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The challenge of cartilage integration: understanding a major barrier to chondral repair

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Abstract

Articular cartilage defects caused by injury frequently lead to osteoarthritis, a painful and costly disease. Despite widely used surgical methods to treat articular cartilage defects and a plethora of research into regenerative strategies as treatments, long-term clinical outcomes are not satisfactory. Failure to integrate repair tissue with native cartilage is a recurring issue in surgical and tissue engineered strategies, seeing eventual degradation of the regenerated or surrounding tissue. This review delves into the current understanding of why continuous and robust integration with native cartilage is so difficult to achieve. Both the intrinsic limitations of chondrocytes to remodel injured cartilage, and the significant challenges posed by a compromised biomechanical environment are described. Recent scaffold and cell-based techniques to repair cartilage are also discussed, and limitations of existing methods to evaluate integrative repair. In particular, the importance of evaluating the mechanical integrity of the interface between native and repair tissue is highlighted as a meaningful assessment of any strategy to repair this load-bearing tissue.

Impact statement

The failure to integrate grafts or biomaterials with native cartilage is a major barrier to cartilage repair. An in depth understanding of the reasons cartilage integration remains a challenge is required to inform cartilage repair strategies. In particular, this review highlights that integration of cartilage repair strategies is frequently assessed in terms of the continuity of tissue, but not the mechanical integrity. Given the load bearing nature of cartilage, evaluating integration in terms of interfacial strength is essential to assessing the potential success of cartilage repair methods.

Introduction

For an individual, damaged articular cartilage in the knee can see loss of joint function, pain and the likely development of osteoarthritis [1, 2]. From a health economy standpoint, osteoarthritis presents a significant financial burden, estimated at 1 to 2.5% of gross national product for countries including the USA, Canada and France [3]. In 2015, lost labour force participation alone due to arthritis was estimated to cost the Australian economy $1.8 billion [4].

Knee injuries in adolescence and young adulthood (such as those incurred playing sport) increase the risk of developing osteoarthritis at a younger age than is otherwise seen in the population [5, 6]. The burden of osteoarthritis for individuals within working age is significant, with high levels of pain and reduced function impacting day-to-day life and placing limitations on individuals' capacity to work [7]. One of the possible causes of post traumatic osteoarthritis (PTOA) is an injury that occurs directly in the articular cartilage. Following joint trauma involving articular cartilage, strategies to repair and regenerate the affected cartilage in the acute setting are a potential way to halt or slow the progression of PTOA, with the goal of re-establishing the mechanical properties of the tissue and appropriate load distribution [8].

Current clinical treatments of articular cartilage injury fail to provide long-lasting repair, where "failure" is defined as a poor Lysholm score (a clinical assessment of a patient's knee symptoms and function) or the patient requiring a knee replacement [9, 10]. Gold standard surgical approaches include microfracture, mosaicplasty, and autologous chondrocyte implantation. Microfracture largely results in the production of fibrocartilage, which does not possess the same mechanical properties as the original hyaline cartilage, and may also see the formation of subchondral cysts [11, 12]. Fibrocartilage is unable to withstand joint loads and resist wear like hyaline cartilage, and can degenerate after 24 months, potentially requiring revision surgery [13, 14]. Numerous synthetic and naturally derived biomaterials are also under development as acellular implants, or to support the delivery of cells to regenerate cartilage tissue [15].

Both surgical repair methods and tissue engineering approaches frequently see failure of the graft or newly formed tissue to integrate with native cartilage [16]. Vertical integration with the subchondral bone is also necessary to repair osteochondral defects and occurs more readily than integration with cartilage, though this does not guarantee a healthy repair of the subchondral bone, with signs of the onset of osteoarthritis sometimes observed (e.g. cysts and sclerotic thickening) [16, 17]. A failure of lateral or basal integration with cartilage remains a significant challenge as it frequently leads to degradation of the surrounding tissue over time, and is the focus of this review [10, 16].

A clear understanding of the difficulties in achieving integration is essential to focus current research efforts to repair articular cartilage defects. This review will summarise the current understanding of why integration is lacking in cartilage repair, and recent strategies under development to overcome this problem.

1. Articular cartilage – a heterogeneous tissue with limited capacity for self-repair

Articular cartilage provides the knee joint with a smooth, low friction, load bearing surface. It enables efficient movement of the joint, cushions loads up to 3.5 times body weight and provides resistance to wear, supporting longevity of the joint [18]. Cartilage is avascular and aneural, and contains few cells (chondrocytes), which make up only 1 to 2% of its total volume [19]. Articular cartilage is largely water (over 70%) with two main components comprising its dry weight: type II collagen and proteoglycans, mainly aggrecan [19]. Collagen fibrils form a solid network with proteoglycan aggregates embedded throughout. Negatively charged glycosaminoglycan (GAG) side chains of aggrecan imbue the tissue with a fixed charge density. The electrostatic repulsion of the negatively charged proteoglycans is constrained by the tensile properties of the collagen network, and the small pores between aggrecan molecules give rise to cartilage's low permeability [20]. The fixed negative charge of proteoglycans is balanced by mobile counterions, which result in an osmotic pressure that hydrates and swells cartilage tissue and is key to its resistance to compressive loads [21].

When damaged, articular cartilage has limited ability for self-repair. This is particularly the case for partial-thickness and full-thickness chondral defects (Figure 1), where blood and reparative bone marrow cells from the subchondral bone are not available as in osteochondral defects [22].

Articular cartilage tissue is inhomogeneous, with the concentration and arrangement of its extracellular matrix (ECM) molecules varying through its depth, as do the density and morphology of cells (Figure 1). The depth-dependent variations confer different mechanical properties to the tissue, which is classified into four distinct zones [19]. At the surface of articular cartilage is the superficial zone, where water content and permeability are at their highest, and proteoglycan concentration its lowest [23]. Collagen fibres are tangential to the surface of cartilage in the superficial zone, and perpendicular in the deep and calcified zones of cartilage [19]. Collagen is thought to be oriented randomly through the middle or transitional zone, surrounding chondrocytes in a network [23]. Cartilage ECM also varies with position over the knee joint – a thick deep zone is seen in the centre of the cartilage covering the tibia, with thinner superficial and middle zones relative to other areas of the joint [24].

