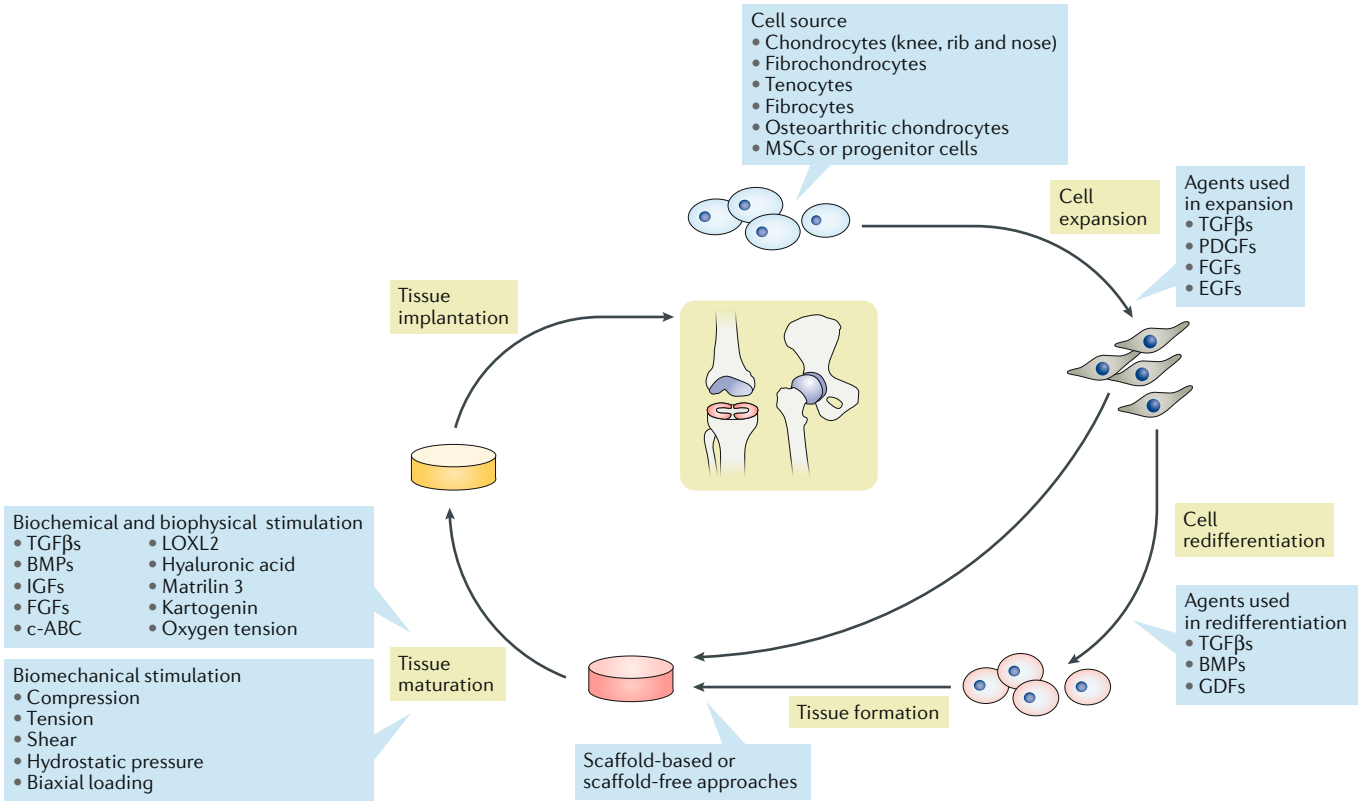

Fig. 3 | **Advances in tissue engineering strategies for articular cartilage and meniscus.** Engineered implants go through several stages of development that can be modified or enhanced by the addition of appropriate stimuli. The source of cells is important, as many cells dedifferentiate in culture. Alternative cell sources currently being trialled include non-articular chondrocytes, tenocytes, fibrocytes, osteoarthritic chondrocytes and stem cells or progenitor cells. Growth factors such as transforming growth factor βs (TGFβs), platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), epidermal growth factor (EGF), bone morphogenetic proteins (BMPs) and growth and differentiation factors (GDFs) are used to effectively expand and help to redifferentiate cells before neotissue formation. Scaffold-based and scaffold-free methods can be used to engineer articular cartilage and menisci, and biochemical and biophysical factors such as TGFβs, BMPs, insulin-like growth factors (IGFs), FGFs, chondroitinase ABC (c-ABC), lysyl oxidase-like 2 (LOXL2), hyaluronic acid, matrilin 3, kartogenin and variations in oxygen tension are used to promote the maturation of engineered tissues. Similarly, biomechanical stimulation by, for example, compression, tension, shear, hydrostatic pressure and biaxial loading, can be used to improve the functional properties of the neotissue. MSC, mesenchymal stem cell.

cartilage has achieved compressive and tensile moduli of ~0.32 MPa (REF.[100]) and ~8 MPa (REF.[99]), respectively, which are within the ranges of values for native articular cartilage (0.1–2 MPa and 5–25 MPa, respectively)[101]. Similarly, scaffold-free engineered menisci have compressive and tensile moduli of ~0.12 MPa (REF.[102]) and ~5 MPa (REF.[103]), respectively, compared with the ranges of values for native tissue of 0.1–0.15 MPa and 10–30 MPa, respectively[88]. Thus, scaffold-free methods have the potential to circumvent challenges associated with scaffolds and to produce biomechanically functional implants.

Advances in scaffold-based and scaffold-free approaches have also focused on the recapitulation of native tissue architecture[104–107]. For example, stiffness gradient hydrogels (0.005–0.06 MPa) derived from poly(ethylene glycol) and chondroitin sulfate yield constructs with stiffness-dependent glycosaminoglycan gradients that mimic the glycosaminoglycan gradient found in articular cartilage between the superficial and deep zones[104]. In another study, bi-layered poly(ε-caprolactone) scaffolds with porous layers and aligned fibrous layers supported the development of zonal arrangement of engineered cartilage[105]. Collagen density and the alignment of porous collagen scaffolds can also be tailored via biaxial compression[106], which might be useful for engineering anisotropy in the meniscus. Scaffold-free approaches have also been used to generate zonal tissue and anisotropy; for example, anisotropic menisci with zonal variations have been produced using the self-assembling process[107]. These studies[104–107] suggest that recapitulating zonal and anisotropic properties of cartilage and menisci might be necessary to impart native functional properties to a tissue-engineered product.

Anisotropy
Having directionally dependent properties.

