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Systematic Comparison of Biomaterials-Based Strategies for Osteochondral and Chondral Repair in Large Animal Models

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Joint repair remains a major challenge in orthopaedics. Recent progress in biomaterial design has led to the fabrication of a plethora of promising devices. Pre-clinical testing of any joint repair strategy typically requires the use of large animal models (e.g., sheep, goat, pig or horse). Despite the key role of such models in clinical translation, there is still a lack of consensus regarding optimal experimental design, making it difficult to draw conclusions on their efficacy. In this context, the authors performed a systematic literature review and a risk of bias assessment on large animal models published between 2010 and 2020, to identify key experimental parameters that significantly affect the biomaterial therapeutic outcome and clinical translation potential (including defect localization, animal age/maturity, selection of controls, cell-free versus cell-laden). They determined that mechanically strong biomaterials perform better at the femoral condyles; while highlighted the importance of including native tissue controls to better evaluate the quality of the newly formed tissue. Finally, in cell-laded biomaterials, the pre-culture conditions played a more important role in defect repair than the cell type. In summary, here they present a systematic evaluation on how the experimental design of preclinical models influences biomaterial-based therapeutic outcomes in joint repair.

1. Introduction

The limited healing capacity of cartilage means that any traumatic injury or pathological damage to the joint surface can progress to an advanced degenerative state[1] and osteoarthritis (OA). Current treatments for early stage osteochondral degeneration include debridement, autologous chondrocyte implantation (ACI), microfracture (MF), subchondral-drilling, mosaicplasty, and allografting, while more aggressive late-stage OA often requires arthroplasty. Whilst short-term improvements are evident following ACI and MF, long-term effects are inconsistent.[1,2] Similarly, although joint replacements demonstrate great success, they are not a permanent solution and many patients will require a second, often less successful, revision arthroplasty years later.[3]

It is clear that alternative approaches for osteochondral regeneration are required. Tissue engineering (TE) represents one potential option for the treatment of chondral and osteochondral lesions.[4] TE aims to restore the structure and function of native cartilage and bone, typically combining biomaterials (providing structural support and/or cues for cell growth and differentiation) with cells to restore the native tissue.[5,6] Despite many innovative biomaterial-based technologies developed to address osteochondral regeneration in recent years, only a handful of products have received regulatory approval and an even smaller number are currently under

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Table 1. Inclusion and exclusion criteria applied in literature search.

Inclusion criteria	Exclusion criteria
English language	Studies investigating joints other than knee
Large animal model, that is, pig, goat, sheep, and horse	Articles investigating meniscal repair
Biomaterial-based treatments, for example, scaffolds, hydrogels, fibrin glue	Biomaterial-based treatments incorporating growth factors, genetic material, (platelet-rich) plasma, or other biological fluids
Low risk of bias	
Literature published between January 2010–December 2020	Literature published prior to 2010, or later than December 2020

investigation in human studies.[7] This is partly due to the stringent review process a new device must face to receive regulatory approval. Prior to human evaluation, preclinical data must establish sufficient scientific rationale for clinical investigation. Efficacy, durability, favorable toxicology profiles, and dose responses (where applicable) should be evident.[8] To demonstrate proof-of-concept during the final stages of product development, regulatory agencies such as the Food and Drug Administration (FDA) also recommend evaluation in large animal models[9] whereby macroscopic, histological, biomechanical, and biocompatibility tests are used to thoroughly assess safety and efficacy.[10] It is essential that preclinical findings are wholly reliable and accurate to successfully progress product development and clinical translation. Ultimately, the reliability of experimental findings depends on how rigorously and robustly the study was designed. There are a large number of potential sources of bias that might result in an experimental outcome or study conclusion that deviates from the truth—some examples are selection bias which can result from lack of randomization or attrition bias due to unjustified missing results.[11] This risk of bias (RoB) can be managed by implementing rigorous experimental planning.[12]

A deeper understanding of preclinical study design in osteochondral defects (OCD), such as informed choice of animal model, optimization of defect size and location and use of appropriate control, is paramount to help investigators accurately validate findings and maximize study impact, ultimately enhancing the next generation of product development while remaining cognizant of clinical testing and regulatory approval requirements, and reducing the RoB. Herein we review a variety of studies, published within the period of January 2010 to December 2020, which employed large animal models in preclinical testing to investigate biomaterials for cartilage/osteochondral repair—including both cell-free and cell-seeded approaches. Specifically, we performed a RoB assessment to identify studies with superior experimental design allowing for more robust, clinically relevant conclusions. We have determined a series of key factors that should be taken into consideration in order to standardize large animal preclinical study design, and which consequently will advance the effective translation of novel therapeutics to the clinic.

