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Review article

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Failure of cartilage regeneration: emerging hypotheses and related therapeutic strategies

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Abstract

Osteoarthritis (OA) is a disabling condition that affects billions of people worldwide and places a considerable burden on patients and on society owing to its prevalence and economic cost. As cartilage injuries are generally associated with the progressive onset of OA, robustly effective approaches for cartilage regeneration are necessary. Despite extensive research, technical development and clinical experimentation, no current surgery-based, material-based, cell-based or drug-based treatment can reliably restore the structure and function of hyaline cartilage. This paucity of effective treatment is partly caused by a lack of fundamental understanding of why articular cartilage fails to spontaneously regenerate. Thus, research studies that investigate the mechanisms behind the cartilage regeneration processes and the failure of these processes are critical to instruct decisions about patient treatment or to support the development of next-generation therapies for cartilage repair and OA prevention. This Review provides a synoptic and structured analysis of the current hypotheses about failure in cartilage regeneration, and the accompanying therapeutic strategies to overcome these hurdles, including some current or potential approaches to OA therapy.

Sections

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Key points

- Multiple pathways can cause cartilage regeneration to fail following injury, thereby leading to a cascade of events that ultimately results in a degenerative disease state.
- Various hypotheses for why cartilage regeneration fails exist relating to a lack of regeneration-competent cells, pathological mechanical changes, non-resolving inflammation and metabolic switches.
- Treatment strategies should not only consider the potential mechanisms underlying the initial failure in cartilage regeneration but also the stage of disease progression.
- The cause of entry into the cascade of events that prevent cartilage regeneration might not necessarily be the target point of exit of an ideal treatment strategy.

Introduction

Articular cartilage, typically described as 'hyaline' cartilage, facilitates load transmission and smooth articulation of bones more durably than any synthetic analogue, but has a limited capacity to regenerate[1]. Therefore, cartilage defects often fail to heal and progressively induce degenerative changes, ultimately leading to osteoarthritis (OA). The incidence and prevalence of cartilage defects are currently unknown[2], in part owing to the difficulty in identifying individuals with cartilage defects unless they are symptomatic and require treatment. However, the prevalence of knee OA is well studied and has been increasing steadily over the past 30 years, currently affecting 5% of the world population[3]. The increased prevalence of OA is only partly explained by population ageing and an increasing prevalence of obesity[4,5]. In 2022, OA was described by the FDA as a 'serious disease', enabling an accelerated approval pathway for new treatments[6].

Adequate, robustly effective approaches for the regeneration of cartilage injuries are imperative to prevent or delay the onset of OA. Despite extensive research and development, no surgery-based, material-based, cell-based or drug-based treatment can predictably and durably restore the structure and function of hyaline cartilage. This lack of treatments urges us to rethink why articular cartilage fails to regenerate. Fundamental research is required to mechanistically understand the causes of failed cartilage regeneration, as this knowledge is the strongest asset to control such processes and unlock opportunities for successful breakthroughs in cartilage restoration. Indeed, the development of next-generation therapies for cartilage repair and OA prevention, or the adoption of existing ones, should be inspired and guided by the formulation of hypotheses about why cartilage cannot efficiently regenerate.

Therefore, the purpose of this Review is to offer a synoptic, structured analysis of the field, whereby different possible hypotheses about cartilage regeneration failure are introduced, as well as some related therapeutic strategies. The primary objective of this Review is to analyse cartilage regeneration, which is defined as an induced reparative response that involves the formation of new cartilage in a defect. However, the transition from a focal, isolated cartilage lesion to end-stage, full-joint degenerated cartilage (as occurs in OA) is a continuum and several underlying biological processes (for example, related to inflammation or catabolism) might have common underlying features. For this reason, this work does not exclude from the discussion some of the approaches currently under assessment for cartilage regeneration in the context of OA.

Cellular failure

The low cell density of cartilage[7], in combination with the low proliferative capacity of mature chondrocytes[8], has often been proposed as the underlying cause of the limited regenerative potential of cartilage. In fact, the cell density and in vitro proliferative capacity of articular chondrocytes decreases with age (being notably lower after 30 to 40 years of age)[9], and cartilage cell density also decreases in the setting of post-traumatic OA[10]. Moreover, chondrocyte apoptosis is considered a hallmark of cartilage degeneration, but whether chondrocyte apoptosis is a cause or result of the disease is unclear[11]. Interestingly, compared with larger mammals, smaller mammals have a larger chondrocyte density[12] and greater cartilage regeneration capacity[13]. Cells with progenitor-like characteristics are present in healthy and osteoarthritic cartilage[14–16]. These cells have a high proliferation capacity and chondrogenic potential[17,18], but somehow, the progenitor-like cells generally do not manage to efficiently regenerate cartilage defects[18]. Hence, there is a strong rationale to enable cartilage regeneration through the delivery or inducing the recruitment of competent cells into cartilage defects, as targeted by some of the strategies discussed in this section and illustrated in Fig. 1.

Cell delivery

Autologous chondrocyte implantation (ACI) involves the delivery of de-differentiated culture-expanded chondrocytes into cartilage defects[19] and this approach leads to clinical improvement in approximately 85% of cases. However, this procedure often results in fibrocartilage formation or cartilage hypertrophy[20]. Furthermore, the complex logistics of this approach, such as the necessity for two surgeries, and the associated high patient burden, delayed rehabilitation and high costs, are considerable drawbacks of this treatment[21–23]. Cartilage progenitor cells (for example, from bone marrow or the synovial membrane) have been used to replace chondrocytes in ACI or matrix-assisted ACI techniques. Progenitor cell implantation results in effective filling of the defect in caprine[16], equine[24] and human pilot studies[18], and using these progenitor cells instead of chondrocytes allows for faster culture expansion[16,18]. However, the contribution of the delivered cells in forming and sustaining new cartilage, at a site where environmental conditions are not optimal for supporting chondrogenic differentiation, remains controversial. Various scaffold materials have been developed and applied to support delivered cells to the cartilage defect, functioning as enhanced configurations of the endogenous fibrin scaffold of the clot that naturally forms in microfractures[25]. Although various composite hydrogels and porous scaffolds are under investigation for their utility in matrix-assisted ACI[25], the biological or mechanical properties required to predictably improve clinical outcomes are not yet established[26].