Engineering articular cartilage

Cell sources. Although chondrocytes are the obvious choice for use in engineering articular cartilage, the scarcity of chondrocytes necessitates cell expansion in vitro, which results in rapid dedifferentiation[108]. Although, to date, there is no evidence that dedifferentiated cells can be redifferentiated in vivo, the results of some studies have suggested that redifferentiation can be

accomplished in vitro[109,110]. For example, culturing either in vitro expanded chondrocytes or MSCs under 3D culture conditions supplemented with transforming growth factor-β1 (TGFβ1), growth and differentiation factor 5 (GDF5) and bone morphogenetic protein 2 (BMP2), collectively termed aggregate redifferentiation, resulted in increased expression of the chondrogenic genes *SOX9*, *ACAN* and *COL2A1* compared with untreated cells[111]. Alternative cell sources include chondrocytes from non-articular cartilages; for example, costal (rib) chondrocyte-derived neocartilage has compressive properties on a par with those of native articular cartilage[109]. HOX-negative nasal chondrocytes are thought to possess greater self-renewal capacity than articular chondrocytes[112] and a nasal chondrocyte-based articular cartilage product (N-TEC) is currently in clinical trials for articular cartilage repair in Europe[113]. In addition, constructs engineered using osteoarthritic chondrocytes have yielded neocartilage containing type II collagen and lubricin, but not type I collagen or type X collagen, which are indicative of chondrocyte dedifferentiation and hypertrophy[114]. Thus, non-articular and osteoarthritic cartilage might yield viable cells for use in articular cartilage repair.

Adult MSCs derived from adipose tissue, bone marrow, synovium or skin have been extensively investigated for use in cartilage tissue engineering. Bone marrow-derived MSCs and umbilical cord blood-derived MSCs are already used to create engineered cartilage repair products, and dermis-derived MSCs and precursor cells have chondrogenic differentiation potential[115,116]. Other types of MSCs and progenitor cells are emerging as candidates for use in tissue engineering. For example, peripheral blood-derived MSCs and endothelial progenitor cells have both been used to fill osteochondral defects in rabbits[117,118]. In a non-controlled, clinical pilot study with 15 participants, adult $CD146^+$ cartilage progenitor cells formed hyaline-like cartilage when implanted into knee articular cartilage defects[119]. After 12 months, the improvement in the International Knee Documentation Committee (IKDC) score was 52% and the improvement in the Lysholm score was 71% compared with preoperative scores[119]. Notably, hypertrophy frequently occurs in MSCs during in vitro chondrogenic differentiation[120], indicating the possibility that MSC-derived neocartilage might progress towards endochondral ossification[121], resulting in neotissue that is not suitable for cartilage repair and regeneration. Thus, despite promising early data, the long-term (>1 year) durability of MSC-derived tissues remains to be investigated.

Biochemical stimuli. Growth factors have long been recognized as important factors in neocartilage formation[122], but other molecules are emerging as potential modulators of engineered cartilage. In the past few years, hyaluronic acid has been shown to stimulate chondrogenesis and reduce hypertrophy in bone marrow-derived MSCs[123] and in a co-culture of adipose-derived MSCs and chondrocytes[124]. Similar effects have also been shown for the addition of matrilin 3 to cultures of bone marrow-derived MSCs[125]. The addition of kartogenin induced chondrogenic differentiation in MSCs and reduced type II collagen breakdown by 1.8-fold in a mouse model of OA[126]; however, the therapeutic dose and long-term in vivo efficacy of kartogenin have yet to be determined, limiting its use[127]. Biophysical stimuli such as glycosaminoglycan-depleting enzymes (such as chondroitinase ABC) or crosslinking agents (such as lysyl oxidase-like 2 (LOXL2)) have also been used to increase collagen content and to form collagen crosslinks, leading to improved tensile properties in neocartilage[128–130]. In fact, a regimen of TGFβ1, chondroitinase ABC and LOXL2 applied after aggregate redifferentiation generated neocartilage with tensile modulus and ultimate tensile strength values approximately twice those of untreated neocartilage[99]. Oxygen tension also has an important role in chondrogenesis and in improving neotissue functional properties. In one study, hypoxia upregulated *LOX* expression in chondrocytes by 18-fold, leading to an increase in tensile stiffness of neocartilage by ~80% compared with neocartilage formed under normoxic conditions[131]. Overall, these studies suggest that novel biochemical and biophysical stimuli should be used for effective neocartilage formation.

Biomechanical stimuli. Biomechanical stimuli such as compression, shear and hydrostatic pressure are important for cartilage homeostasis and are already used to improve the properties of engineered cartilage[132]. One advance in the use of biomechanical stimuli in tissue engineering has been the application of these stimuli to non-articular chondrocytes. Passive axial compression applied to costal chondrocytes increased the instantaneous modulus of engineered constructs by up to 92% compared with unstimulated neocartilage constructs[133]. Tension has also been trialled as an additional stimulus to improve the biomechanical properties of neocartilage. Tension stimulation of scaffold-free neocartilage treated with TGFβ1, chondroitinase ABC and LOXL2 resulted in increases of almost six-fold in tensile modulus and strength[99]. After in vivo implantation, these constructs had 90% of the collagen content and up to 94% of the tensile properties of native tissue[99]. A combination of compression and shear has also been tested, and resulted in a substantial increase in type II collagen production by chondrocytes in engineered neocartilage[134]. The results of these studies suggest that biomechanical stimulation has a pivotal role in engineering functional cartilage tissue in vitro. Understanding biomechanical stresses in the native environment of the joint, as well as their effects on both the generation of robust neotissue in vitro and the generated tissue in vivo, is important for achieving clinical translation of engineered cartilage.

Engineering menisci

Cell sources. Although meniscal fibrochondrocytes might seem to be an obvious choice for engineering the meniscus, co-culturing these cells with others might be required to achieve the best results. Similar to chondrocytes, meniscal fibrochondrocytes dedifferentiate when expanded[135], a fact that has led to the investigation of MSCs from the bone marrow[136], synovium[137] and adipose tissue as alternative cell sources[138]. In a 2017 study,

International Knee Documentation Committee (IKDC) score
A scoring system used to measure symptoms, sports and daily activities, current knee function and function before injury.

COL1A1, *COL2A1*, *ACAN* and *SOX9* were induced in tonsil-derived MSCs, and the feasibility of using these cells to repair meniscus defects was shown in rabbits[139]. Co-culture of synovium-derived stem cells and meniscus cells at a ratio of 1:3 increased glycosaminoglycan production by ~82% compared with stem cell monoculture and by ~33% compared with meniscus cell monoculture[140]. These findings echo those of studies investigating the formation of neomenisci using co-cultures of chondrocytes and differentiated cells[141] (such as tenocytes, ligament fibrocytes or meniscus fibrochondrocytes). For example, neomenisci formed using 50% articular chondrocytes and 50% meniscal fibrochondrocytes contain 700% more glycosaminoglycan and 90% more collagen than neomenisci formed using fibrochondrocytes alone[97]. The identification of new cell sources, as well as the optimization of co-culture systems, will both be important for overcoming the hurdles of cell culture for meniscus tissue engineering.