2. Experimental Section

2.1. Search Criteria

The research articles were retrieved by using PubMed (www.ncbi.nlm.nih.gov/pubmed/). Search terms were adapted from Leenaars et al.[13] Briefly, the following search terms were defined: "cartilage & osteochondral", "tissue engineered", "animals", a full description of the search terms is available in the Supporting Information. Search filters adapted from Hooijmans et al.[14] were applied. **Table 1** illustrates the full list of inclusion/exclusion criteria enforced. Briefly, low RoB publications focused on evaluating the treatment of knee cartilage defects with biomaterials, both cell-free and cell-seeded, in goat, horse, goat, and pig models were included. Exclusion criteria included studies investigating meniscal lesions or joints other than the knee. Studies employing biomaterials incorporating growth factors, other biomolecules or genetic material were also excluded. However for a detailed insight into gene-activated scaffolds on cartilage repair we would like to direct the reader to the following articles by Kelly et al.[15] and Chen et al.[16] Additional publications are also recommended for a more thorough review of regenerative biomaterials for OCD repair[17] and biofabrication-based techniques for cartilage repair.[18]

2.2. Risk of Bias Assessment

Methodological quality was assessed according to **Table 2**. RoB analysis was performed utilizing modified versions of the Office of Health Assessment and Translation (OHAT)[11] and Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)[19] tools. Selection, detection, attrition, and reporting bias was scored independently by L.B.F. and K.L.B. using the questions below. "Yes" and "not applicable" answers were scored 0 (indicating low RoB), "unclear" scored 0.5 (indicating some RoB), and "no" scored 1 (indicating high RoB). An overall score ≤ 2 signified low RoB. Scores above 2 suggested high RoB, and these papers were excluded. Where there were differences in opinion between assessors, results were discussed until consensus was reached.

3. Factors Influencing Outcome when Investigating Articular Joint Repair in Large Animal Models

3.1. Risk of Bias

A total of 616 independent studies were identified (PubMed) including 329 using pigs, 72 goat, 140 sheep, and 75 horse models. 543 articles were removed upon application of exclusion criteria (Table 1), leaving 73 articles remaining to evaluate RoB. 41 articles were deemed to have low RoB (Table S1, Supporting Information) and were further evaluated. From those 41 articles, 26 studied OCD repair and 15 studied cartilage only defects (Table S2, Supporting Information).

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Table 2. Questions asked to determine risk of bias. Further details on the RoB analysis are described in Table S1, Supporting Information.

Bias Domain	Signalling question	Response
Selection	Was there a sample allocation sequence provided?	Yes/Not Applicable/No/Unclear
Detection	Was there a blinded assessment of outcome?	Yes/No/Unclear
Attrition	Were all outcomes included and if not, was there an explanation?	
Reporting	Are reports free of selective outcome reporting?	
	Was an appropriate validated outcome used?[10]	
Other	Was ethical approval included?	
	Were there no potential conflicts of interest?	

3.2. Animal Models

All new treatments are tested in animal models to ensure safety, feasibility, and efficacy before trialling in human patients. Factors including animal species and age are important to ensure comparability to human pathologies and healing processes. Osteochondral defect size and anatomical location are also crucial. While there is currently no perfect preclinical model to simulate joint defects, pigs, goats, sheep, and horses exhibit the closest human resemblance. Note that the aim of this review is not to discuss the pros and cons of each animal in depth, for this we direct the reader to Moran et al., and others.[20–22] However, we would like to outline some important characteristics of each model, highlighting potential sources of error in each case. Of the 41 studies analyzed, there were 17 in pigs, 12 in sheep, 10 in goat, and 2 in horse models used.