To bypass the challenges of ensuring that cells reach a differentiated phenotype following implantation, as well as to protect the cells from inflammatory insults by including their extracellular matrix, various cartilage tissue engineering strategies have emerged and are a subject of ongoing debate[27]. For example, researchers have used nasal septum-derived chondrocytes to reproducibly engineer autologous hyaline-like cartilage grafts. This approach has shown promising clinical effectiveness[28], even in advanced stages of cartilage degeneration[29]; however, the results need to be verified in suitably powered controlled

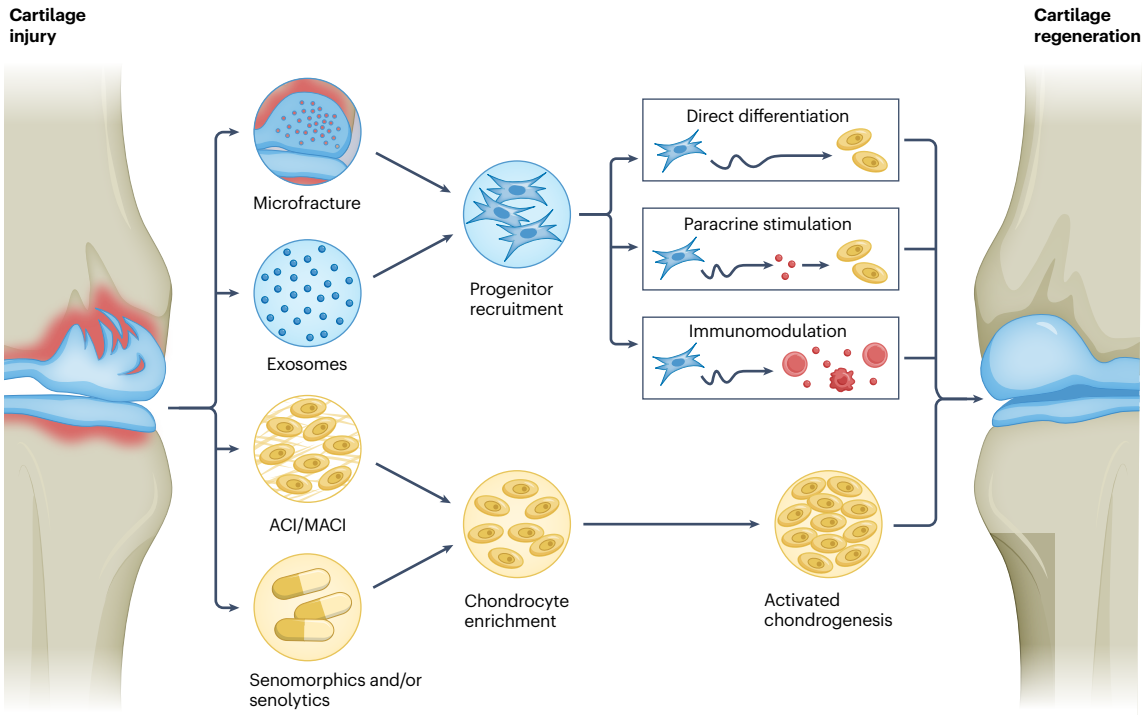

Fig. 1 | Postulated mechanisms of cellular-based strategies for cartilage regeneration. The ultimate goal of cell-based strategies for cartilage regeneration is to enrich the density of cells competent in activating or undergoing regeneration. These strategies involve either the recruitment of progenitor cells or the enrichment of chondrocytes. The recruitment strategy involves techniques such as microfracture (to promote recruitment of progenitor cells from the bone marrow to fill the defect) or exosome delivery (to promote the homing of progenitor cells that reside nearby). The recruited progenitor cells promote cartilage regeneration by modulating the inflammatory milieu, by inducing chondrogenesis through paracrine signalling, or by directly differentiating into chondrocytes. The chondrocyte enrichment strategy involves cell-delivery techniques such as autologous chondrocyte implantation (ACI) or matrix-assisted autologous chondrocyte implantation (MACI) and the use of agents that target senescence (such as senomorphic and senolytic drugs) to increase the longevity of the chondrocytes. The original version of this figure was created with BioRender.com.

and prospective trials. Moreover, the drawbacks of a two-stage surgery remain. An alternative approach that uses a single-stage procedure is the use of allogeneic mesenchymal stromal cells (MSCs) complemented with rapidly digested autologous chondrons (which includes the pericellular matrix of the isolated chondrocytes to protect the allogeneic MSCs); studies show that this approach has led to good outcomes in patients for up to 5 years[30,31].

Other single-stage techniques that deliver cells and tissues to cartilage defects are currently under investigation. For example, researchers have applied fragmented cartilage chips to cartilage defects, often in combination with orthobiologics[32]. Furthermore, the use of allogeneic, particulated juvenile articular cartilage has shown some clinical benefit in the short term[33]. The ease of these procedures and the simplified regulatory framework required for minimally manipulated products have pushed these treatments towards clinical practice, although results from clinical trials and mid-term to long-term follow-up data are lacking.

Apart from regenerating a neo-cartilage that mimics native characteristics, the integration of the neo-cartilage into the surrounding cartilage is crucial to provide normal stress distribution upon loading to prevent treatment failure in the short term and long term[34,35]. The hypocellularity of the surrounding cartilage, owing to cell death caused by the injury, might be counteracted through the use of engineered cartilage grafts that have a high cellularity and integrative potential[36] or through the use of these grafts in combination with various local treatments (including controlled enzymatic digestion approaches[37]) to enhance the migration of resident cells. These strategies could enhance the integrative potential of the neo-cartilage, developed by the cell delivery methods described above.

Cell recruitment

Microfracture is a method for treating cartilage injury that involves perforation of the subchondral bone to promote the filling of the defect with cells from the bone marrow, particularly MSCs with chondrogenic differentiation potential[38]. Although this technique has been used in the clinic for decades, microfracture typically leads to the formation of fibrocartilage instead of hyaline cartilage, which has inferior mechanical stability[20,39]. Therefore, microfracture has been abandoned as the gold standard for the treatment of cartilage defects over 2 cm^2 (ref. 40). Autologous matrix-induced chondrogenesis is an extension of this technique that combines microfracture with the use of an exogenous scaffold to contain the recruited progenitor cells at the prepared bed of the cartilage defect[41]. Various acellular materials are also available for treating lesions that extend to the subchondral bone; for example, MaioRegen or Agili-C™ are approved for clinical use to provide

immediate mechanical stability to the lesion while guiding cellular recruitment and repopulation of the defect[42–44].

To recruit resident progenitor cells from surrounding tissues towards the cartilage defect following injury to promote cartilage regeneration, the use of the secretome of MSCs or purified MSC-derived exosomes represent a potential cell-based but cell-free alternative[45]. The MSC secretome and associated exosomes include several chemokines and other signalling molecules that have the capacity to recruit endogenous progenitor cells[45]. The recruited progenitor cells could have immunomodulatory effects, could facilitate regeneration and chondrogenesis through paracrine stimulation, or could directly differentiate into chondrocytes[46,47] (Fig. 1). This method has resulted in favourable responses in animal models of cartilage injury[48]. The potential of this therapy needs further exploration in terms of large-scale cell isolation and characterization to enable safe clinical application[45]. The development of further approaches aimed at the activation of resident progenitor cells requires further understanding of the origin and fate of these cells[49]. With a deeper understanding of the role of cartilage progenitor cells in embryonic development and pathophysiology, more specific and effective cues to activate endogenous cells, possibly with off-the-shelf products, could be designed and adopted. Various fundamental studies are currently ongoing to address these questions.

Targeting senescence

Physiological age-related changes in chondrocytes result in accumulation of oxidative stress that disrupts cartilage homeostasis, in turn leading to increased stiffness and altered biological and biomechanical properties of the cartilage[50]. Similarly, ageing, by itself, is a confounding factor in the evaluation of the response to any treatment method[51]. Senescence, a cell state typically associated with ageing and degeneration, is hypothesized to have an important role in the inability of the cartilage to regenerate following injury.