The highest density of chondrocytes is found in the superficial zone of cartilage, where the cells have a flattened morphology parallel to the surface of the tissue. Chondrocyte morphology becomes round through the middle zone of cartilage, with a random arrangement shifting to more orderly columns in the deep zone of cartilage [19]. Chondrocytes are responsible for the synthesis and degradation of the ECM in cartilage, though remodelling of the tissue is limited. Cells are bound in small spaces in the ECM known as lacunae, with matrix molecules diffusing out into the tissue [16, 18, 25, 26]. Superficial zone chondrocytes are less metabolically active than deeper chondrocytes, and secrete a protein key to lubricating the surface of cartilage (lubricin), whereas middle and deep zone chondrocytes synthesize a greater quantity of matrix [27].

The mechanical behaviour of cartilage is dependent on interactions between interstitial fluid and the ECM macromolecules aggrecan and collagen [21]. Its behaviour is commonly described by two mechanisms – the intrinsic viscoelasticity of cartilage's solid matrix, and poroelasticity due to solid-fluid interactions [20, 21, 23, 28]. The viscoelastic behaviour of cartilage depends on the time-dependent rearrangement of ECM molecules in response to loads, whilst poroelastic behaviour is due to the movement of interstitial fluid through small pores between aggrecan molecules [20].

When compressed, interstitial fluid is squeezed out of pores between aggrecan molecules, and as the fluid moves through these pores, energy is dissipated as a frictional drag force. The low permeability of cartilage gives rise to high fluid pressure that protects the solid matrix and chondrocytes within the tissue from stresses and strains experienced in physiological loading, as these are largely borne by the fluid phase [21]. Though the fluid phase plays a significant role in initial load bearing, research suggests collagen in the superficial zone deforms under load and also contributes to the instantaneous response of cartilage [29]. Under sustained load, osmotic pressure due to the fixed charge density of GAGs is found to contribute significantly to, and even dominate, the mechanical response of cartilage at equilibrium [21, 29]. Under impact loading, cartilage is understood to behave as a single incompressible solid phase, with no time for fluid to flow and dissipate loads under rapid loading conditions [23].

The heterogeneity of cartilage leads to mechanical variation in the tissue. In particular, depth-dependent architecture may result in greater fluid support in the superficial zone of cartilage where tensile properties are at their greatest, providing protection for the middle and deep zone matrix from large stresses [30-32]. Models to study cartilage frequently account for depth-dependent variation, but few consider location-dependent variation in the tissue structure, which may be necessary for accurate estimation of some mechanical parameters [24]. Biphasic theory has commonly been used to model cartilage as a solid and fluid phase, though triphasic theory that accounts for the contribution of osmotic pressure may provide greater insight to the mechanical response of cartilage [21, 33].

Stress and strain distributions throughout the tissue, and how chondrocytes experience this mechanical environment, are important for maintenance of cartilage matrix. Chondrocytes have a stiffness three orders of magnitude less than bulk cartilage, and are surrounded by pericellular matrix of intermediate stiffness, that can act to both propagate mechanical signals or shield chondrocytes from them [27, 34]. Under loading chondrocytes may experience increased hydrostatic pressure and deformation, as well as fluid flow, and changes to pH and osmolarity associated with increases in fixed charge density as proteoglycans are forced together [35]. Mechanotransduction of these signals into a biochemical response by the cells can see enhanced synthesis of ECM molecules or a pathologic response depending on the nature of the load [35].

Important to supporting cartilage function is the synovial fluid circulating within the joint. Synovial fluid contains hyaluronic acid and lubricin which lubricate the surface of cartilage. It also serves as a source of nutrients for chondrocytes, containing electrolytes, oxygen and glucose which diffuse into cartilage, and allows for the removal of waste metabolites [19, 36]. The anti-adhesive environment created by synovial fluid may also impede integration however, as has been illustrated in an in vitro model of cartilage repair where lubricin (also referred to as proteoglycan 4), but not hyaluronic acid, was found to inhibit adhesive strength between two cartilage surfaces [36].

Biomaterials used in tissue engineering approaches to repair articular cartilage tend to have mechanical properties orders of magnitudes lower than cartilage (which experiences loads between 0.5-7.7MPa) [37, 38]. Additionally, these biomaterials fail to match the structural complexity of articular cartilage. Mimicking the viscoelasticity, low permeability and zonal variation of cartilage ECM is a significant challenge in developing biomaterials for cartilage repair [25].

Effective integration and repair are achieved when ECM is continuous from the defect to the native tissue, with cellularity similar to normal cartilage [39]. At a glance, it is clear why seamless integration is difficult to

achieve – chondrocytes have limited ability to migrate to the defect to rebuild the complex, heterogeneous structure of cartilage and maintain the mechanical integrity of the tissue. A deeper discussion of these mechanisms follows, focusing on the importance of both chondrocytes (Section 2) and the mechanical environment (Section 3) in understanding the challenge of integration.

2. Chondrocytes in damaged cartilage

2.1 Cartilage defects are lined by a zone of dead cells

A margin of tissue surrounding chondral defects has been observed to have lower cellularity than healthy cartilage tissue [11, 16, 40]. When partial-thickness cartilage defects were created in rabbit and miniature pig models, lower cell density was seen in the weeks and months following surgery within 100µm of the defect edge, compared to tissue far from the defect [40]. In a study of microfracture in rabbits, areas of low cell density near the defect edge were observed, as well as the formation of chondrocyte clusters [16]. Chondrocyte clusters are frequently seen accompanying areas of cell death due to injury, and are considered a marker of degeneration and osteoarthritis [41]. While these clusters may represent an attempt at intrinsic repair, ultimately the products of increased biosynthetic activity and abnormal differentiation of cells in clusters is understood to contribute to ECM degradation, calcium deposition, inflammation of the joint and further deterioration of cartilage [41, 42].

Debridement of the defect, whilst necessary to remove dead tissue, can result in further chondrocyte death in neighbouring tissue [40]. The degree of damage, however, is dependent on the instruments and methods used [43-45]. When blunt tools (such as a curette) are used, chondrocyte death via necrosis occurs from the mechanical disruption to the tissue and force applied by the instrument itself. This is subsequently accompanied by a region of apoptosis that can extend up to 400µm inwards radially, as observed in the superficial zone of cartilage [43, 44, 46]. The use of a sharp scalpel has been found to be less damaging, with dead cells isolated largely to the very edge of the defect [39, 43].

Due to the lack of an intrinsic wound healing response, articular cartilage is unable to resorb damaged tissue [10]. Dead cells and their surrounding ECM then become a physical barrier to the formation of continuous, hyaline cartilage. Cells supplied to the area via a graft or scaffold must integrate with this area of damaged cartilage, which will progressively deteriorate with time [43]. The use of a sharp scalpel to freshen and prepare the defect before implanting a graft or biomaterial may therefore be necessary and preferable to the use of a blunt curette, ideally exposing a population of viable cells at the defect edge that are capable of generating ECM molecules and integrating with the implant.