Biochemical stimuli. Growth factors including members of the TGFβ family, fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs) and epidermal growth factor (EGF) have shown efficacy in improving extracellular matrix production in engineered meniscus[88]. The addition of TGFβ1 and FGF2 stimulated collagen synthesis in meniscus constructs by 144% and 60%, respectively, compared with untreated constructs, although only TGFβ1 was effective in stimulating glycosaminoglycan production[142]. Growth factors have also been used to induce lubrication in engineered menisci; the use of insulin-like growth factor 1 localized lubricin to the neotissue surface and resulted in a coefficient of friction of ~0.2 (REF.[143]). Zonal development can also be engineered using growth factors. Modulating the release of TGFβ3 and connective tissue growth factor using 3D-printed scaffolds resulted in MSC-derived menisci with zone-specific *COL1A1* and *COL2A1* expression, as well as zone-specific production of type I and type II collagen[144]. Other biochemical stimuli can also aid the production of engineered menisci with improved functional properties. Treatment of neofibrocartilage implants with a combination of TGFβ1, LOXL2 and chondroitinase ABC increased collagen crosslink formation by 3.8-fold compared with untreated implants[103]. Upon implantation, the tensile strength of the interface of native meniscus and treated neofibrocartilage increased by 745% compared with the in vitro properties of untreated implants[103]. By contrast, changes in oxygen tension have yielded mixed results for engineering menisci. A 2017 study showed increased *ACAN* and *COL2A1* expression, as well as proteoglycan and type II collagen production by expanded human meniscus fibrochondrocytes under hypoxic conditions[145], whereas a 2013 study showed that normoxic conditions resulted in increased expression of *COL2A1* and *ACAN*, as well as the production of type II collagen and aggrecan by expanded human fibrochondrocytes compared with hypoxic conditions[146]. Therefore, modulation of oxygen tension as a biochemical stimulus might hold promise for meniscus engineering[130], but further investigations are needed to identify optimal culture conditions.

Biomechanical stimuli. The meniscus functions under compression, which results in the development of tensile hoop stress, therefore both of these mechanical forces are important for meniscus engineering. For example, using a compressive regimen of 10% strain at 1 Hz (which also results in tension), the collagen content, circumferential tensile modulus and radial tensile modulus of neomeniscus constructs can be increased compared with unstimulated constucts[147]. Over the past few years, studies of the development of biomechanical stimuli for meniscus engineering have focused on replicating the native zonal arrangement and matrix-level organization. For example, application of sinusoidal hydrostatic pressure between 0.55 and 5.03 MPa at 1 Hz for 4 h per day to aggregates of human fibrochondrocytes resulted in a substantial difference in type II collagen production between inner and outer zone meniscus fibrochondrocytes[148], providing support for the use of this stimulus to help recapitulate zonal architecture. A bioreactor applying 5–10% compressive strain was used to produce neomenisci with a fibrous collagen matrix in the outer zone that was similar in alignment to native tissue[149]. Investigations into how biomechanical stimuli can induce anisotropy in other engineered fibrocartilages have also been informative for meniscus engineering. For example, the application of passive axial compression during culture promoted anisotropic collagen organization similar to that seen in native tissue in tissue-engineered temporomandibular joint discs[150]. In addition to recapitulating native tissue biochemical and biomechanical properties, it is important to mimic other native features such as anisotropy and zonal organization because these structural features are necessary for meniscus function.

Clinical studies

The technologies used to produce cell-based repair products for articular cartilage repair have been reviewed elsewhere[151]. This section focuses on the clinical applications of articular cartilage and meniscus repair products in development (TABLE 1) and promising results from clinical trials of these products (TABLE 2). Acellular, scaffold-based products are not discussed. Additional clinical studies that have been performed under Institutional Review Board approval and in accordance with the principles of the Declaration of Helsinki, but not as part of registered clinical trials, are listed in Supplementary Table S1.

The majority of engineered cartilage products in the clinical pipeline, such as NOVOCART 3D and NeoCart, are manufactured using expanded autologous chondrocytes (TABLE 1). Because chondrocytes dedifferentiate upon in vitro expansion, products derived from expanded chondrocytes are likely to have inferior biomechanical properties to those of native tissue. Strategies such as the application of hydrostatic pressure have been developed to recover the chondrogenic phenotype. These strategies have resulted in articular cartilage repair implants that produce early-stage clinical improvements, but the long-term success and durability of these implants remains to be seen.

RevaFlex and CARTISTEM are both manufactured using allogeneic cells (TABLE 1). In a phase I/II

Table 1 | **Cell-based tissue-engineered products for articular cartilage and meniscus repair**

Product name (company)	Cell or tissue source	Seeding density	Biomaterial or scaffold	Stimuli	Time between operations (time in culture)	No. of patient operations	Refs
Articular cartilage							
BioCart II (ProChon Biotech)	Autologous chondrocytes (passage number unknown)	0.4×10^6 cells plus 0.1×10^6 cells/cm^2 of scaffold	Freeze-dried fibrin–hyaluronan	Autologous serum and FGF2	3–4 weeks (3–4 days in 3D culture)	2	172,173
BioSeed-C (BioTissue SA)	Expanded autologous chondrocytes (passage number unknown)	20×10^6 cells per scaffold	Fibrin, polyglycolic acid, polylactic acid and polydioxanone	Autologous serum	4–5 weeks	2	174–176
BST-CarGel (Piramal Healthcare (Canada))	Autologous whole peripheral blood	3:1 ratio of autologous whole peripheral blood to biomaterial	Dissolved chitosan in glycerophosphate buffer	Unknown	n/a	1	177
CaReS (Arthro Kinetics Biotechnology)	Primary autologous chondrocytes	Unknown	Type I collagen hydrogel	Autologous serum	2 weeks (10–13 days in 3D culture)	2	178
Cartilage autograft implantation system (CAIS) (DePuy Mitek)	Autologous cartilage fragments	1–2 mm minced cartilage dispersed onto scaffold	Absorbable co-polymer of 35% polycaprolactone and 65% polyglycolic acid with a polydioxanone mesh	Unknown	n/a	1	179
Cartipatch (TBF Genie Tissulaire)	Expanded autologous chondrocytes (passage 3)	10×10^6 cells/ml of hydrogel	Agarose–alginate	Autologous serum	6-7 weeks	2	180,181
CARTISTEM (Medipost)	Expanded, allogeneic, umbilical cord blood-derived MSCs (passage number unknown)	500 µl of hydrogel per cm^2 of defect area, 5×10^6 cells/ml of hydrogel	Hyaluronic acid hydrogel	Fetal bovine serum	n/a	1	153
co.don Chondrosphere (co.don AG)	Expanded, autologous chondrocytes (passage number unknown)	10–70 spheroids/cm^2 of defect area or ~3×10^6 cells/cm^2 of defect area	Scaffold-free	Autologous serum	~5–10 weeks	2	182,183
HYALOFAST (Anika Therapeutics)	Autologous BMAC	2 ml BMAC per scaffold	Benzyl ester of hyaluronic acid (HYAFF-11)	Unknown	n/a	1	184
HYALOGRAFT C (Anika Therapeutics)	Expanded autologous chondrocytes (passage 1 or passage 2)	$1.5–4 \times 10^6$ cells per scaffold	Benzyl ester of hyaluronic acid (HYAFF-11)	Autologous serum and TGFβ1	4 weeks (2 weeks in 3D culture)	2	185–188
INSTRUCT (CellCoTec B.V.)	Autologous, primary articular chondrocytes and bone marrow-derived cells	Unknown	Poly((ethylene oxide) terephthalate-co-poly(butylene) terephthalate)	Unknown	n/a	1	189
NOVOCART 3D (Aesculap Biologics)	Expanded autologous chondrocytes (passage 1)	0.5–3 × 10^6 cells/cm^2 of scaffold	Type I collagen and chondroitin sulfate	Autologous serum	3 weeks (2 days in 3D culture)	2	190
NOVOCART Inject (TETEC AG)	Expanded autologous chondrocytes (passage number unknown)	Unknown	In situ polymerized injectable albumin–hyaluronic acid hydrogel	Autologous serum, BMP2 and insulin	Unknown (3–4 weeks in 2D culture)	2	157