Senescence is characterized by three major features: cell growth arrest, apoptotic resistance and a senescence-associated secretory phenotype[52,53]. The senescence-associated secretory phenotype has a deleterious effect on the surrounding cells and tissue by spreading the senescence phenotype and promoting cell stress. Interestingly, the transplantation of senescent cells into the knee joint can accelerate cartilage degeneration and the development of OA[54]. By contrast, selectively eliminating senescent cells in models of OA can delay or prevent disease development[55]. These findings suggest that senescence is causally linked to the inability of cartilage to regenerate. Studies have explored two main approaches to target senescence: senomorphic compounds, which modulate the secretome effect of senescent cells, decreasing and reverting their harmful effects; and senolytic drugs, which selectively remove senescent cells, allowing local tissue renewal and reinstatement of homeostasis[56]. In cartilage, both approaches have shown promising results in preclinical models[55]. Clinical studies are exploring the use of fisetin (a senolytic drug) for treating or alleviating OA progression[57]. Despite the optimism surrounding this new drug category, the first phase II trial of a senolytic compound (UBX0101) failed to reach the expected end point[58]. Thus, more studies are required to test the promise of senolytic compounds, including defining the optimal dose[59,60].

Mechanical failure

Abundant evidence suggests that mechanical changes within a joint contribute to cartilage loss[61–64]. Damage to articular cartilage through trauma or chronic degeneration alters the load-bearing contact area of the joint. These mechanical changes can lead to abnormal joint loading, the release of alarmins from osteoclasts, the activation of fibroblasts and macrophages and the production of pro-inflammatory mediators, ultimately inducing cartilage injury[65,66] (Fig. 2). The resulting pathological changes involve a progressive breakdown of the extracellular matrix and lead to fibrillation of the collagen network and synovial inflammation[67]. Such changes are particularly evident in joint areas that would otherwise be infrequently loaded[62,67]. Abnormal loading patterns can be aggravated by changes in the subchondral bone, which at later stages of degeneration can become sclerotic and stiff[68]. Previous research has shown that mechanical alterations can reduce the regenerative capacity of cartilage[69,70]. These cumulative mechanical changes are often further compounded with age-related metabolic changes and age-induced stiffness in the cartilage extracellular matrix, resulting in larger biological abnormalities[50]. As such, treatments that prevent subchondral bone sclerosis could be a potential pathway for protecting the regenerative potential of pathological cartilage[69].

The chondrocyte response to mechanical conditioning depends on multiple factors, such as loading magnitude, frequency and duration[61]. Thus, although abnormal mechanical exposure has been associated with the onset of degenerative changes[68], physiological cyclic compression can enhance chondrogenesis, extracellular matrix production and the ability of the tissue to regenerate[71]. Indeed, mechanical stimulation is one of the most important physiological stimuli for activating important signalling molecules associated with chondrocyte metabolism and cartilage homeostasis[63,64,68]. Hence, it is reasonable to hypothesize that a mechanical abnormality can not only induce cartilage failure but also reduce the efficiency of cartilage healing. From this perspective, different approaches to restoring appropriate mechanical loading have been integrated into cartilage regenerative strategies, as detailed in this section and illustrated in Fig. 2.

Joint realignment

On a gross anatomical scale, changes to the mechanical alignment accelerate cartilage degeneration, ultimately deforming the joint and effecting the joint kinematics and function[67]. On the basis that mechanical failure can hinder cartilage regeneration, researchers have developed a procedure known as high tibial osteotomy that involves cutting and reshaping the bone to surgically realign the joint; this technique has proven successful at correcting the load across a valgus or varus knee joint[72]. The use of osteotomies for rescuing cartilage from degeneration is thus recommended by the Osteoarthritis Research Society International as a joint-preserving treatment in young and symptomatic patients with OA[73]. This procedure has also been shown to enable the regeneration of cartilage tissue and slow down the progression of joint degeneration[74,75].

Joint distraction

Knee joint distraction is an alternative, less commonly practised surgical procedure to high tibial osteotomy that reduces loading forces on the joint by distracting it with an external fixation device (typically for a duration of 6 weeks). This intervention can increase the thickness of cartilage and improve collagen type II synthesis, suggesting that this approach facilitates the cartilage regeneration processes[76–78]. However, studies have reported that a large (>50%) incidence of pin tract infections can occur following knee joint distraction[78]. Although these cases are usually treated successfully with oral antibiotics[79], the lasting success of knee joint distraction remains unclear, as only small randomized controlled trials with short follow-up times have been reported to date (Table 1).