2.2 Resident chondrocytes have limited ability to migrate

Chondrocytes in mature cartilage are understood to have limited ability to migrate to the site of a defect, as they are contained in pericellular matrix and may be further blocked by a layer of damaged tissue [16]. The presence of chondrocytes lining the defect is thus a barrier to repair when cell death or abnormal metabolism and clustering occurs, however viable chondrocytes at the surface are essential for integration and the regeneration of seamless ECM. This has been illustrated in vitro, where migration of implanted chondrocytes or endogenous cells has been observed to increase the interfacial strength between cartilage and repair tissue [47-49]. Supporting this is evidence that exposure (via culture medium) to inhibitors of three signalling proteins associated with chondrocytes' migratory ability (Src, PLCγ1 and ERK1/2) suppressed the migration of implanted chondrocytes compared to controls in an in vitro model [48]. The interfacial strength was higher (approximately 70kPa compared to 40kPa) when samples were not pre-treated with inhibitors of these signalling proteins, suggesting that the presence of cells at the interface is associated with stronger integration [48]. These results were supported by significantly higher glycosaminoglycan (GAG) and total collagen contents in the integration zone compared to the integration zone of samples pre-treated with inhibitors [48].

It has been demonstrated that in some circumstances, chondrocytes may migrate. Evidence of nasal chondrocyte migration from cartilage tissue into cell-free scaffolds was seen in an in vitro model using

bovine nasal cartilage explants and collagen scaffolds [47]. A degree of integration, as measured by the development of interfacial strength, suggested migration of resident cells from the cartilage into the scaffold, or some diffusion of ECM molecules to facilitate integration [47]. Nasal chondrocytes are reported both to proliferate at a faster rate than articular chondrocytes and have greater chondrogenic potential [50-52]. Though nasal cartilage serves a vastly different function in the body, it is also made of hyaline cartilage, and nasal chondrocytes have shown responsiveness to mechanical cues in vitro [53, 54]. Nasal chondrocytes may be a favourable source of adult chondrocytes for cell-based articular cartilage repair and were demonstrated as safe and feasible in a first-in-human trial of articular cartilage repair with engineered grafts [52, 55]. Methods to validate the purity and potency of nasal cartilage biopsies, and avoid contaminating cells from neighbouring tissues, have also been developed to aid clinical translation [52, 56]. However, a pre-clinical goat model of cartilage repair using the same approach showed promising filing of the defect at 6 months yet found fixation of the implant was an issue [57].

Conversely, an in vitro study of bovine cartilage pre-treated with trypsin to deplete its proteoglycan content did not see migration of endogenous cells [58]. Hydrogel-encapsulated chondrocytes cultured in the cartilage explant rings were observed to infiltrate the cartilage following trypsin pre-treatment, however endogenous chondrocytes remained within their lacunae [58].

A study of adult human articular cartilage explants also saw no significant cellular outgrowth [59]. Explants were “cleansed” to remove cells from the surface, and after four weeks of culture no cells could be observed on the surface when compared to non-cleansed controls [59]. Chemotactic factors, including transforming growth factor-β1 (TGF-β1), were also applied and still no cellular outgrowth was observed in cleansed explants, nor a significant increase in the number of cells on the surface of native cartilage [59]. Additionally, in vivo chondrocyte migration and intrinsic regeneration of cartilage have only been reported when combined with penetration of the subchondral bone and access to reparative cells from bone marrow [60]. A study of partial-thickness chondral defects in an adult sheep model showed no cellular

outgrowth and insufficient integration after 26 weeks when cartilage grafts alone were transplanted into chondral defects, without bone marrow stimulating techniques [60]. Despite evidence of chondrocyte migration in vitro, at present there is little evidence of in vivo migration of chondrocytes in mature cartilage [41, 60]. Strategies to repair chondral defects therefore cannot simply rely on endogenous chondrocytes colonising areas of acellular cartilage at the defect edge or migrating into a graft unaided.

2.3 Chondrocyte metabolism in injured cartilage is altered

Not only limited by an inability to migrate, surviving chondrocytes in injured cartilage undergo significant changes which may impact their ability to synthesise ECM molecules needed for repair, and therefore further contribute to the deterioration of tissue.

An in vitro study following the creation of partial-thickness defects (via scalpel) in bovine cartilage explants found changes to chondrocytes at the defect edge [61]. When explants were cultured in medium containing 10% synovial fluid or 10% foetal calf serum, cells adopted abnormal morphology, average cell volume more than doubled (compared to chondrocytes cultured in serum-free medium or those at a distance from the injury), and the formation of chondrocyte clusters was observed [61]. Such changes influence matrix synthesis and are associated with the progression of osteoarthritis [62-64]. The authors suggested a mechanism whereby inflammatory factors present in synovial fluid or foetal calf serum may permeate the damaged matrix and impact nearby chondrocytes (whereas chondrocytes surrounded by intact matrix further from the defect retained normal morphology) [61]. This penetration of synovial fluid into damaged matrix takes on further significance in the context of a traumatic joint injury, which sees a sustained increase in inflammatory mediators in the synovial fluid [65].This study also observed that raising the osmolarity of the medium had a “protective effect”, avoiding the changes to chondrocyte morphology, volume and the formation of clusters in the presence of foetal calf serum [61]. In fact, irrigation with hyperosmotic solutions during surgery may be beneficial owing to this chondroprotective effect [66].

Mechanotransduction pathways are an important signalling mechanism for regulating matrix metabolism in cartilage [67]. Whilst moderate dynamic loading has a positive effect by increasing the synthesis of matrix molecules, injurious compression (i.e. compressive loads well above those normally experienced in vivo) can cause changes to the volume and morphology of chondrocyte organelles that play a central role in the biosynthetic function of the cells (such as the nucleus and endoplasmic reticulum) and ultimately decrease the synthesis of matrix molecules [67, 68].

Following impact loading of canine articular cartilage discs, an increase in denatured collagen was observed, and fibronectin and proteoglycan synthesis were altered over 10 days of culture (changes consistent with the development of osteoarthritis) [69]. Additionally, chondrocytes in the superficial zone of cartilage appear to be more vulnerable to cell death under injurious loading than middle or deep zone chondrocytes (independent of depth-dependent variations in compressive strain) [70]. A proposed mechanism for chondrocyte death due to impact loading is rupturing of the cell membrane under the high strain rates, as opposed to gradual unfolding of the "ruffled" cell membrane that is thought to occur during physiological loading, and has been observed in bovine osteochondral explants [71].