3.2.1. Species Selection: Cartilage Thickness

Common pigs are relatively large and difficult to handle, therefore most cartilage studies utilize smaller miniature pigs (termed "minipigs") because they are easier to handle, and their joint size, weight, and cartilage thickness (0.5–1.5 mm in the medial femoral condyle)[23,24] are still relatively close to that of humans (1.69–2.55 mm[25]). Additionally, the bone apposition rate and trabecular thickness of the minipig are similar to that of human bones.

Sheep also present similar knee anatomy to humans, however there is some controversy regarding cartilage thickness, with some studies reporting just 0.45 mm[26] on the ovine femoral condyle, while others report up to 1.68 mm.[27,28] This discrepancy translates to variability in defect volume, which can subsequently lead to variable study outcomes, making it difficult to identify significant experimental differences. With thinner cartilage generally, sheep are perhaps more amenable for studying osteochondral defects, rather than evaluating cartilage alone. Additionally, the size and anatomy of the sheep knee facilitates second-look arthroscopy which is beneficial for follow-up studies.

Goats also allow for relatively easy second-look arthroscopy, and they generally have thicker cartilage than sheep (0.8–2 mm), making them suitable for both chondral and osteochondral regeneration. This is quite important since partial-thickness defects are more representative of human pathology.[29] However, like sheep, goats demonstrate significant variability in cartilage thickness which can lead to inconsistent results. While goats and sheep allow us to investigate small lesions similar to those in certain human patients (0.5–1 cm^2),[9] both animal models are complicated by stiff subchondral bone plates which if disrupted, can result in postoperative cyst formation.[30]

Horses, the largest model, have, on average, 1.5–2.0 mm thick articular cartilage—measured in three locations on the femoral trochlea and two locations on the medial femoral condyle.[31] Cartilage thickness, the presence of a vertically-loaded stifle joint during gait, and the possibility to study full or partial thickness defects, make horses a very suitable preclinical model for biomaterial investigation.[20] However, it is notable that the majority of studies in horses use trochlear defects—where cartilage thickness is at lowest end of the range—given the great technical challenge entailed upon continuous static loading of the weight-bearing medial condyle.[22] Consequently, the lateral trochlea of the femur, where loading is intermittent, is the most common location for cartilage defects in equine models.

3.2.2. Species Selection: Skeletal Maturity of the Host

With a view to clinical translation, another key consideration when choosing a preclinical model is skeletal maturity. Cartilage repair capacity declines significantly with age, being nearly non-existent in adult humans; similarly, cartilage thickness decreases with age.[33] The density and structure of the subchondral bone and the proliferative capacity of resident cells may also vary as time progresses, and so animal age is an important factor to consider when investigating OCD, MF, or debridement in these models.[9] Since the use of skeletally immature animals may greatly overestimate the repair potential, it's important to note that pigs reach skeletal maturity around 18 months;[33,34] sheep and goats between 2–3 years; and horses between 2–4 years. In this review, we found that sheep and horse studies primarily employed mature animals (**Figure 1**). In goat studies, at least 50% of the animals were mature (20% did not specify animal age). Interestingly, studies in pig predominantly used immature animals (70.6%).

Animal skeletal maturity is also critical when investigating cell-seeded devices, especially those employing autologous cells (including 83% of the cell-based therapeutics evaluated in this review). Differentiation and proliferation potential varies with age for both chondrocytes and mesenchymal stem cells (MSCs)—the primary cell types used in cartilage/osteochondral repair

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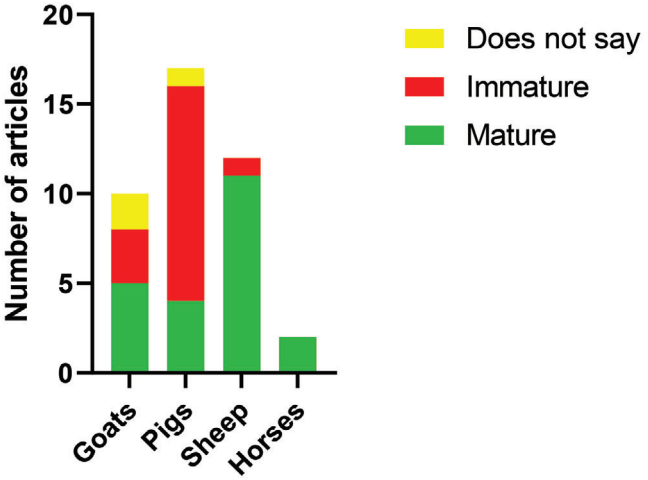

Figure 1. Analysis of skeletal maturity of animals used in preclinical studies for tissue engineering approaches for cartilage regeneration. Percentage of animal studies performed in mature versus immature animal models relative to the total number of studies for that model. Some reports did not specify maturity—recorded as "Does not say."