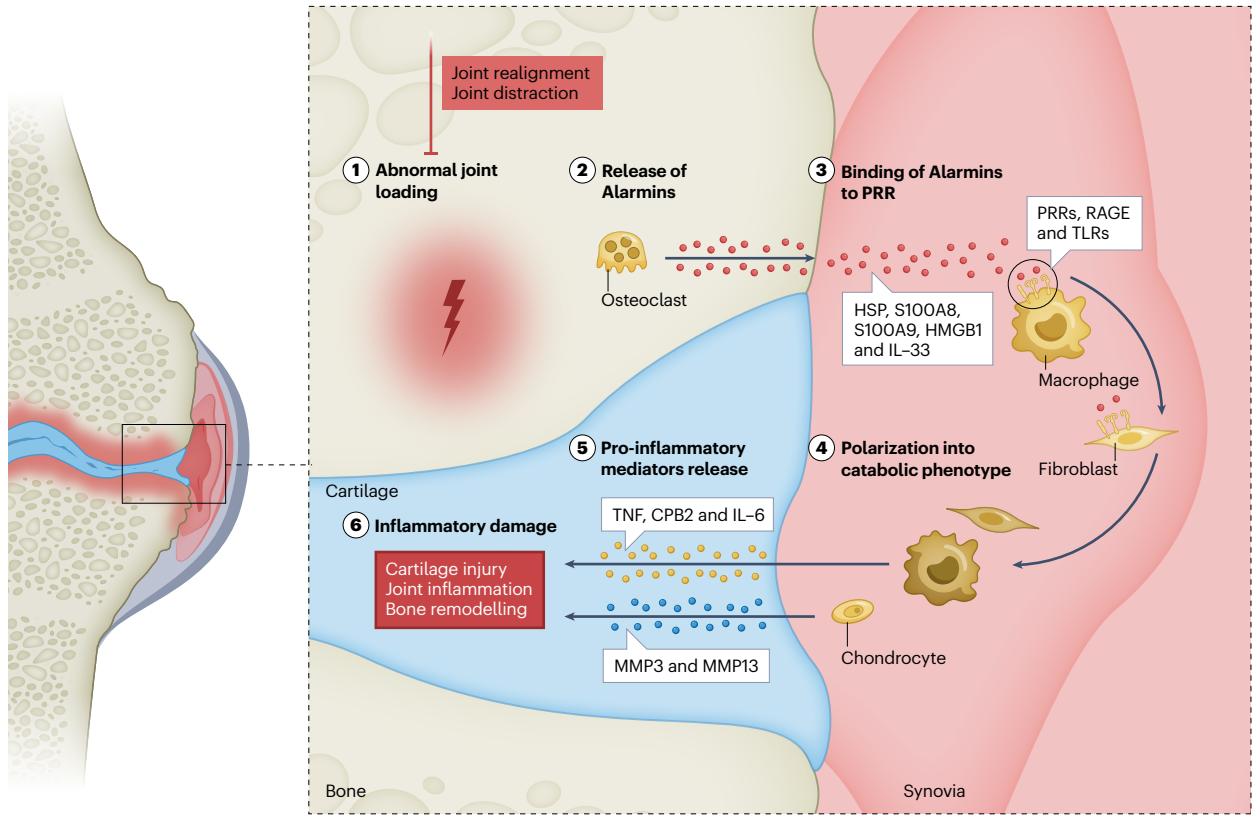

Fig. 2 | Postulated mechanisms of abnormal joint loading aggravating cartilage damage following injury. Mechanical imbalance following injury results in abnormal joint loading forces that activate the osteoclasts to release chemokine signals called alarmins (for example, heat shock proteins (HSPs), IL-33, S100A8, S100A9 and high mobility group box 1 (HMGB1)). These alarmins bind to pattern recognition receptors (PRRs), receptor for advanced glycation endproducts (RAGE) and Toll-like receptor (TLRs) on macrophages, resulting in M1 polarization of macrophages and the subsequent activation of fibroblasts that release pro-inflammatory mediators (such as TNF, carboxypeptidase B2 (CPB2), IL-6, matrix metalloproteinase 3 (MMP3) and MMP13), perpetuating cartilage injury and joint inflammation. The joint realignment and distraction strategies might counteract this cascade by restoring physiological joint loading. The original version of this figure was created with BioRender.com.

Although high tibial osteotomy and knee joint distraction have shown good clinical outcomes, our current understanding of the long-term regenerative properties of both procedures remains limited. Whether the cartilage regenerated post-intervention is fibrocartilage or hyaline and whether, if the latter, the development of fibrocartilaginous cartilage is caused by a suboptimal mechanical environment, is also unclear. These important factors warrant further investigation, as the cartilage type affects the durability of its performance. Developing methods to investigate the primary role of mechanical loading on cartilage cells in a native joint, coupled with the use of macroscopic biomechanical modelling to quantify the physical forces that act on defined regions, will be crucial to refining these surgical techniques, defining the ideal modes for implementation in patients and possibly enhancing their regenerative effect.

Inflammatory stress

Inflammation creates an environment that is detrimental to the optimal functioning of chondrocytes and cartilage regeneration[80]. Inflammatory stress impairs chondrocyte viability and matrix synthesis by these cells, and induces matrix catabolism through promoting the production of various matrix metalloproteinases (MMPs), interleukins (including IL-1α and IL-1β), and aggrecanases (such as a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) and ADAMTS5), resulting in characteristic histological features of cartilage degeneration[80]. The complexity of the inflammatory process, including the involvement of multiple inflammatory mediators, poses a challenge for therapeutically targeting key inflammatory signals that prevent the natural regenerative mechanisms[81]. Along with an increased understanding of these various inflammatory pathways, an increasing list of therapeutic targets is emerging that includes various protein kinases, sirtuins, neurotrophins and alarmins, as well as more well-established targets such as pro-inflammatory cytokines and matrix-degrading enzymes, that are being tested to counteract the inflammatory environment[81–84]. However, none of these targets has been clinically validated as an effective target for enabling cartilage regeneration. The strategies that are currently being investigated in this domain are illustrated in Fig. 3 and discussed in the next section.

Pharmacological therapy

Although inflammation is a common hallmark of cartilage degeneration[85], agents that selectively target inflammation have so far failed to restore cartilage homeostasis and activate regeneration.

Table 1 | Different hypotheses for why articular cartilage regeneration fails and potential therapeutic solutions

Hypothesis	Strategy	Intervention	Stage in translation	Limitation
Cellular failure	Cell delivery	ACI or MACI	Phase III clinical trial[22]	Two-stage procedure, high cost, complex logistics and availability issues
		ACPI	Pilot clinical study[18]	
		Processed cartilage with or without biologic drugs	Clinical use[30,32,33]	No randomized trials or mid-term to long-term follow-up data; clinical trial underway[154]
	Cell recruitment	Microfracture	Clinical use[40]	Lower success rate for defects >2 cm^2
		AMIC	Clinical use[41]	
		Cell-free scaffolds	Clinical use[42]	No randomized trials or mid-term to long-term follow-up data
		Progenitor recruitment	Lineage tracing studies and basic research[49]	Lack of clinical validation
		Exosomes	Preclinical in vivo study[155]	Difficult large-scale production, isolation and characterization
	Targeting senescence	Senomorphics	Preclinical in vivo study[147]	Low stability, low bioavailability and unexplored therapeutic protocols for dosage, starting timepoint, and duration of the treatment
		Senolytics	Preclinical in vivo studies (navitoclax and a combination of quercetin and dasatinib[55,59]); phase I clinical trials (fisetin and quercetin[57,153]); and phase II clinical trial (UBX0101[58])	Unexplored therapeutic protocols for dosage, starting timepoint and duration of the treatment
Mechanical failure	Joint realignment	High tibial osteotomy	Phase III clinical trials[72]	Only a current option for young, symptomatic patients
	Joint distraction	Knee joint distraction	Phase III clinical trials[78]	Only a current option for young, symptomatic patients; has a reportedly high rate of pin-tract infection
Inflammatory stress	Pharmacological therapy	Anti-inflammatory therapy	Phase I clinical trial (ADAMTS inhibitor[156]); phase II clinical trials (cathepsin K inhibitor[157], Wnt signal modulator[158] and MEPE derivative[159]); and phase II–III clinical trials (inhibitors of IL-1α and IL-1β[160,161]); preclinical in vivo study (MMP13 inhibitor[162])	Only evaluated for their symptomatic analgesic benefits in patients with osteoarthritis following cartilage injury (rather than earlier stages of injury)
		Pro-regenerative therapy	Preclinical in vitro study (tankyrase inhibitor[163]); phase I clinical trials (kartogenin[164] and ANGPTL3 derivative[165]); and phase III clinical trial (recombinant FGF18)[166]	Only test in patients with osteoarthritis following cartilage injury (rather than earlier stages of injury); lack of local delivery mechanisms
		SYSADOAs	Phase III clinical trial (glucosamine sulphate)[167]	Only evaluated for their symptomatic analgesic benefit in patients with osteoarthritis following cartilage injury (rather than earlier stages of injury)
	Gene therapy	Viral vector approaches	Phase I clinical trial (AAV-mediated delivery of IL-1 receptor antagonist cDNA)[168]; phase II clinical trial (delivery of TGFβ1[169])	Immunogenicity and cytotoxicity associated with the use of viral vectors.
		Non-viral approaches	Preclinical in vivo studies (FuGene 6-mediated delivery of IL-10 transgene[170], chitosan-graft-polyethylenimine nanoparticles deliver DNA to the joint[171], nanomicelle system for delivery of *RUNX1* mRNA[172] and scaffold-mediated delivery of *SOX5*, *SOX6* and *SOX9* (ref. 173))	Low stability and limited efficiency in delivering the drug to the target site
	Cellular therapy	MSCs	Phase III clinical trial[45]	Two-stage procedure, high cost, complex logistics and availability issues

Table 1 (continued) | Different hypotheses for why articular cartilage regeneration fails and potential therapeutic solutions