Table 1 (cont.) | **Cell-based tissue-engineered products for articular cartilage and meniscus repair**

Product name (company)	Cell or tissue source	Seeding density	Biomaterial or scaffold	Stimuli	Time between operations (time in culture)	No. of patient operations	Refs
Articular cartilage (cont.)							
NeoCart (Histogenics)	Expanded autologous chondrocytes (passage number unknown)	12×10^6 cells/ml collagen solution	Bovine type I collagen	Hypoxia and hydrostatic pressure	6–12 weeks	2	191–193
N-TEC (BIO-CHIP)	Expanded autologous nasal chondrocytes (passage number unknown)	50×10^6 cells per membrane	Type I and type III collagen membrane (Chondro-Gide)	• Autologous serum, FGF2 and TGFβ1 (expansion) • Autologous serum, insulin and ascorbic acid 2-phosphate (3D culture)	≥7 weeks (2 weeks in 2D culture and 2 weeks in 3D culture)	2	194
RevaFlex (ISTO Technologies)	Expanded allogeneic juvenile chondrocytes (passage number unknown)	Unknown	Scaffold-free	Unknown	n/a	1	152
Meniscus							
Chondrogen (Mesoblast)	Expanded allogeneic adult bone marrow-derived MSCs (passage 2)	25×10^6 or 75×10^6 cells/ml of sodium hyaluronate	Sodium hyaluronate	Fetal bovine serum (expansion)	n/a	1	159
Cell Bandage (Azellon)	Expanded autologous bone marrow-derived MSCs (passage 1)	1×10^6 cells/cm^2 of scaffold	Collagen sponge from bovine corium	Fetal bovine serum and FGF (expansion)	>2 weeks (6 h in 3D culture)	2	158

Acellular, scaffold-based products are not included. The term 'chondrocytes' refers to articular chondrocytes unless otherwise specified. The sponsors and products listed here might since have been acquired by other companies. BMAC, bone marrow aspirate concentrate; BMP2, bone morphogenic protein 2; FGF, fibroblast growth factor; MSC, mesenchymal stem cell; n/a, not applicable; TGFβ1, transforming growth factor β1.

study, chondral defects treated with RevaFlex had grossly 'normal or nearly normal' cartilage repair (as measured by the International Cartilage Repair Society (ICRS)-Cartilage Repair Assessment System) with no signs of immunological response after 1 year in 66.7% of patients treated[152]. In a study in South Korea, treatment of chondral lesions with CARTISTEM improved clinical outcomes compared with preoperative scores and there were no signs of bone or tumour growth up to 7 years after surgery[153]. CARTISTEM has completed a phase I/IIa study in the USA[154]. The successful clinical outcomes of allogeneic therapies to date open up a new avenue for eliminating donor site morbidity and the extra surgical step of tissue harvest when treating cartilage lesions.

Although engineered cartilage products in the clinical pipeline are primarily indicated for knee defects, several products have also been used in the hip (TABLE 2). Treatment of acetabular chondral defects with BST-CarGel improved International Hip Outcome Tool (iHOT) scores by 46% in a retrospective case series of 37 patients[155]. In a prospective study of 13 patients, treatment of acetabular chondral delamination (average defect size 3.7 cm^2) with BST-CarGel resulted in over 90% filling by volume of each chondral defect after 2 years[156]. In another study, the application of either NOVOCART 3D Inject or co.don Chondrosphere to acetabular cartilage defects (average size 2.21 cm^2) produced substantial improvements in activity and quality of life and reduced pain after a mean of 19 months[157].

Compared with articular cartilage, few clinical trials have been carried out with engineered meniscus products (TABLE 2). For example, Cell Bandage, which is composed of autologous bone marrow-derived MSCs embedded in a collagen sponge, is placed between the torn edges of the meniscus and the defect is sutured closed. It is thought that the MSCs embedded in Cell Bandage release growth factors that promote defect repair[158]. In a first-in-human study, Cell Bandage improved IKDC scores by ~40 points, the Tegner–Lysholm score by ~40 points and the range of motion (ROM) score by ~10 degrees at 12 months after surgery, and these results were maintained at 24 months[158]. In another study, Chondrogen injections containing 50 million or 150 million allogeneic bone marrow-derived MSCs also substantially decreased patient-reported visual analogue scale pain scores for up to 24 months[159]. Although meniscus repair products are not as numerous as articular cartilage products and fewer clinical trials have been performed, preliminary clinical data suggest positive outcomes for cell-based therapies.

International Cartilage Repair Society (ICRS)-Cartilage Repair Assessment System
A tool used to macroscopically evaluate the quality of cartilage repair tissue.

International Hip Outcome Tool
A tool used to measure symptoms, functional limitation, work-related concerns, sports and recreational activities, and social, emotional and lifestyle concerns using a visual analogue scale.

Tegner–Lysholm score
A patient-reported score of the effect of knee pain and stability on daily life.

Range of motion (ROM) score
A measurement of the range of flexion and extension of a joint.