Injurious loading of bovine cartilage explants also saw cell death and a drop in biosynthetic activity for the remaining cells three days after injury [68]. Interestingly, chondrocytes in injured cartilage had significantly less response to subsequent dynamic loading compared to controls – in normal cartilage this dynamic loading would see an upregulation in matrix synthesis [68]. Proposed explanations for this failure to respond positively to dynamic loading were changes to the cells themselves, or that damage to the ECM potentially interrupted normal mechanotransduction signalling [68].

A comparison of in vivo animal studies illustrated negative effects of injurious loading in the long-term [72]. Where an overloading event was followed by normal weight bearing, different studies consistently

observed the deterioration of cartilage in the months following injury, specifically including the loss of chondrocytes, chondrocyte clustering and loss of proteoglycans [72].

Given resident chondrocytes cannot be relied on to migrate to and remodel the edge of a cartilage defect, many cartilage repair strategies thus involve supplying stem cells or adult chondrocytes, or releasing reparative cells from bone marrow via microfracture. Strategies to elicit migration of endogenous cells have also been considered, including enzymatic digestion of cartilage matrix or the use of chemotactic agents [26]. Recent approaches under development to facilitate integration are described in Section 4.

Whilst viable cells are essential to grow tissue and repair cartilage defects, what happens to this nascent tissue at the defect edge when the joint is loaded? And what loads are beneficial to the regenerating tissue, and not excessive or damaging?

3. Mechanical conditions surrounding the defect

3.1 Integration is dependent on new ECM linking with the surrounding tissue

Integrative repair is characterised by the formation of a collagen network in the defect or repair tissue, and importantly, this network linking up with the existing matrix in the non-damaged cartilage [73, 74].

In cartilage ECM the formation of collagen crosslinks, covalent bonds between collagen molecules, are catalysed by the enzyme lysyl oxidase and increase with maturity of the tissue [18]. Bovine cartilage explants cultured in pairs in a medium containing a known inhibitor of lysyl oxidase (β-aminopropionitrile) failed to develop any adhesion between the two cartilage surfaces after 14 days [73]. In the presence of the inhibitor, the interfacial strength was virtually non-existent (0.1 ±0.1kPa) and significantly lower than in the absence of inhibitor (36.3 ±3.3kPa, $p<0.001$) [73]. Integration was inhibited without the action of lysyl oxidase to potentially form covalent crosslinks, suggesting that these are a fundamental component of integrated cartilage repair.

Culturing constructs in medium containing lysyl oxidase has been shown to enhance integrative repair between cartilage explants and both cartilage and engineered tissue in vitro (Figure 2). Discs of engineered tissue or native cartilage were press-fit into bovine articular cartilage rings and cultured under static conditions in medium containing lysyl oxidase, and were well integrated morphologically (engineered or native tissue is flush against the outer ring with no visible gaps) [75]. However, biomechanical evidence of integration was only observed for engineered tissue constructs in contact with native cartilage, which saw a significantly higher tensile stiffness and failure strength for lysyl oxidase-treated samples compared to controls [75].

Treatment with lysyl oxidase has also improved the mechanical properties of self-assembled neocartilage grown from human articular chondrocytes, when combined with TGF-β1 and chondroitinase-ABC [76]. Further investigation is required to understand how lysyl oxidase could be translated for in vivo use, in particular, how it could be applied or delivered to a defect to assist integration of engineered constructs with native cartilage.

Further evidence has shown that the strength of the interface between repaired and native cartilage is dependent on the deposition of collagen at the interface [77]. A positive correlation was found between the interfacial strength of two bovine cartilage surfaces and the deposition of collagen, as quantified by incorporated [^{3}H]proline [77]. After 14 days of culture in apposition, there was also no statistically significant difference between the interfacial strength of live:live cartilage pairs, and live:decellularized cartilage pairs (29.3 ±4.2 kPa and 32.2 ±5.1kPa respectively) [77]. Though incorporated [^{3}H]proline levels were significantly lower in decellularized cartilage than the opposing live cartilage with which it was paired, radiolabel incorporation levels were still significantly higher than the background level observed in decellularized cartilage only pairs. This evidence suggested that matrix components that appeared in decellularized cartilage were produced in live cartilage, and transported to the boundary (there was no evidence that cells had migrated to the interface themselves) [77].

It is clear collagen crosslinking is necessary for integration to ensure physical links between repair and host tissue. This is not likely sufficient to recreate a continuous and functional matrix however, which is also dependent on proteoglycan content to restore the viscoelastic properties of healthy cartilage.

3.2 Loads are abnormally distributed across repair and native tissue

Damage to cartilage invariably sees disruption to its ability to dissipate mechanical loads. Pairs of bovine cartilage explants were held opposed and uniaxially compressed to study contact mechanics between the two cartilage surfaces [78]. Full-thickness defects were created in one explant of each pair to study the impact of a focal defect on deformation of the surrounding tissue and surface sliding [78]. Authors observed that at peak displacement in the presence of a defect, axial and shear strains at the edge of the defect were significantly higher than intact samples [78]. Significant effects were also observed in the cartilage opposing the defect, and though stress relaxation saw axial strains quickly return to equilibrium levels seen in intact samples, higher lateral and shear strains persisted [78].

Heterogeneous strain profiles were also seen in a study of cartilage defects in porcine knee explants in vitro, repaired with agarose [79]. Measured under compression, strain profiles varied both laterally (across the interface of cartilage and agarose) and through the depth of the cartilage or agarose. Lateral normal strains, parallel to the surface of cartilage, were significantly higher for agarose measured at the boundary with native cartilage, than agarose further from the defect edge or the native cartilage itself (by 4 times and 3.5 times respectively) [79]. Native cartilage also recovered more slowly in response to loading compared to agarose, owing to differences in viscoelastic properties between the tissue and the gel. The authors suggest that the interface thus experiences “residual stress” in bridging the two [79]. Though matching the time-dependent mechanical properties of hydrogels and engineered tissue to cartilage may be important, the stiffness or elastic modulus of the biomaterial tends to be the focus of material comparisons in the literature. Further investigation is required to characterize the viscoelastic properties

of hydrogels and engineered tissues, to understand how they compare to cartilage and how the viscous properties may play a role in cell differentiation and the development of functional neocartilage [80-82].

Measurements in both studies were performed under only uniaxial compressive loads (whilst the knee joint is of course capable of a multiaxial range of motion). However, these results illustrate the significant changes to the biomechanics of cartilage in the presence of a defect, and the imbalance in the distribution of loads between implanted scaffolds and native cartilage [78, 79].