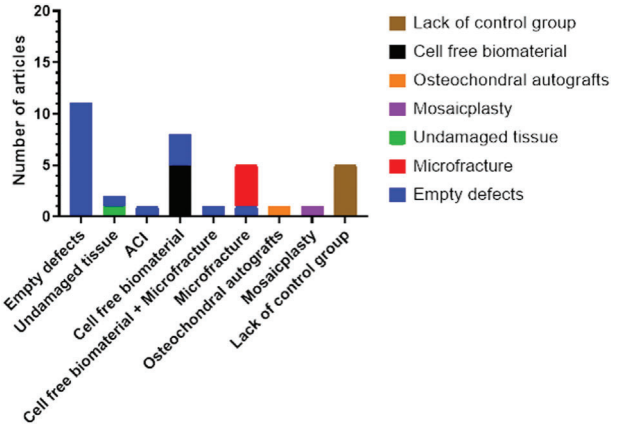

Figure 2. Comparators used in preclinical studies fortissue engineered (osteo-)chondral regeneration approaches. Each bar representsthe number of articles using the comparator listed in the X-axis alone or incombination with the comparator listed in the plot legend.

devices;[33] additionally it has been shown that cell age (passage) may influence the chondrogenic potential of the extracellular matrix (ECM) or the biomaterial-based device itself.[35]

3.2.3. Defect Size

Defect size is another crucial parameter. Critical size defects are defined as those in which spontaneous repair does not occur. Critical size varies between models according to species and skeletal maturity. In adult pigs, 6 mm defects have traditionally been considered critical,[36] although Vasara et al.[37] report spontaneous healing of this size defect in the skeletally immature animal. In sheep, defects up to 7 mm have been reported to heal spontaneously after 6 months,[38] with defects >8 mm considered critical.[39] In goats, 6 mm defects do not heal after 6 months, so are considered critical.[40] In horses, 9 mm defects are deemed critical,[31] however some studies have reported that 4 mm defects do not heal spontaneously.[41]

Taking into consideration the many differences observed among and between species, including skeletal maturity/age and inter- and intra-animal disparities in critical size, an empty (untreated) defect should always be included as an internal control in individual animals to negate any natural variations in cartilage thickness, which can influence study outcomes more than variability in skeletal maturity.[28,41]

3.3. Selecting an Appropriate Control

Choice of comparator group in clinical/preclinical trials is determined according to product type and the associated regulatory requirements. For cell-laden biomaterials intended to repair cartilage only defects, ACI has traditionally been the preferred control, although it is expensive and technically challenging. Regulatory agencies also accept MF as a control in specific circumstances, generally for small focal defects where MF is considered first-line human therapy.[7] Moreover, MF controls are recommended by some regulatory agencies such as the Food and Drug Administration (FDA), in smaller sheep/goat models investigating certain biomaterial-based devices and autologous grafts.[7] Extended guidance for comparator groups also indicates debridement, mosaicplasty, autogenous perichondral/periosteal or osteochondral allografts in specific circumstances.[8]

Figure 2 illustrates the different controls reported in this review. The most commonly used control was an empty/unfilled defect (51.2%), either alone or with an additional control. MF was used in 13% of studies (83% of which were cartilage-only defects), while mosaicplasty, ACI, and osteochondral autografts were included as comparator groups in 2.5% of studies.