Table 2 | **Clinical trials of cell-based tissue-engineered products for cartilage and meniscus repair**

Product (company)	Clinical status	Study location	No. of patients	Clinical indication	Comparator	Outcomes	Refs
Articular cartilage							
BioCart II (ProChon Biotech)	Phase II (status unknown)	USA and Israel	40 (estimated)	Single, contained cartilage defect on the femoral condyle of the knee (1.5–7.5 cm^2, depth up to 6 mm)	Microfracture	Results not published	195
BioSeed-C (BioTissue SA)	Phase III (ongoing)	Germany	80	Focal, contained, full-thickness cartilage defect on the lateral and medial condyles of the knee (Outerbridge grade III–IV)	chondrotissue (BioTissue SA)	Results not published	196
	Non-interventional study (completed 2016)	Germany	76 (target)	Focal cartilage defects on the femoral condyles, trochlea and patella of the knee (>2 × 2 cm and Outerbridge grade III–IV) that have been previously treated with BioSeed-C	None	Results not published	197
BST-CarGel (Piramal Healthcare (Canada))	Phase IV (terminated)	Canada and Europe	5	Single, focal, full-thickness cartilage defect on the femoral condyle of the knee (1.5–3 cm^2 and ICRS grade III–IV)	Microfracture	Results not published	198
	Phase III (status unknown)	Unknown	50 (estimated)	Focal chondral defects of the hip (>2 cm^2)	Microfracture	Results not published	199
	RCT (completed 2011)	Canada, South Korea and Spain	80	Focal cartilage defect on the medial femoral condyle of the knee (grade III–IV, unknown scoring system)	Microfracture	• Improved lesion filling and quality of repair tissue superior to microfracture alone at 12 months • Equivalent WOMAC scores and comparable safety outcomes between groups at 12 months	177
	Observational study (completed 2014)	Canada and Spain	67	Focal cartilage defects on the femoral condyle of the knee (ICRS grade III–IV or Outerbridge grade III–IV)	Microfracture	• Improved lesion filling and quality of repair tissue superior to microfracture alone at 5 years • No difference in WOMAC scores and comparable safety outcomes between groups at 5 years	200
Cartilage autograft implantation system (CAIS) (DePuy Mitek)	Phase III (status unknown)	Singapore	36 (estimated)	Full-thickness cartilage defect on the femoral condyle or trochlea of the knee (2–10 cm^2)	Microfracture	Results not published	201
	Clinical trial (terminated)	USA and Canada	75	One or two focal chondral defects (1–10 cm^2, depth up to 6 mm) or a non-osteochondritis dissecans lesion between grades I and III or an osteochondritis dissecans lesion between grades I and IV	Microfracture	Results not published	202
CARTIPATCH (TBF Genie Tissulaire)	Phase III (terminated)	Belgium	40	Isolated femoral osteochondral defect (2.5–7.0 cm^2, maximum depth of 10 mm, ICRS grade III–IV)	Microfracture	Results not published	203
	Phase III (completed 2013)	Belgium	64	Single femoral osteochondral defect (2.5–7.0 cm^2, maximum depth 10 mm, ICRS grade III–IV)	Microfracture	Results not published	204

Table 2 (cont.) | **Clinical trials of cell-based tissue-engineered products for cartilage and meniscus repair**

Product (company)	Clinical status	Study location	No. of patients	Clinical indication	Comparator	Outcomes	Refs
Articular cartilage (cont.)							
CARTIPATCH (TBF Genie Tissulaire)	Phase III (completed 2013)	France	47	Isolated femoral osteochondral defect (2.5–7.5 cm^2, ICRS grade III–IV)	Mosaicplasty	• Decreased IKDC score compared with mosaicplasty at 24 months • Decreased O'Driscoll score compared with mosaicplasty at 24 months	181
	Phase II (completed 2006)	France	17	Isolated chondral or osteochondral defect on the femoral condyles of the knee (1–5 cm^2, ICRS grade III–IV)	None	• Increased IKDC score at 24 months compared with baseline • 81% defect fill observed by MRI at 24 months	180
CARTISTEM (Medipost)	Phase I/II (completed 2017)	USA	12	Single, focal, full-thickness cartilage defect of the knee (≥2 cm^2, ICRS grade III–IV)	None	Results not published	154
	Phase III (completed 2015)	South Korea	103	Cartilage defect of the knee (2–9 cm^2, ICRS grade IV)	Microfracture	Results not published	205
	Phase III (completed 2011)	South Korea	104	Cartilage defect of the knee (2–9 cm^2, ICRS grade IV)	Microfracture	Results not published	206
	Phase I/II (completed, date unknown)	South Korea	7	Full-thickness cartilage defects of the knee (>2 cm^2, Kellgren–Lawrence grade III and ICRS grade IV)	None	• Maturing repair tissue by arthroscopy reported at 12 weeks • Improved VAS pain score and IKDC score at 24 months compared with pre-transplantation scores • Regenerated cartilage detected by MRI at 36 months • Improved outcomes stable and no signs of osteogenesis or tumorigenesis at 7 years	153
co.don Chondrosphere (co.don AG)	Phase III (active, not recruiting)	Germany and Poland	102	Isolated single chondral defect on the femoral condyle of the knee (1–4 cm^2, depth up to 6 mm, ICRS grade III–IV)	Microfracture	Results not published	207
	Phase II (completed 2018)	Germany	75	Isolated single chondral defect or osteochondritis dissecans lesion on the femoral condyle, trochlea, tibia or retropatella (4–10 cm^2, depth up to 6 mm, ICRS grade III–IV)	Different doses of co.don Chondrosphere	No substantial differences in the incidence of adverse events reported between the different doses	208
HYALOFAST (Anika Therapeutics)	Prospective study (recruiting)	USA and Europe	200 (estimated)	Cartilage defect on the femoral condyle or trochlea (1–6 cm^2, ICRS grade III–IV)	Microfracture	Results not published	209
INSTRUCT (CellCoTec B.V.)	Prospective study (completed 2014)	Europe	40	Cartilage defect on the femoral condyle and trochlea of the knee (modified Outerbridge grade III–IV)	None	• Graft delamination reported in two patients leading to treatment failure in one patient • ~90–100% defect filling at 24 months • Improved VAS pain score and IKDC score at 24 months compared with baseline • Improved KOOS at 12 months compared with baseline • Histological presence of hyaline cartilage in 72% of tissue samples and fibrocartilage and hyaline cartilage in 97% of tissue samples • Presence of repair tissue detected by MRI at 12 months	189

Table 2 (cont.) | **Clinical trials of cell-based tissue-engineered products for cartilage and meniscus repair**