Similarly, finite element modelling of a cartilage defect filled with a tissue engineered construct, found that a difference in mechanical properties (with constructs weaker than cartilage) created stress concentrations in the native cartilage close to the defect [83]. The tissue engineered construct was also predicted to experience increased compressive strains 33% higher than intact cartilage, reaching levels of strain which could impact cell height and volume (and potentially the behaviour of implanted cells) [83].

The presence of a defect and subsequent mismatch in mechanical properties between an implant and native cartilage causes significant variation in the mechanical environment compared to intact cartilage. The heterogeneous distribution of loads across the interface from repair tissue into native cartilage is seen at the macroscale and may also be felt at a cellular level. This abnormal environment may influence mechanotransduction pathways and thus the biosynthetic activity of chondrocytes, or if areas of stress concentration reach excessive levels, damage the structure of the ECM itself or cause cell death.

3.3 Mechanical loading disrupts matrix formation at a poorly integrated interface

Many in vitro studies of cartilage repair and integration focus only on static culture conditions that do not consider physiologically relevant factors such as the synovial fluid, and in particular, omit an essential component of the in vivo environment, mechanical loading. Additionally, when evaluating the degree of integration, many studies are often limited to reporting continuity of the tissue in terms of histology or biochemical content, which may not correlate with mechanically strong integration [74, 84].

Stress concentrations experienced by repair and host cartilage tissue were found to disrupt matrix formation in vitro [85]. A scaffold-cartilage explant cultured under dynamic loading for two weeks had significantly lower interfacial strength and GAG content compared to controls cultured under no loading [85]. A finite element model described the discontinuity in stress from the host cartilage to the scaffold interface, and suggested that micromotion caused by mechanical loading, as well as changes in stress and fluid pressure, prevented the matrix formation that was otherwise observed without loading [85].

Abnormal distribution of loads at the boundary between host and repair tissue may disrupt normal cellular processes. In the presence of a focal defect, computational modelling has shown cellular deformation near the defect, including changes to the actin cytoskeleton and higher nuclear stresses, which have been shown to affect chondrocyte gene expression [86].

Scaffolds seeded with cells prior to 28 days of static culture in cartilage explants exhibited some initial integration and development of interfacial strength [87]. Confined loading was then seen to have a positive impact on integration strength [87]. After two weeks of a sinusoidal 1N loading regime, confined scaffold-cartilage explants achieved push-out strengths of 50.2 ±1.6kPa, significantly higher than those without any loading [87]. However, under a load of magnitude 6N, push-out strength was similar to unloaded controls and significantly lower than for the 1N loaded samples [87]. 6N loaded samples also exhibited only partial gap filling based on micro-CT analysis and 3D reconstruction, as compared to the completely filled gap seen for 1N loaded samples [87].

Finite element modelling of the system found that the interfacial strength after 28 days culture (prior to loading) was the main factor associated with strength after loading - not discontinuities in compressive and shear stress between the scaffold and cartilage [87].

The type of mechanical load provided has an impact on chondrocyte metabolism, in addition to the magnitude [88]. In healthy cartilage, intermittent hydrostatic pressure promoted the expression of type II

collagen and aggrecan. In osteoarthritic cartilage intermittent hydrostatic pressure also up-regulated expression of these matrix proteins, whereas shear stress down-regulated their expression, and saw an increase in the release of nitric oxide (and chondrocyte apoptosis) [88].

Furthermore, the type of mechanical load may also impact integration of an implant. Push-out strength increased when scaffolds seeded with cells implanted into osteochondral defects were cultured in a spinner bioreactor, where magnetic stirring provided only shearing and not compressive forces [89]. Greater diffusion of nutrients was suggested as one possible reason for the improved integration [89].

Mechanical stimulation by low-intensity ultrasound has also been shown to regulate chondrogenesis in vitro and improve integration [90, 91]. Following 56 days of continuous low-intensity ultrasound treatment, the interfacial strength of ring/core bovine cartilage explants as measured by a push-out test was almost 4 times higher than untreated controls (0.19±0.08 MPa compared to 0.05±0.03 MPa, p=0.005) [91].

Recreating the complex loading conditions experienced by cartilage in vitro is challenging. A recently developed multi-axial loading system, using a ceramic ball for dynamic compressive and sliding loads, may support future insights into the impact of the mechanical environment on the development of neocartilage and integration [92].

Focal defects cause abnormal stress and strain distributions across the interface of repair and native cartilage. This compromised biomechanical environment can impact cell behaviour (which may have already been damaged by injury to the native cartilage). Though mechanical stimulation is an important part of regulating the turnover of matrix in healthy cartilage, without any physical link between repair and native tissue, loading may cause micromotion and inhibit the formation of matrix at the boundary. It appears that applying a load to an already partially integrated interface may benefit further integration, up to a limit. What this loading threshold is, its characteristics (i.e. compression, shear, or a combination of them), and the prerequisite amount of interfacial strength that is needed require further investigation.

This is extremely important from the standpoint of rehabilitation post surgery: knowing the specifications of load that is beneficial for graft-cartilage integration versus detrimental will be paramount in driving the type of rehabilitation regime required.

Possible strategies to overcome this problem are discussed in Section 4, including the use of bioadhesives to secure a scaffold, or in situ bioprinting for better defect filling and contact with native cartilage.

4. Current strategies for cartilage repair

Several biomaterial-based cartilage repair strategies have advanced to clinical trials, including both acellular hydrogel matrices to support and fill chondral defects, and expanded autologous chondrocytes seeded onto synthetic or natural scaffolds before implantation [93, 94].

One approach employed a bioadhesive layer to secure a polyethylene glycol (PEG) hydrogel in cartilage defects following microfracture, with promising results at 24 months in terms of tissue thickness and knee function scores [95]. However, delamination of cartilage tissue from the subchondral bone was observed in approximately 28% of patients by 24 months [95].

A five year follow-up of a chitosan scaffold saw significantly greater defect filling by MRI analysis compared to microfracture treatment alone [96]. Significantly improved clinical scores were also observed at one and five years, though no difference was demonstrated between the two treatments, with the same clinical improvement seen following microfracture treatment alone [96].

Larger and longer term follow up of these and other approaches will be informative, given existing surgical treatments like microfracture see improvement in knee function over the short term (1-2 years), with deterioration often observed in subsequent years [9].