Hypothesis	Strategy	Intervention	Stage in translation	Limitation
Metabolic stress	Enzyme modulation	Hexokinase and PFK1 inhibitor	Preclinical in vivo study[122]	Short half-life, lack of local delivery mechanisms and systemic effects
	Mitochondrial modulation	Biogenesis	Preclinical in vivo study[125]	
		Dynamic homeostasis	Preclinical in vitro study[126]	
		Renewal	Preclinical in vivo study[127]	
	Antioxidant therapy	Enzymes	Preclinical in vivo study[139]	Unexplored therapeutic protocols for dosage, starting timepoint and duration of the treatment
		Non-enzyme compounds	Preclinical in vivo study[142]	
		Diet	Phase II clinical trial[145]	Controversial results in clinical trials

AAV, adeno-associated virus; ACI, autologous chondrocyte implantation; ACPI, autologous chondrocyte progenitor implantation; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AMIC, autologous matrix-induced chondrogenesis; ANGPTL3, angiopoietin-like protein 3; FGF18, fibroblast growth factor 18; MACI, matrix-induced autologous chondrocyte implantation; MEPE, matrix extracellular phosphoglycoprotein; MMP13, matrix metalloproteinase 13; mRNA, messenger ribonucleic acid; MSCs, mesenchymal stromal cells; PFK1, phosphofructokinase 1; SYSADOA, symptomatic slow-acting drugs for osteoarthritis; TGFβ1, transforming growth factor β1.

This failure might be because targeting inflammatory endpoints does not address the initial trigger or upstream event that initiated the inflammatory cascade. Indeed, no anti-inflammatory therapy has yet emerged as a disease-modifying OA drug[86]. Although various repurposed existing drugs, including hydroxychloroquine, TNF inhibitors and corticosteroids, have shown no disease-modifying effect, newer formulations of existing drugs such as bisphosphonates[87] and triamcinolone acetonide[88] are being explored to extend the short-lived symptomatic benefits of these drugs towards disease modification. Potential anti-inflammatory drugs and their clinical progress are presented in Table 1.

Although NSAIDs lack disease-modifying properties, NSAIDs can have synergistic chondroprotective effects when combined with another group of drugs known as symptomatic slow-acting drugs for osteoarthritis, including glucosamine sulphate[89]. Newer candidates for disease-modifying OA drugs that also exhibit chondroprotective properties, including kartogenin, tankyrase inhibitors, recombinant fibroblast growth factor 18 and angiopoietin-like-3 derivatives such as LNA043, have shown promising results in preclinical studies and clinical trials[90]. NSAIDs are now being designated for the acute management of inflammatory symptoms, whereas slow-acting drugs for osteoarthritis are being used for maintenance therapy[91]. Beyond the identification of new compounds or druggable pathways, it remains to be tested whether a pharmacological approach can be more effective if introduced in earlier phases of cartilage failure, to block the degeneration processes that would otherwise progressively take over upon the onset of injuries or abnormal mechanical loading. In this context, strategies for local and sustained drug delivery would also be required to bypass adverse effects typically associated with extended systemic exposure to anti-inflammatory therapies.

Gene therapy

In parallel to the traditional, systemically delivered, small-molecule or antibody-based approaches to targeting inflammatory drivers, gene therapy strategies focus on establishing a sustained local synthesis of gene products and modulating the expression of candidate genes towards articular cartilage restoration[92,93]. Although viral vectors have been commonly employed to transfer the therapeutic genes of interest to the chondrocytes, utilization of non-viral vectors including polymers and liposomes is also on the rise[92]. Transforming growth factor β1 (TGFβ1) and IL-1 receptor antagonist (IL-1RA) remain the commonly employed candidates for overexpressing via gene therapy[93]. For ex vivo gene transfer approaches, the genes of interest are overexpressed in chondrocytes that are then transplanted, whereas for in vivo gene transfer approaches, vectors (such as adeno-associated virus vectors and non-viral vectors) are employed to directly transfer the gene to the chondrocytes in vivo[93].

Gene therapy has been used to enhance the chondrogenic differentiation of MSCs[94] and to augment the benefits of microfracture by inducing the expression of chondrogenic genes in the MSCs that enter the defect from the marrow, either through in vivo expression of transgenes[95] or through implantation of ex vivo genetically modified bone marrow clots that contain MSCs (known as 'gene plugs')[96]. Using a systems biology approach, chondrocytes have also been re-programmed to express anti-inflammatory biologic drugs in response to the presence of a pro-inflammatory cytokine, thus providing a negative feedback system to control the inflammatory environment[97]. Emerging gene therapy strategies use various categories of RNA therapeutics, such as small interfering RNAs, microRNAs and antisense oligonucleotides as non-viral approaches in gene therapy[92]. This class of RNA therapeutics shares with other non-viral approaches the limitation of low stability and limited efficiency in delivering the drug to the target site, which remains the prime focus of ongoing research[98,99]. Various viral and non-viral approaches used in gene therapy and their stages in clinical translation are shown in Table 1.

Cellular therapy

MSCs from various sources, such as from the bone marrow or adipose tissue, have been utilized to ameliorate the inflammatory cascade that prevents effective cartilage regeneration[100]. MSCs possess immunomodulatory, especially anti-inflammatory, properties and have been shown to restrain the activation of lymphocytes, pro-inflammatory macrophages and other immune cells typically involved in preventing tissue regeneration[46]. Delivery of MSCs, or MSC-derived exosomes as a surrogate, into an inflamed joint milieu has shown promise in mediating effective cartilage regeneration in phase III clinical trials[45,101]; this approach can selectively enhance M2 polarization of macrophages, as well as the expression of chemokines and immunosuppressive factors such as indoleamine 2,3-dioxygenase, TGFβ1, prostaglandin E_2 and IL-10 (ref. 46). However, MSCs are typically injected upon diagnosis of OA and their effect on cartilage regeneration at earlier stages following injury is still a subject of debate. Moreover, the therapeutic