Product (company)	Clinical status	Study location	No. of patients	Clinical indication	Comparator	Outcomes	Refs
Articular cartilage (cont.)							
NOVOCART 3D and NOVOCART 3D Plus (Aesculap Biologics, TETEC AG)	Phase III (recruiting); NOVOCART 3D	USA	30 (estimated)	Patients in whom microfracture failed in a previous trial	None	Results not published	210
	Observational study (active, not recruiting); NOVOCART 3D	Germany	81	Localized, full-thickness cartilage defect of the knee (2.5–10 cm^2, ICRS grade III–IV)	None	Results not published	211
	Phase III (recruiting); NOVOCART 3D	USA and Canada	233 (estimated)	Isolated cartilage defects on the femoral condyle of the knee (2–6 cm^2)	Microfracture	Results not published	212
	Phase III (active, not recruiting); NOVOCART 3D Plus	Europe	263	One or two cartilage defects on the femoral condyle and/or the trochlea of the knee (2–6 cm^2, ICRS grade III–IV)	Microfracture	Results not published	213
NOVOCART Inject and NOVOCART Inject Plus (TETEC AG)	Phase III (recruiting); NOVOCART Inject Plus	Europe	100	One or two focal cartilage defects on the femoral condyle, trochlea, patella or tibial plateau of the knee (4–12 cm^2, ICRS grade III–IV)	None	Results not published	214
	Non-interventional study (recruiting); NOVOCART Inject	Germany	245 (estimated)	'Insulated' full-thickness cartilage defects of the knee (2.5–10 cm^2, ICRS grade III–IV)	None	Results not published	215
	Observational study (active, not recruiting); NOVOCART Inject	Germany	21	'Insulated' full-thickness cartilage defects of the hip (1.5–10 cm^2, ICRS grade III)	None	Results not published	216
NeoCart (Histogenics)	Phase III (active, not recruiting)	USA	245	Cartilage defect of femur and/or trochlea of the knee	Microfracture	Results not published	217
	Phase II (completed 2014)	USA	30	Cartilage defect on the femoral condyle of the knee (ICRS grade III)	Microfracture	• No difference in adverse event rates between groups • Greater improvement in KOOS, IKDC and VAS pain scores at 6, 12 and 24 months compared with microfracture • Improved MOCART scores at 24 months compared with scores at 3 months • Improved KOOS, SF-36 and IKDC scores at 5 years compared with baseline • Decreased VAS pain score and improved range of motion at 5 years compared with baseline	191,218
	Phase I (completed, date unknown)	USA	8	Full-thickness cartilage defect on the femoral condyle of the knee (grade III, unknown scoring system)	None	• Improved VAS pain score at 12 months compared with baseline • Improved IKDC score and range of motion at 24 months compared with baseline • Six patients with 67–100% defect filling, one patient with 33–66% defect filling and one patient with <33% defect filling as determined by MRI • No arthrofibrosis or implant hypertrophy found	192

Table 2 (cont.) | **Clinical trials of cell-based tissue-engineered products for cartilage and meniscus repair**

Product (company)	Clinical status	Study location	No. of patients	Clinical indication	Comparator	Outcomes	Refs
Articular cartilage (cont.)							
N-TEC (BIO-CHIP)	Phase II (recruiting)	Europe	108 (estimated)	One or two localized cartilage defects on the femoral condyle and/or trochlea of the knee (2–8 cm^2, ICRS grade III–IV)	N-CAM (BIO-CHIP)	Results not published	113
	Phase I (completed 2018)	Switzerland	10	One or two cartilage defects on the femoral condyle and/or trochlea of the knee (2–8 cm^2, ICRS grade III–IV)	None	• No adverse events • Defect filling with repair tissue variable • Improved KOOS and IKDC scores at 24 months compared with pre-operative values • Approaching 'ideal level' of glycosaminoglycan content determined by ΔR_1 ($R_1 = 1/T_1$) and water and collagen contents 'similar to those in native tissue' at 24 months	194
RevaFlex (ISTO Technologies)	Phase III (terminated)	USA	14	One or two cartilage defects on the femur of the knee (≤5 cm^2)	Microfracture	Results not published	219
	Phase I/II (completed, date unknown)	USA	9	Up to two cartilage defects on the femoral condyle or trochlea of the knee (1–5 cm^2, ICRS grade III–IV)	None	• Improved patient-reported outcome measures at 12 months • Cartilage repair graded as grossly normal/near normal in 66.7% of patients at 12 months • Maturation of the implant (determined by defect filling and quality of repair tissue) observed by MRI at 12 months	152
Meniscus							
Chondrogen (Mesoblast)	Phase I/II (completed 2011)	USA	55	Following meniscectomy	Placebo (hyaluronan)	Results not published	220
	Phase I/II (completed 2008)	USA	55	Following meniscectomy	Placebo (hyaluronan)	• Three patients with >15% increase in meniscus volume in group receiving 50×10^6 cells, 0 in the control group and 0 in group receiving 150×10^6 cells at 24 months • Decreased VAS pain score and increased Lysholm score in all treatment groups at 24 months compared with baseline	159
Cell Bandage (Azellon)	Phase I (ongoing)	Europe	10	Meniscus tear that would otherwise be treated by meniscectomy (white–white zone)	None	Results not published	221

Acellular scaffold-based products are not included. The term 'Europe' refers to trials that took place in three or more European countries; if a trial took place in fewer than three European countries, all countries are listed. The sponsors and products listed here might since have been acquired by other companies. ICRS, International Cartilage Repair Society; IKDC, International Knee Documentation Committee; KOOS, Knee Injury and Osteoarthritis Outcome Score; MOCART, magnetic resonance observation of cartilage repair tissue; R_1, longitudinal relaxation rate; RCT, randomized controlled trial; SF-36, short form-36; T_1, longitudinal relaxation time; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Challenges to clinical translation

Cell sourcing

Obtaining sufficient numbers of autologous cells remains a major limiting factor to the translation of engineered articular cartilage and meniscus products (FIG. 4a). As previously noted, sourcing cells from non-articular cartilages, such as costal cartilage, might be a solution to the lack of autologous chondrocytes, although passaging might still be necessary with these cells. Expression of *COL1A1* and *COL2A1* by these cells decreases after just one passage[108], but although this collagen expression profile is undesirable for engineering articular