A range of cartilage repair strategies evaluated using in vivo animal studies over the past five years are summarised in Table 1. Animal models continue to be an important method for assessing the success of articular cartilage repair strategies, given the challenges of replicating physiological conditions in vitro. Of

course, each of these models has its own limitations as a stand in for the human knee joint, e.g. different gait mechanics (murine and lapine models), spontaneous healing (lapine models), higher joint loading conditions (equine models) [11, 97]. Further trade offs exist in terms of cost, logistics and ethical concerns associated with housing larger over smaller animals, and considering the variability across these models, care must be taken in comparing results across different animal studies [97, 98]. Given these limitations, the development of ex vivo models utilising human tissue may reduce the reliance on expensive animal models in future, and allow for a broader range of analyses (by employing non-destructive imaging techniques, as well as traditional histology) [99].

In situ forming hydrogels, and an expandable scaffold, have been used to improve filling of a defect, or combined with an adhesive component to improve fixation [100-105]. Several methods employ a bi-phasic or bi-layered approach to support regeneration of the subchondral bone and cartilage, by implanting a rigid scaffold in combination with a softer gel phase or layer [106-110]. Multi-layered scaffolds or gradient scaffolds also exist to replicate the zonal architecture of cartilage down to the subchondral bone [111, 112].

Factors to induce chondrogenesis or recruit endogenous cells are also often employed. Chondro-inductive factors used include TGF-β1, bone morphogenic protein 2 [113, 114], the ECM protein matrilin-3 [115] and the small molecule kartogenin [116, 117]. Homing of cells has also been induced via scaffolds functionalised with affinity peptides [107, 108, 118].

Many of the studies listed employ short-term animal studies for proof-of-concept, and may not cover a timespan sufficient to evaluate integration and ultimately the potential long-term success of the repair method. In a large study of microfracture treatment in rabbits, though successful development of hyaline cartilage was seen at early timepoints, fibrillation, fissuring and degeneration of the tissue was seen from 24 weeks [11].

To assess integration, most studies are also limited to macroscopic observations, histology and biochemical assays. Although these measures are important in understanding the nature of the repair

tissue produced, tissue that appears continuous may not correlate to mechanically strong and well-integrated tissue [84].

Of the studies listed, roughly half assess the biomechanical properties of the repair tissue, though variation in the mechanical test performed and protocols used can make direct comparison of mechanical outcomes across the studies difficult [119]. Furthermore, even fewer studies assess the interfacial strength of the boundary between repair and host tissue. Assessment of this integration strength is also not standardised. Methods include a push-out test using an indenter to measure the maximum force required to dislodge the repair tissue, or a tensile test of dumb-bell shaped slices of repaired cartilage, where the interface of repair and host tissue is at the centre [84, 112].

Testing following treatment of cartilage defects in a porcine model with scaffold-free cultured constructs found that interfacial strength was significantly dependent on both the treatment, and the depth of the tissue (with superficial zone samples the weakest) [84]. Though repair tissue near the centre of the defect and at the interface with native tissue were not observed to be significantly different in terms of histological scores, all specimens failed at the integration boundary [84]. This study highlights the importance of mechanical quantification of the interfacial strength between repair and host tissue to understand if the interface is not only continuous morphologically, but physically linked.

Conclusion

Long-term repair of articular cartilage via surgical methods or tissue engineered techniques remains elusive. A major barrier to successful repair is integration with native cartilage tissue. Chondrocyte death in the tissue surrounding a cartilage defect, and the immobility and potentially altered behaviour of chondrocytes in the adjacent tissue, necessitate repair methods that deliver viable cells to the interface. Whether through biomaterials seeded with cells or improved strategies to elicit chondrocyte migration, viable chondrocytes at the edge of a cartilage defect are essential for integration. Given the sensitivity of chondrocytes to the mechanical environment, altered mechanical conditions caused by the defect are a

significant obstacle to overcome in cartilage repair. The abnormal distribution of loads across the interface from repair to host tissue can impact the behaviour of chondrocytes and their ability to remodel ECM and link with native cartilage. Adhesion to provide initial fixation of a scaffold and support long-term integration may be key for any regenerative attempt in this field. Many promising tissue engineering approaches exist to overcome these problems, and given the primary function of cartilage as a load bearing tissue, methods that assess the biomechanical integrity of the developing interface are essential to evaluate integration and the potential long-term success of these cartilage repair methods.