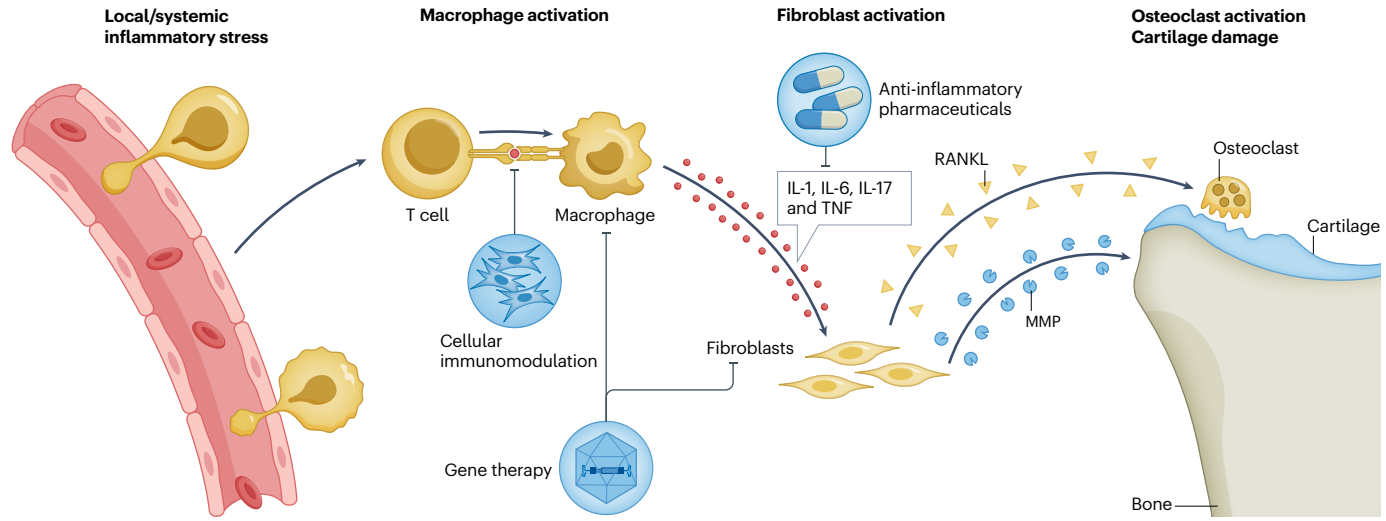

Fig. 3 | Inflammatory events involved in cartilage regeneration and associated therapeutic strategies. Inflammation in the joint is a common outcome of several processes including cellular insufficiency, mechanical imbalance or metabolic imbalance, and ultimately prevents cartilage regeneration. Joint inflammation results from chemotaxis of immune cells to the joint and M1 polarization of the macrophages, leading to the activation of fibroblasts through pro-inflammatory cytokines (such as IL-1, IL-6, IL-17 and TNF). These molecules aggravate cartilage damage, for example, through promoting receptor activator of nuclear factor-κB ligand (RANKL)-mediated activation of osteoclasts and the production of matrix metalloproteinases (MMPs). Anti-inflammatory strategies are aimed at preventing immune activation through anti-inflammatory pharmaceutical compounds, cell-instructed immunomodulation or genetic modification of cells. The original version of this figure was created with BioRender.com.

efficacy of injection of the MSCs into the joint space compared with local recruitment of resident progenitor cells (discussed in the earlier section on cellular failure) is called into question[102]. Also, this strategy needs to address the challenges in identifying the quality attributes for immunomodulatory MSCs and biomarkers of response in patients, to increase the robustness and predictability of clinical outcomes[103,104].

Metabolic stress

Articular cartilage is an avascular tissue that receives oxygen and nutrients that cross the synovial barrier into the synovial fluid and diffuse into the cartilage[105]. Although most tissues have an oxygen tension of ~13%, oxygen tension is about 5% at the surface and 1% in the deepest regions of cartilage[105]. Generally, most of the energy in chondrocytes comes from glycolysis, and only about 25% of the energy requirement comes from oxidative phosphorylation[106]. An appropriate balance between oxygen and glucose uptake, plus redox control from oxidative phosphorylation, are essential for chondrogenesis, differentiation modulation and cell survival[107]. Thus, chondrocytes rely on unique molecular mechanisms, such as hypoxia-inducible factor 1 (HIF1) regulation, mitochondria dynamics, redox control and metabolic regulation, to adapt to their physiological low-oxygen and low-nutrient micro-environment[108].

During physiological mechanical loading, metabolic homeostasis is critical for maintaining the function of cartilage[108]. However, when the cartilage is damaged, the local oxygen tension is no longer finely controlled and the energy demand increases, compromising the optimal microenvironment required for chondrocyte function and regenerative capacity[109]. In these scenarios, a metabolic shift occurs that is characterized by dysregulation of the glycolytic cascade, leading to lactate accumulation and promoting the acidification of the local microenvironment[108]. Consequently, this shift inhibits matrix synthesis and facilitates cartilage degeneration[110]. Moreover, the failure to obtain oxygen and glucose compromises the upstream formation of ATP, limiting cell function and survival[111]. Furthermore, such a metabolic shift disrupts mitochondrial homeostasis and exposes the chondrocytes to reactive oxygen species (ROS)-induced stress[108]. This hyperoxide state activates downstream critical survival pathways such as AMP-activated protein kinase (AMPK) signalling, mechanistic target of rapamycin (mTOR) signalling and the cytokine response, affecting matrix remodelling and cell survival[112].

In acute and reversible situations, chondrocytes can survive these metabolic changes and return to their homeostatic condition after the stress is resolved[112]. However, extensive cartilage defects lead to a chain of events that have deleterious consequences, accelerating cartilage degeneration in the long term[108]. An important marker of this irreversible effect is a shift from aggrecanase activity towards MMP activity in proteoglycan and collagen type II turnover[113], which can be controlled by metabolic changes under stress conditions[114–116]. Additionally, ageing, obesity and type II diabetes mellitus are all associated with disruption of metabolic homeostasis and are linked to poorer cartilage regeneration outcomes[117]. Therefore, several approaches and therapeutic solutions targeting cartilage metabolism are currently being explored[118] (Fig. 4).

Enzyme modulation

Most of the reactions in the glycolytic pathway are reversible, and changes in the enzymes that catalyse these reversible reactions will define the directionality or the metabolic flux in the cell[108]. Three important enzymes control the flow through the glycolic pathway by mediating irreversible reactions: hexokinases, phosphofructokinase 1 (PFK1) and pyruvate kinase[111]. Interestingly, some therapies currently

approved in other contexts, such as 2-Deoxyglucose (a hexokinase inhibitor), metformin (AMP-activated protein kinase) and rapamycin (mTOR inhibitor), show beneficial anti-inflammatory effects by modulating the activity of these glycolytic enzymes[119,120]. Tofacitinib, a drug approved for the treatment of rheumatoid arthritis, downregulates the expression of hexokinase 2, glucose transporter 1 (GLUT1), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) and pyruvate dehydrogenase kinase 1 (PDK1) in synovial fibroblasts, thereby modulating immunometabolism[121]. Furthermore, enzyme modulation using inhibitors of hexokinase 2, pyruvate kinase 2 or lactate dehydrogenase A have shown encouraging results in treating cartilage degeneration in various preclinical models, by reducing glycolytic activity and lowering the expression of cytokines and metalloproteinases[122]. Systemic administration of PFKFB3 inhibitors can suppress cartilage degeneration in a mouse model of collagen-induced arthritis, blocking local glycolysis and preventing immune cell migration and inflammation[123]. Despite these promising results in delaying the onset of arthritis, whether or not modulating these glycolytic pathways also facilitates cartilage regeneration following injury or trauma-induced joint mechanical instability remains unknown.

Mitochondrial modulation

Mitochondrial homeostasis is maintained through three main biological processes: mitochondria biogenesis (mitogenesis), mitochondria dynamics and mitochondria autophagy (mitophagy)[124]. In the context of articular cartilage regeneration, different groups have approached each of these mitochondria processes and successfully restored chondrocyte viability by promoting mitochondrial biogenesis[125], correcting dynamic homeostasis[126] or enabling mitochondrial renewal[127]. In addition, compounds such as hydrogen sulphide, irisin, puerarin, quercetin, procyanidins and fibroblast growth factor 18 have successfully mitigated cartilage inflammation and oxidative stress by modulating the AMPK–SIRT–PGV1α pathway and mitochondria dynamics in preclinical models[128–133]. Similarly, metformin, currently being tested in a randomized clinical trial for the treatment of patients with OA who are overweight, has shown beneficial effects on chondrocytes by promoting mitochondria fusion, fission inhibition and activation of the Parkin-mediated mitophagy pathway in vitro[134]. Finally, in a rat model of OA, mitochondrial transfer results in notable improvements in cartilage regeneration[135]. These data emphasize an apparent relationship between cartilage regeneration and mitochondria homeostasis, which is a promising avenue to explore for enabling native cartilage regeneration in the future.