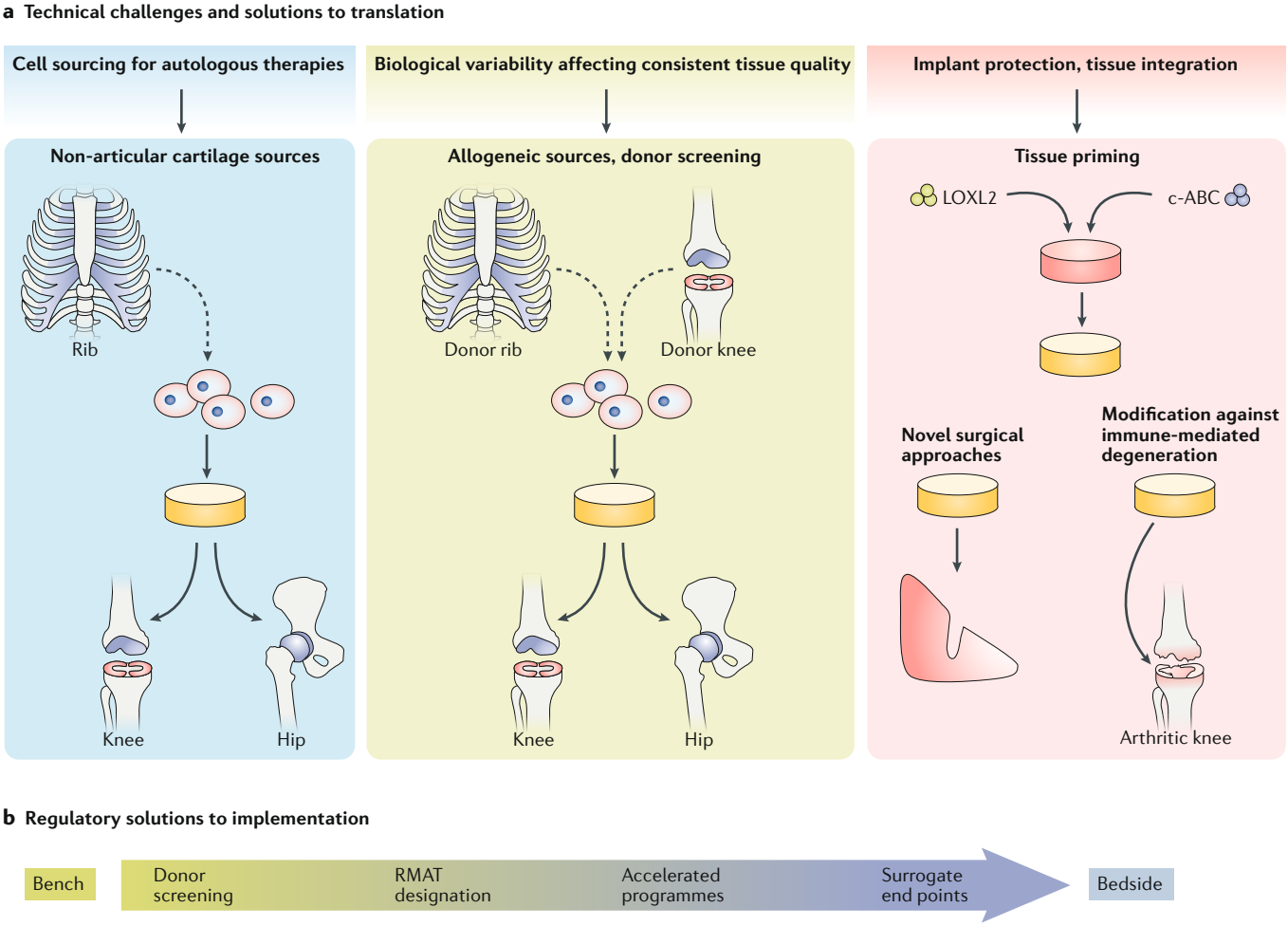

Fig. 4 | **Challenges to the clinical translation of engineered cartilage and meniscus products. a** | The main technical challenges to clinical translation include obtaining sufficient numbers of autologous cells, the effects of biological variability on the consistent production of high-quality engineered tissues and integration of the engineered tissues once implanted in vivo. Potential solutions and avenues of further investigation include: cells sourced from non-articulating cartilage (such as costal (rib) cartilage); allogeneic approaches, including extensive screening to identify appropriate donors; modification of engineered tissues to withstand immune-mediated degeneration within an inflamed joint; priming of engineered tissues with chondroitinase ABC (c-ABC) and lysyl oxidase-like 2 (LOXL2) for enhanced integration; and novel in vivo implantation methods that protect tissue-engineered implants. **b** | Regulatory challenges to clinical translation include the long time-frames and high costs associated with clinical trials. It is hoped that solutions such as the Regenerative Medicine Advanced Therapy (RMAT) designation, other FDA programmes that enable accelerated review and approval of applications, and the use of surrogate end points will help overcome these challenges.

cartilage, passaged cells that express *COL1A1* might still be useful in meniscus tissue engineering because native meniscus contains ~80% type I collagen in the red–red zone[88]. Furthermore, a spectrum of engineered cartilages from hyaline to fibrous can be engineered from costal chondrocytes by modulating their redifferentiation after passaging[160]. Innovative use of cells and non-articular cartilage cell sources has the potential to greatly alleviate the scarcity of cells for autologous articular cartilage and meniscus therapies.

Biological variability

Biological variability between donors makes the consistent production of high-quality autologous neotissue difficult to achieve (FIG. 4a). Not all donors possess cells capable of forming robust neotissue. For example, chondrocytes sourced from 64–80-year-old donors exhibited variable expression of chondrogenic genes at passage two[161]. In cells from one group of donors, *COL2A1* expression increased when the cells were cultured as a microtissue compared with monolayer culture, whereas in cells from another group of donors, *COL2A1* expression did not increase upon microtissue culture[161]. Using allogeneic cells would reduce problems related to donor variability during manufacturing, but the allogeneic implants would need to be well tolerated by the recipient. Several cartilage repair products already include allogeneic cells or tissues (TABLE 1). Lending further credence to this approach, healing of temporomandibular joint disc defects using allogeneic neocartilage has been achieved in mini-pigs[162]. In that study, costal chondrocyte-generated neocartilage implants were well

Tribological properties
Functional properties relating to friction and lubrication of tissues.

tolerated immunologically and resulted in a decrease in OA[162]. Although there is increased concern about disease transmission with the use of allogeneic approaches, tissue banks already provide allogeneic cells and tissues for transplantation in accordance with FDA guidance on donor screening and testing[163]. Thus, the use of well-characterized allogeneic cells might avoid disease transmission while mitigating the intractable problem of biological variability.

Achieving biomimicry

Insofar as the functions of articular cartilage and the meniscus are to distribute loads and enable frictionless joint movement, tissue engineering efforts should reflect these functions. Advances have been made in improving the robustness of engineered cartilage towards native tissue values; however, considerable efforts are still required to engineer tribological properties and durability into neocartilage and neomenisci to achieve biomimicry. It has been well documented that a functionality index (FI) enables comparison of the quality of engineered tissues relative to healthy native tissues[100,109,150]. However, to be more powerful, the FI should be modified to reflect the relevant salient properties of each target tissue, such as including the coefficient of friction for articular cartilage or an anisotropy index for the meniscus. Although complete biomimicry (FI = 1) in engineered cartilage has traditionally been the goal of tissue engineering approaches, a 2018 study[162] in which the implantation of engineered cartilage with an FI of 0.42 resulted in the complete healing of temporomandibular joint disc defects raises the question as to the degree of biomimicry necessary to achieve regeneration. It remains to be seen whether the achievement of biomimicry, especially with respect to biomechanical properties, imparts long-term durability to neotissue in vivo. Furthermore, no data exist to definitively show that the repair of articular cartilage and meniscus damage delays or halts the progression of OA. The ability of small defect repairs to stop OA progression would be difficult to assess in a well-controlled, randomized clinical trial owing to the need to include a no-treatment study arm and the long time-frames involved. Although evidence exists that neotissue with an FI of <1 elicits successful healing and that complete biomimicry might not be necessary[162], data on the long-term outcomes of using such an approach are lacking. Thus, it will be instructive to continue examining the degree of biomimicry necessary to ensure satisfactory long-term healing outcomes.