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Fig. 1

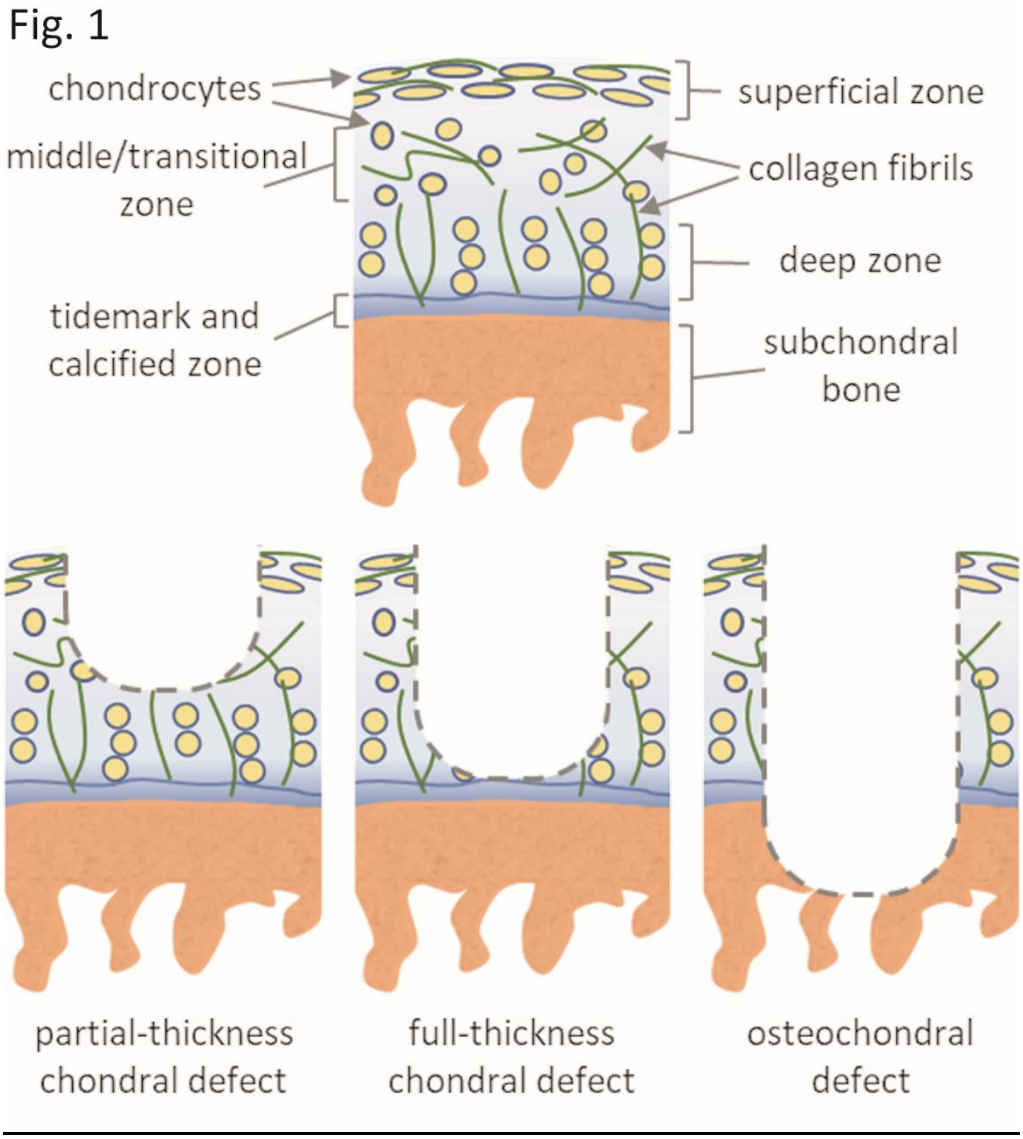

Figure 1

The composition of articular cartilage varies with depth of tissue (top), including orientation of collagen fibrils and morphology of chondrocytes. Cartilage defects are classified as partial-thickness or full-thickness chondral defects (bottom, left and middle), or osteochondral defects (bottom, right) which penetrate the calcified cartilage or subchondral bone.

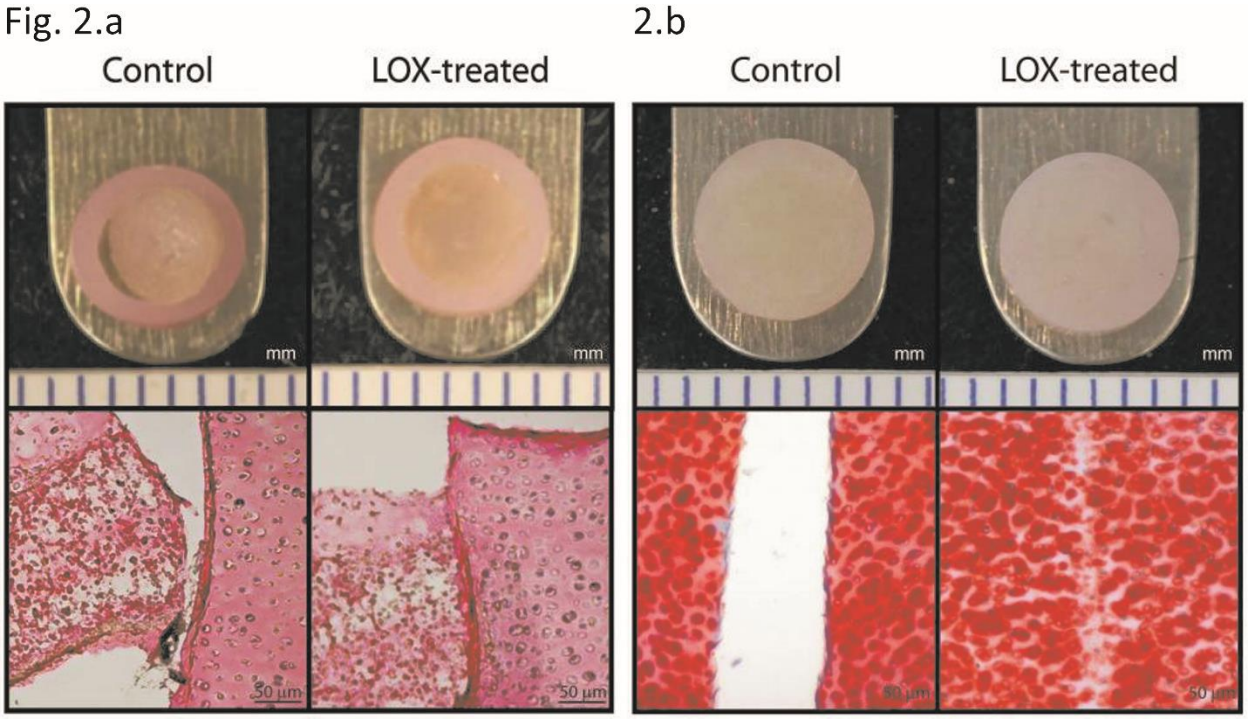

Figure 2

Construct-to-native cartilage assemblies (A) and native-to-native cartilage assemblies (B) following 14 days integration time. Gross observations (top) saw greater continuity than untreated controls for construct-to-native cartilage assemblies treated with lysyl oxidase, but no difference for native-to-native cartilage assemblies. Histology with picrosirius red (bottom) clearly illustrated differences in continuity compared to controls for both assemblies following treatment with lysyl oxidase (scale bar 50µm). Figure is reproduced from Athens et al and is licensed under CC BY 4.0 [56].

Study	Repair method	Cell source	Animal model, defect type	Length of study	Outcome	Biomechanical assessment of neocartilage?
[84]	scaffold-free tissue-engineered constructs derived from synovial mesenchymal stem cells	pig synovial mesenchymal stem cells	Pig model, chondral	24 weeks	cartilaginous tissue formed and well integrated, morphologically	Tensile test for integration strength - integration strength was significantly lower than native cartilage, and significantly dependent on the layer of tissue (superficial/middle/deep)
[100]	in situ bioprinting of gelatin methacrylamide (GelMa) and hyaluronic acid methacrylate (HAMA) hydrogel and cells	sheep mesenchymal stem cells	Sheep model, chondral	8 weeks	formation of hyaline-like cartilage and better macro/microscopic characteristics than controls, but poor lateral integration	Indentation - instantaneous Young's modulus, equilibrium modulus and maximum stress did not detect statistically significant differences, at low sample size with large standard deviation
[101]	gelatin-hydroxyphenylpropionic acid (Gtn-HPA) conjugate hydrogel seeded with cells	rabbit chondrocytes	Rabbit model, osteochondral	12 weeks	formation and integration of hyaline-like cartilage was superior for medium stiffness hydrogel, compared to low or high stiffness hydrogels	-