3.3.1. Empty Defect

Empty defect controls should be considered essential in determining the degree of success of a new therapy. Not only are they useful to assess self-healing capacity, if used as an internal control in the same animal, individual variability can also be assessed, negating any controversy regarding "critical size", genetics, or skeletal maturity. Empty defects also allow us to control for anatomical and biomechanical differences in different locations of the knee which can influence intrinsic healing capabilities. For example, differences in gene expression, glycosaminoglycan (GAG) content, cell morphology, and matrix composition have been described in different locations within the joint.[42–50] It's therefore critical to ensure that empty defects are created in locations mirroring the defects being treated (i.e., in the contralateral limb) in order to obtain the most accurate comparison of regeneration and to maximize the statistical power of your study; that is, by minimizing the individual and location variance, also minimize the standard error within the treatment groups which then facilitate to discern the role of the treatments on the therapeutic outcome. An interesting approach is to utilize historical empty defects—from previous studies—as controls. This approach has some benefits such as the reduction of animals, which not only

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reduce cost but more importantly complies with the 3Rs principle. On the other hand, researchers must be very careful with maintaining as close as possible parameters such as skeletal maturity, defect size and location, among others that might influence the suitability of their comparator. Most of the studies reviewed (≈84%) confirmed empty defects were located in the same position as the treated group, although not all utilized bilateral models, thus the empty control was performed in a different animal, separate from the treatment group. 3 studies analyzed defects in two different locations which were randomly allocated to control or treatment groups.

3.3.2. Microfracture and ACI

Choice of MF versus ACI control primarily depends on the treatment being investigated. Care must be taken regarding damage aetiology and treatment mechanism of action for each study. MF and ACI are typically control groups for cartilage-only defects. The majority of studies reviewed using MF controls (with the subchondral bone unaltered) evaluated regeneration of the cartilage layer only. However, McCarrel et al.[51] used a MF control for an OCD repair biomaterial-based study. While they successfully demonstrated cartilage regeneration similar to the MF-control using a bone marrow-saturated biphasic graft, an osteochondral allograft control may have been more relevant in this case.

Just one study included ACI as comparator (together with an empty defect).[52] They showed that a decellularized biomaterial-based device significantly improved cartilage regeneration in a full-thickness porcine defect compared to both controls. Interestingly, when gene expression analysis was performed after 6 months, they noted significant differences in the genetic profile between controls and the native cartilage, with trends suggesting chondrocyte hypertrophy and cartilaginous calcification in the controls, illustrating clear differences in terms of the quality of newly formed tissue compared to the native undamaged tissue.

3.3.3. Native Undamaged Tissue Control

It is well known that achieving full repair of any articular cartilage or osteochondral defect is extremely challenging, indeed, to our knowledge, no TE-based approaches have ever resulted in full restoration of the defect to the levels of undamaged native tissue. However, we consider the use of native undamaged controls valuable, in that they may provide an extra level of knowledge and comparison to ultimately aid in assessing the true level of tissue regeneration achieved. From the studies assessed in this review, one study emphasized the importance of such controls as a positive comparator: they investigated articular cartilage regeneration in a minipig, using a type-I collagen (Col-I) scaffold seeded with articular chondrocytes, compared to an unseeded scaffold. They utilized an empty defect control, but also compared results to undamaged native cartilage tissue from ten healthy animals where no surgery was performed. There was no significant difference between the acellular treatments compared to the cell-seeded group, or between the acellular treatment groups compared to the native cartilage control obtained from the untreated healthy animals. However, the cell-seeded group had significantly poorer histological results compared to the native cartilage. So, despite the fact there were no direct differences between cell-seeded and cell-free scaffolds, by incorporating a healthy control group as an additional comparator, Schwarz et al.[53] identified important differences between the treatment groups. It is important to acknowledge that to have a group of untreated animals is costly and it certainly causes some ethical concerns. An alternative approach, used by 19.5% of the studies evaluated in this review, is to take samples of undamaged tissue in the animal's contralateral limb. This strategy allows the researchers to compare the newly formed tissue against undamaged tissue in the same animal, thus avoiding inter-animal variability and negating ethical and "3Rs" concerns, as the undamaged tissue is obtained from the same animal utilized for the treatment group.[54–61]

3.4. Defect Location

A strong relationship between healing capacity and defect location has been observed,[20] suggesting defect site can easily influence study outcomes when evaluating osteochondral and chondral repair devices. Different regions of the joint experience different load-bearing stresses. Similarly, surface geometry can also influence healing.[10]

The studies analyzed in this review exhibited several variations in defect location (**Figure 3**A). The majority of studies used the femoral condyle (71.41%), followed by the trochlea of the femur (19.05%). Moreover when analyzing further, according to each animal model, the femoral condyle was preferred in all animals except horses. In horse models, condylar defects are technically complex to perform, and healing is extremely challenging due to the continuous loading, thus the trochlea is the most common defect location in the horse (Figure 3B).