Antioxidant therapy

ROS production contributes to failure of cartilage repair and is one of the main consequences of metabolic dysfunction[109]. Indeed, drugs used to limit cartilage damage such as NSAIDs, hyaluronic acid and glucosamine sulphate, have antioxidant effects on chondrocytes[136–138]. Thus, research is underway on different approaches to mitigating the inflammatory and matrix degradation effects of ROS on chondrocytes. The various strategies being explored include promoting the activity of antioxidant enzymes (such as super-oxide dismutase, catalase and the glutathione family of proteins)[139,140]; using non-enzymatic molecules with antioxidant activity (such as vitamin E[141], vitamin C[142], flavonoids[143] and chelants[144]); and prescribing antioxidant diets[145]. However, despite promising mechanistic results, human clinical trials of vitamin E supplementation, vitamin C supplementation or antioxidant diets have led to controversial results[146]. Additional studies exploring the optimal dose for these treatments might help to improve the effects of antioxidant compounds to aid in cartilage regeneration.

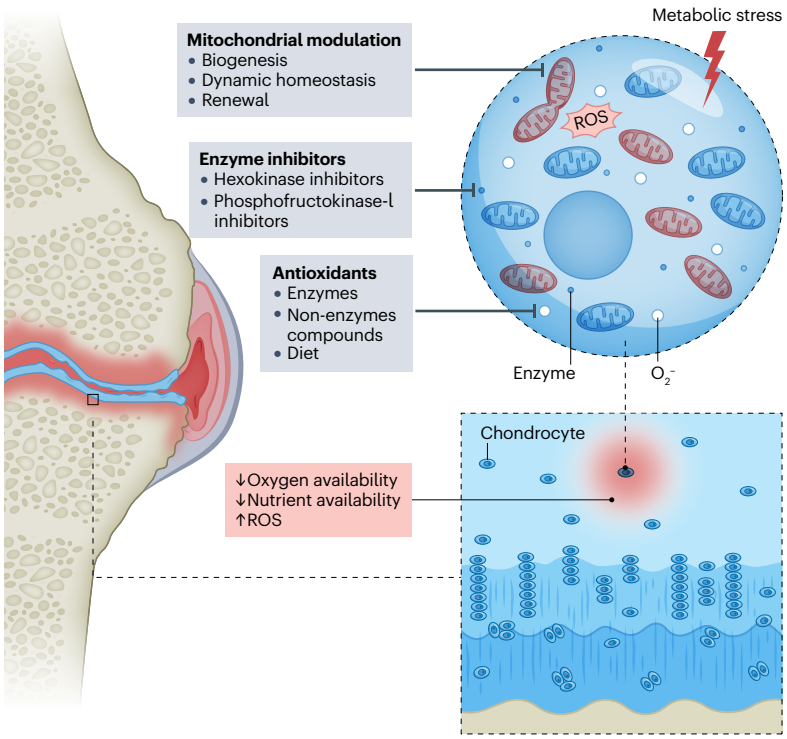

Fig. 4 | Strategies for targeting the metabolic changes that negatively affect cartilage regeneration. Metabolic stress in articular cartilage results in reduced oxygen and nutrient availability, as well as increased concentration of reactive oxygen species (ROS). Strategies to restore metabolic homeostasis to aid in cartilage regeneration involve modulation of mitochondrial activity, enzyme inhibition and antioxidant therapies. The original version of this figure was created with BioRender.com.

Challenges and future perspectives

This Review outlines various hypotheses of why cartilage is unable to regenerate and describes the current status of different associated therapeutic strategies (Fig. 5). Most of the treatment strategies discussed are focused on the knee joint; other load-bearing joints (such as the hip, ankle and finger joints) might have site-specific biological and mechanical requirements and thus regenerative approaches for these sites deserve analogous attention and further investigation. The presented analysis indicates that despite the vast amount of research in cartilage regeneration, the success of translating possible therapies into clinical practice remains limited. One important challenge in identifying solutions lies in the fact that multiple pathways can cause cartilage regeneration to fail following injury, thereby leading to a cascade of events that ultimately results in degenerative disease states such as OA.

From a molecular, cellular and mechanical perspective, cartilage response to injury and its regenerating mechanisms differ in acute and chronic settings[147]. Indeed, inflammation and immune cell activation are properly activated in the regeneration process of acute cartilage injuries, but can become deleterious if they are not resolved and proceed into a chronic pathological state[148]. Similarly, cell fate pathways such as autophagy, senescence or metabolic shifts can contribute to restoring homeostasis during acute repair responses but can also inhibit cartilage regeneration if prolonged[149]. This stage specificity is also mirrored in the fact that the efficiency in cartilage regeneration following the application of competent cells diminishes as the damage becomes chronic, and advanced alterations need to be targeted by alternative or additional strategies[150,151].

Therefore, the 'entry points' in the cascade of events towards cartilage failure, which are based on the hypotheses outlined in this Review, might not necessarily reflect the major pathological challenge at a given point in time. As a result, the 'exit strategy' might have to address a target that is different from the primary cause of failure (Fig. 6). For example, the lack of regeneration following cartilage injury might initially be the consequence of pathological loading. However, the subsequent events in the cascade such as metabolic dysregulation and inflammation might become the dominant determinants for catabolism and degeneration.

The definition of a treatment strategy for cartilage injuries, as well as the development of innovative treatments, are challenged by the complexity of cartilage biology and repair mechanisms that occur in a dynamically evolving system, with multiple interacting factors[152]. The corresponding variety of clinical phenotypes and molecular endotypes of different individuals limits a one-fits-all solution and might even require combined therapeutic approaches. Various patient-related factors including age, sex and comorbid conditions must be considered to enable tailored and effective regenerative treatment. This information should be combined with the identification of suitable patient biomarkers that are able to capture the concomitant processes of cartilage degradation, neo-matrix formation, intra-articular inflammation and systemic inflammation[153]. Ultimately, the recognition that the outcome of any intervention can only be judged with long-term follow-up assessments, which are economically and logistically challenging, reinforces the need to identify biomarkers as early predictors of long-term treatment effects, with patient pain at the centre of any claimed clinical success.

The animal models that are available to address the biological processes active in cartilage repair and to assess the suitability of possible treatment strategies are limited. Small animal models are valuable tools to investigate the pathogenesis and preliminarily test some hypotheses at low cost. However, the translational value of these insights is limited by the joint anatomy (such as the small size of the joint and the thickness of the cartilage) and greater inherent healing potential compared with humans. Large sized animal models (for example, ovine, porcine and equine species) offer a closer approximation to the human joint; however, efficacy results from these models cannot be directly extrapolated to a clinical scenario and financial, logistic and ethical considerations limit their usage[13]. Critical awareness of the advantages and limitations of disease models and experiments is essential to advance from preclinical hypotheses to viable clinical solutions.