Implant integration and protection

The clinical translation of tissue-engineered products requires many factors to be taken into consideration beyond the manufacture of robust neotissue. Articular cartilage and the white–white zone of the meniscus are avascular, which makes integration of implants into existing native tissue difficult (FIG. 4a). The removal of anti-adhesive glycosaminoglycans and the priming of engineered tissue with collagen crosslinking agents are promising strategies that have shown preliminary success towards improving implant integration. For example, chondroitinase ABC treatment of native articular cartilage plugs before they are press-fitted into an articular cartilage annulus resulted in an integrated assembly with interfacial shear strength of 0.135 MPa, compared with 0.068 MPa in the untreated control[164]. In another study, LOXL2 treatment of similar assemblies of engineered cartilage and native cartilage rings resulted in a 2.2-fold increase in interfacial stiffness[165]. Implant integration can also be affected by postoperative recovery regimens. Unlike humans, animals operated on in preclinical studies will not obey strict rehabilitation regimens and might disrupt implant integration by engaging in impulsive physical activity immediately after surgery. Thus, in both animals and humans, the use of novel tissue-engineered implants might require novel surgical procedures that protect engineered implants and prevent implant displacement. For example, a reproducible intralaminar fenestration technique has been developed that enables engineered neocartilage to be secured into native tissue without directly suturing the implant[162]. Because implant integration, surgical techniques and rehabilitation all contribute to the efficacy of cartilage regeneration, developing appropriate protocols to address these factors should be as much of a priority for researchers as developing the implants themselves.

Inflammation and immunogenicity

Upon implantation, engineered neotissue must also withstand the pro-inflammatory environment of the injured or diseased joint. Chronic joint inflammation (as can be present in OA and RA) can be destructive to tissue-engineered implants and impede their integration and performance. Many studies have examined ways to ameliorate the immune response to ensure the survival of tissue-engineered implants in inflammatory environments, such as joints affected by OA and RA. Macrophage phenotypes can be modulated in vitro to promote healing and to potentially reduce inflammation in OA[166]. Other strategies to reduce inflammation, such as the use of adipose-derived MSCs to reduce matrix metalloproteinase 3 (MMP3) and MMP13 expression, also hold promise[167]. The rejection of allogeneic engineered cartilage and menisci is also a concern. Although articular cartilage is considered to be immune privileged, and fresh allografts (such as osteochondral allografts, DeNovo NT and meniscus allografts) are in current clinical use, the degree of immune privilege an implant has depends on its location within the knee joint and its proximity to the synovium[28]. Meniscus allografts are well tolerated, but it remains to be seen whether allogeneic neomenisci implanted into the vascular red–red zone of the meniscus would elicit an immune response. Osteochondral allografts are frequently used in articular cartilage repair and are well tolerated[33] despite the fact that the subchondral bone is vascularized, lending some support to the idea that red–red zone allografts might be tolerated. However, most irreparable meniscus defects that would require engineered meniscus grafts occur in the white–white zone, which does not contain vasculature. Thus, this area might also possess a degree of immune privilege, similar to articular cartilage, although the exact immune privilege status of the meniscus still

needs further study. Efforts to minimize the immunogenicity of allogeneic and xenogeneic articular cartilage and menisci include decellularization and antigen removal[168–170], but these methods typically create a disrupted matrix and non-viable cells, depriving the neotissue of the capacity for homeostasis, remodelling and integration. A variety of immunological challenges associated with cartilage and meniscus tissue engineering, such as the pro-inflammatory environment of arthritic joints and the antigenicity of allogeneic cells and matrix components, indicate that neotissue should be modified to be able to withstand or modulate the immune response to ensure graft survival and integration.

Regulatory concerns

Several regulatory hurdles surround the translation of engineered cartilage and meniscus products into patients (FIG. 4b). Clinical trials to examine the safety and efficacy of engineered cartilage and meniscus products in large patient populations are costly and time-consuming. Recognizing this, the FDA has announced a new policy framework to expedite the approval of new therapies while preserving public health via a risk-based approach. Special designations, such as the Regenerative Medicine Advanced Therapy (RMAT) designation, have been created to expedite the approval process[171]. Advantages of the RMAT designation include FDA assistance as early as the phase I trial stage, the discussion of potential surrogate or intermediate end points to accelerate approval and eligibility for priority review of marketing applications. The use of surrogate end points might accelerate time to market by shifting some of the burden of proof to post-market follow-up studies. The RMAT designation, as well as other special designations and accelerated programmes[171], might be solutions to reducing the cost and time required to gain marketing approval for engineered articular cartilage and meniscus products.

Conclusions

Current surgical repair techniques for articular cartilage and meniscus pathologies are insufficient to halt the development and progression of OA, which has accelerated the development of alternative tissue engineering strategies. Many advances have been made in cell sourcing and the use of stimuli to engineer neotissue akin to native articular cartilage and menisci, which can potentially provide long-term solutions for cartilage and meniscus healing. For example, the use of cells from allogeneic, non-articulating and/or diseased cartilage might counter the lack of native autologous cells. Although the goal of tissue engineering is to achieve biomimicry, tissue engineering approaches must also aim to create neotissue that withstands joint inflammation, readily integrates into surrounding native tissues and ensures positive outcomes regardless of biological variability and the age of the patient. The progression towards the use of cell-based tissue-engineered therapies in the clinic can be seen in the numerous clinical trials and Institutional Review Board-approved studies that are currently underway. Although most products are primarily indicated for use in the knee, many of the same engineering principles can be translated to the development of products for other joints such as the hip. The establishment of the RMAT designation should accelerate the regulatory process for these products. Rapidly emerging tissue engineering technologies could lead to the development of long-lasting products that are readily available off the shelf for articular cartilage and meniscus regeneration in the not-so-distant future.

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Competing interests
W.E.B. declares she is the Director of Outreach and a social media contributor for Science Cheerleaders, Incorporated. C.A.L. declares she is on the advisory board of Vericel. N.P. declares he is an associate editor of the Arthroscopy Journal. K.A.A. declares he is on the scientific advisory board of Histogenics. K.A.A., J.C.H., H.K. and W.E.B. declare they are listed as co-authors of submitted US patent applications (16/136,894 and 16/137,120). D.W. declares no competing interests.

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