When analyzing the negative outcomes, it is important to note that unfortunately the majority of negative outcomes do not get submitted for publication or published, which then implicates a limitation on the analysis. However, from the studies in this review that reported negative results, the majority of the involved defects are located in the femoral condyle (63.63%), followed by the femoral trochlea (36.37%) (**Figure 4**A). Interestingly, when analyzing rate of device failure/negative results (percentage of negative results relative to the total number of studies using the same defect location), we found that regardless of the animal used, condylar defects have a failure rate of 23.33%, increasing to 50% in trochlear defects. However, variation between defect failure and defect location was also observed within each species (Figure 4B). We identified that biomaterial-based device failure is more common in pig's trochlea, while failures in goat and sheep models were more associated with condylar defects.

The trends observed here are in line with those reported in a retrospective analysis performed in minipigs which aimed to correlate defect location with successful osteochondral regeneration. Jung et al., concluded that medial femoral defects were more strongly associated with improved subchondral bone repair and higher quality hyaline cartilage compared to trochlear defects.[42] Similarly, another caprine study highlighted poorer healing capacity in trochlear defects compared to those in the medial femoral condyle.[40] Several variables may be affecting the

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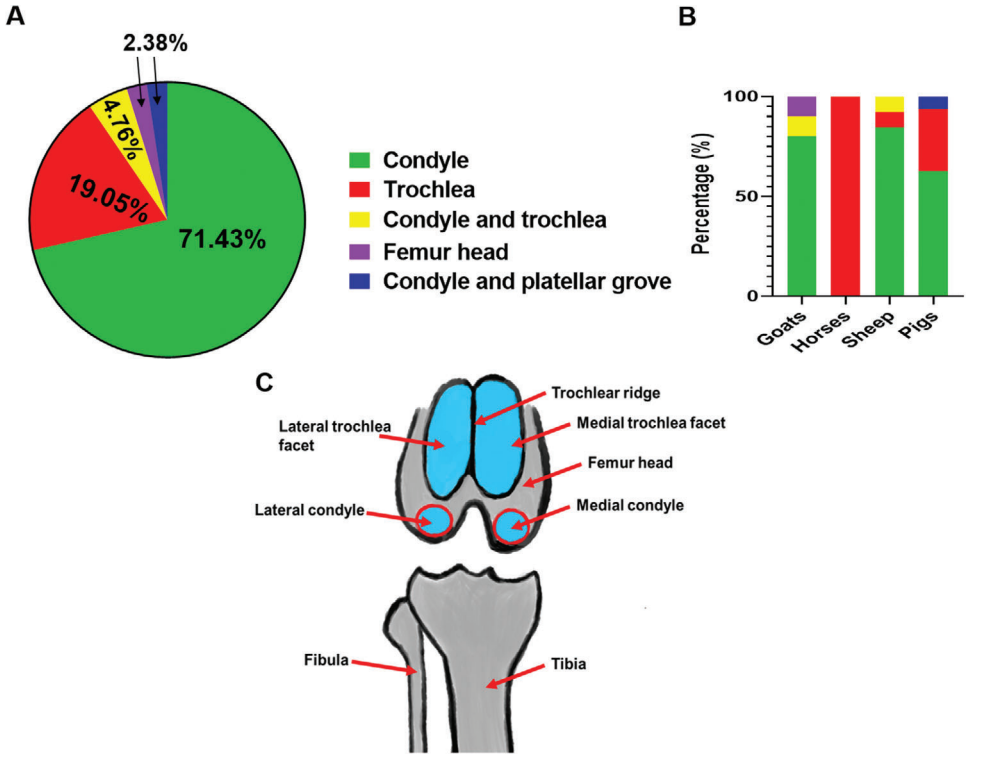

Figure 3. Percentage of studies according to defect location in the femur: A) in all models and B) according to each species. C) Schematic of the knee joint anatomy.