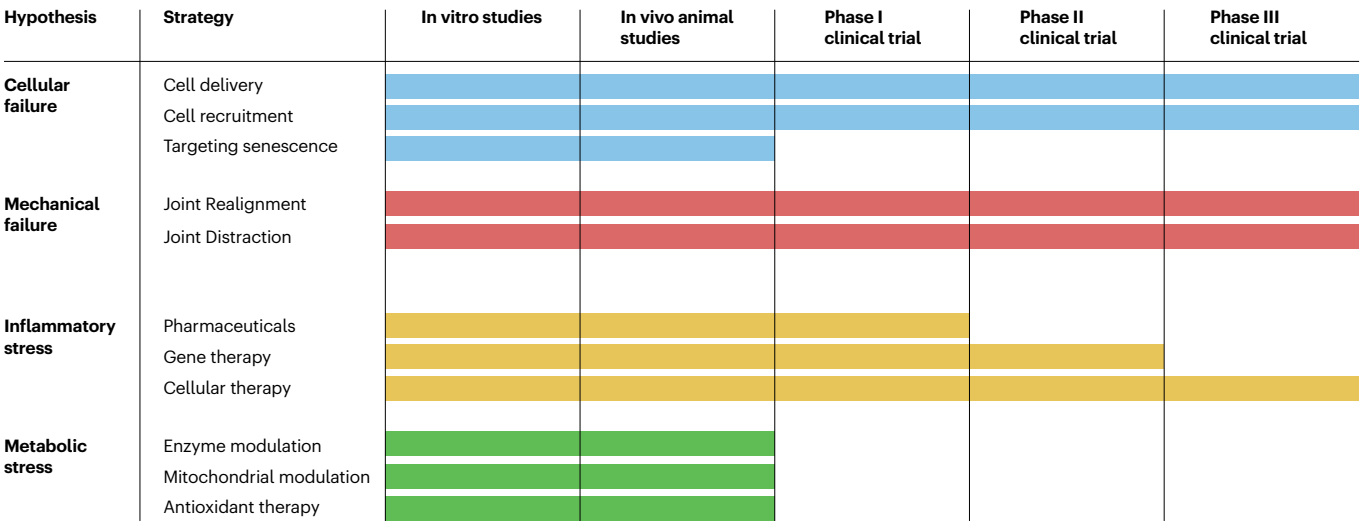

Fig. 5 | Clinical stage of strategies targeting various hypotheses for why cartilage regeneration fails. Various hypotheses are available to explain why cartilage regeneration fails, as summarized in this Review. A number of strategies that target each of these hypotheses are under investigation and are at different stages of clinical development towards clinical translation, as depicted in this figure. Further information on the individual studies and clinical trials of a given therapeutic strategy, in association with the different hypotheses, can be found in Table 1.

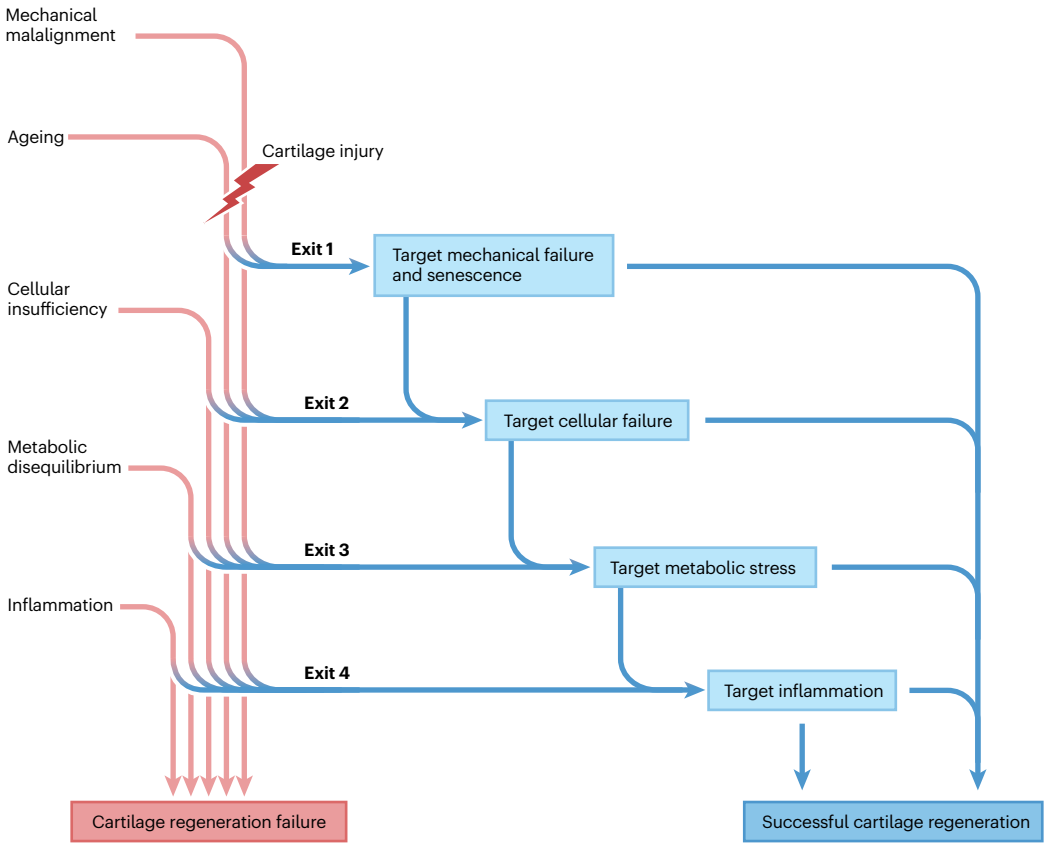

Fig. 6 | Potential entry points and exit strategies relating to cartilage regeneration failure. The various hypotheses for why cartilage fails to regenerate, as summarized in this Review, might have different entry points following cartilage injury. Possible exit strategies for successful cartilage regeneration might involve not only targeting these particular mechanisms but might also depend on the stage of disease progression. Mechanical malalignment and ageing function are potential risk factors that derange joint physiology to make articular cartilage vulnerable to injury. Hence, strategies to exit the cascade of events might initially employ mechanical and anti-senescence therapies. Subsequently, cellular strategies could be introduced to compensate for the reduced density of regeneration-competent cells and metabolic strategies could counteract the associated dysregulated processes that prevent effective cartilage regeneration. At later stages after injury, the lack of resolution of inflammation might require a direct anti-inflammatory intervention.

Conclusion

Various different hypotheses might explain why cartilage fails to spontaneously regenerate, after which a cascade of events follows, ultimately leading to degenerative changes and OA. These hypotheses can be broadly related to cellular failure, mechanical failure, inflammatory stress or metabolic stress. In this Review, we have attempted to logically connect current or envisioned therapeutic strategies, including cellular, genetic, mechanical and pharmacological treatments, with such emerging hypotheses. However, none of the evaluated therapeutic strategies has so far consistently achieved native hyaline cartilage regeneration following injury or disease modification in later stages of cartilage degeneration. A suitable treatment or 'exit strategy' might not necessarily need to address the initial 'entry point' in the cascade of events, but instead might target a different set of factors, depending on the evolution of the disease and the context of the specific patient's characteristics. To move the field forward and address the multidimensional and complex nature of cartilage regeneration, future trials should be designed in a way that tests specific hypotheses and increases our mechanistic understanding of cartilage biology and regeneration failure. In this way, even trials that are not successful at regenerating native cartilage might provide valuable information to identify priorities in the current 'battle of hypotheses' and instruct the development of next-generation treatments.

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