Study	Repair method	Cell source	Animal model, defect type	Length of study	Outcome	Biomechanical assessment of neocartilage?
[102]	4-arm star PEG hydrogel seeded with cells	mouse chondrocytes	Mouse model, osteochondral	12 weeks	formation of hyaline-like cartilage observed integrated with native cartilage	-
[103]	cells encapsulated in methacrylated gellan gum	rabbit autologous adipose stem cells	Rabbit model, chondral	8 weeks	neocartilage formed, filling defect and integrating with native tissue	-
[104]	expandable gelatin scaffold seeded with cells	rabbit chondrocytes	Rabbit model, osteochondral	24 weeks	hyaline-like cartilage tissue formed, and improved integration with host tissue compared to controls	Compression - Young's modulus for treatment significantly higher than controls, and after six months no significant difference with modulus of intact cartilage
[105]	photocrosslinkable PRP hydrogel glue (HNPRP), based on modified hyaluronic acid	-	Rabbit model, osteochondral	12 weeks	filled defects with hyaline-like cartilage formed, with significantly higher macroscopic and histological scores than controls	-

Study	Repair method	Cell source	Animal model, defect type	Length of study	Outcome	Biomechanical assessment of neocartilage?
[106]	biphasic silk fibroin scaffold, fiber-free and fiber-reinforced phases	-	Rabbit model, osteochondral	8 weeks	cartilage and subchondral bone formed, with A. assamensis silk biphasic scaffold performing better than B. mori	-
[107]	decellularized porcine articular cartilage matrix and functionalised nanofiber peptide hydrogel	-	Rabbit model, osteochondral (with microfracture)	24 weeks	hyaline-like cartilage repair and subchondral bone reconstruction	Nanoindentation - significantly higher hardness, contact stiffness and reduced modulus compared to controls, and no significant difference to native cartilage
[108]	demineralized bone matrix integrated with chitosan hydrogel and mesenchymal stem cell affinity peptide	-	Rabbit model, osteochondral	24 weeks	formation of hyaline-like cartilage and superior repair compared to controls	Nanoindentation - significantly higher hardness and reduced modulus than controls, and close to native cartilage
[109]	decellularized horse cartilage-derived matrix, with or without calcium phosphate base	-	Horse model, osteochondral	24 weeks	defect filling was less than in a pilot study (horse model for eight weeks), and repair tissue was inferior though	Micro-indentation - Young's modulus was significantly lower than native cartilage, and there was no difference between scaffolds with or without calcium phosphate base

Study	Repair method	Cell source	Animal model, defect type	Length of study	Outcome	Biomechanical assessment of neocartilage?
					bone part was well integrated when calcium phosphate used	
[110]	silk fibroin layer and silk-nano calcium phosphate layer	-	Rabbit model, osteochondral	4 weeks	formation and integration of hyaline-like cartilage and subchondral bone	-
[111]	multi-layered collagen scaffold	-	Goat model, osteochondral	12 months	formation of hyaline-like cartilage and subchondral bone, and improved healing compared to controls	-
[112]	gradient hydrogel of chondroitin sulphate nanoparticles (chondral zone) and nanohydroxyapatite		Rabbit model, osteochondral	8 weeks	hyaline-like and mineralized neotissue formed	Push-out test for interfacial strength - significantly higher interfacial stress for gradient hydrogel compared to controls

Study	Repair method	Cell source	Animal model, defect type	Length of study	Outcome	Biomechanical assessment of neocartilage?
	(subchondral zone), in two zones of alginate/poly(vinyl alcohol) (PVA)					
[113]	growth factor loaded PLGA microspheres in an alginate-PLGA scaffold (growth factors tested were TGFβ1 and BMP-2)	-	Rabbit model, osteochondral	24 weeks	qualitatively better cartilage formed with loading of 5μg BMP-2	-
[114]	alginate beads containing cells and platelet-rich plasma (PRP)	human adipose mesenchymal stem cells (ADSCs)	Rabbit model, osteochondral	16 weeks	hyaline-like cartilage formed, and repair significantly improved by treatment compared to controls	-
[115]	codelivery of matrilin-3 with cell suspension in hyaluronic acid	human infrapatellar adipose-tissue-derived	Rat model, osteochondral	12 weeks	hyaline cartilage formed and repair significantly improved with matrilin-3 treatment, with complete integration	-

Study	Repair method	Cell source	Animal model, defect type	Length of study	Outcome	Biomechanical assessment of neocartilage?
		mesenchymal stem cells (Ad-MSCs)			seen at a concentration of 140-ng matrilin-3	
[116]	acrylated hyaluronic acid hydrogel with kartogenin-loaded nanoparticles (PLGA)	-	Rabbit model, osteochondral	12 weeks	formation of hyaline-like cartilage tissue and subchondral bone observed, significantly higher histological scoring than controls	Nanoindentation - significantly higher reduced modulus and hardness than controls, and close to native cartilage
[117]	kartogenin (KGN) incorporated polylactic-co-glycolic acid (PLGA)-PEG-PLGA thermogel loaded with cells	bone marrow mesenchymal stem cells	Rabbit model, osteochondral (with microfracture)	12 weeks	Smoother and more integrated hyaline-like cartilage formed compared to controls	Nanoindentation - reduced modulus and hardness were significantly higher than controls, but lower than native cartilage
[118]	silk fibroin and gelatin scaffold, conjugated with bone marrow stem cell-specific-affinity peptide, and seeded with cells	rat bone marrow stem cells	Rabbit model, osteochondral	24 weeks	formation of neo-cartilage with characteristics more similar to native cartilage than controls	Nanoindentation - significantly higher hardness than controls, and reduced modulus closer to native cartilage than controls

Study	Repair method	Cell source	Animal model, defect type	Length of study	Outcome	Biomechanical assessment of neocartilage?
[120]	artificial cartilage particulates (ACPs) - collagen I microspheres seeded with cells and cultured prior to implantation	allogenic rabbit chondrocytes	Rabbit model, osteochondral	12 weeks	neocartilage formed, ACPs cultured for seven days showed better integration	Neocartilage not assessed, however nanoindentation performed prior to implantation
[121]	acellular cartilage sheets (ACSs), implanted with or without cells	pig bone marrow stromal cells (BMSCs)	Pig model, osteochondral	24 weeks	ACSs with BMSCs achieved best cartilage repair	Compression- Young's modulus and maximum compressive strength close to native cartilage for ACSs with BMSCs, and significantly higher than ACSs alone and controls
[122]	PLGA scaffold with BMP-7 loaded PLGA nanoparticles, seeded with cells	rabbit synovium-resident mesenchymal stem cells	Rabbit model, osteochondral	6 weeks	formation of hyaline-like cartilage	-