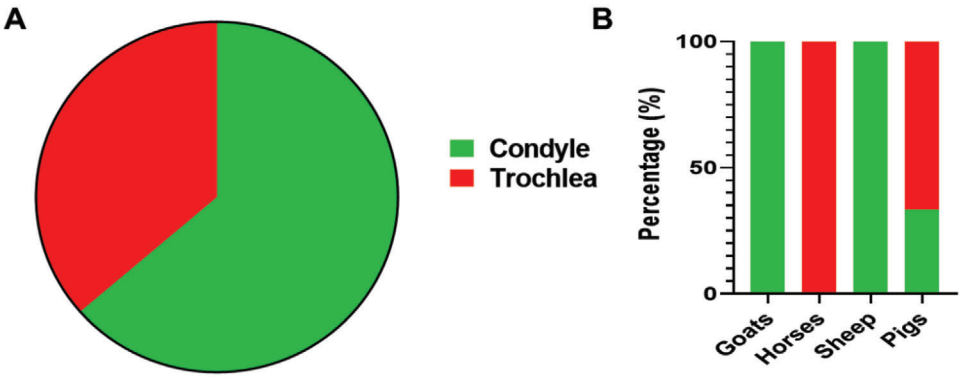

Figure 4. Percentage of studies with a negative outcome per defect location (A). Percentage of studies with a negative outcome per defect location and animal model (B).

outcome of these studies, however the role of mechanical stimuli in tissue regeneration is particularly well documented.[42,62–64] Brown et al.[65] studied mature bovine knees ex vivo and suggested that the inferior healing capacity observed in the femoral trochlea in these animals might be associated with lower mechanical stress (peak rim and radial) and differences in tissue geometry resulting in reduced bone and cartilage formation.[65] Taking into account the differences in mechanical stress between the femoral condyle and trochlea, it becomes extremely important to match defect location with the potential application/site of implantation of the biomaterial-based scaffold, that is, if the scaffold is designed to withstand mechanical compressive loading while promoting tissue repair, then the best location for biomaterial testing is the femoral condyle, which exhibits challenging mechanical compressive stress but better regenerative potential than the trochlea.

When evaluating biomechanical stress, it's important to consider the anatomical and loading differences between different animal models and humans. Very few studies have dedicated efforts to compare this range of species in the literature.[66] Patil et al.[67] did perform a thorough comparison of human versus caprine knee joints, finding major differences in the size and morphology of the trochlear groove, tibial plateau, and menisci. These anatomical differences can be correlated to variations

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observed in joint function. For instance during the normal gait cycle, goat joints are flexed 50–70°, while the human equivalent is flexed up to 30° only.[68] The nature of the bipedal movement characteristic to humans, means there is increased biomechanical stress at the knee joint compared to quadruped animals.

Taking all the evidence into account, and remembering that the ultimate goal of any osteochondral or chondral biomaterial is usually to enhance repair in humans, a complete biomechanical and anatomical study similar to the one performed by Patil et al.[67] comparing the human joint against goat, horse, sheep, and pig equivalents would be hugely beneficial, deepening our understanding of study outcomes and allowing us to optimize experimental design.

3.5. Acute versus Chronic defect

In this review, 73% of all studies employed acute defect models, with only 24% evaluating regeneration in chronic defects.

Acute chondral defects represent less challenging conditions for healing and therefore produce significantly better clinical outcomes compared to chronic defects. While it's known that cartilage rarely heals spontaneously, impromptu repair does occasionally occur with acute inflammatory responses driving the recruitment and activation of resident joint cells which facilitate defect filling.[69,70] However, these conditions are seldom encountered in the clinic, where patients typically present with subacute or chronic lesions. The predominant use of acute models in preclinical studies could therefore account for the discrepancy in treatment performance observed between animal studies and human clinical trials. Despite chronic models being more representative of human defects, financial and ethical concerns associated with multiple animal surgeries represent barriers to executing more accurate preclinical studies.[9]

For the purposes of this review chronic defects are defined as those created in an independent surgery, completed prior to implantation of the biomaterial-based device. Research suggests that in order to achieve a chronic defect most similar to that of human clinical presentations, the defect generated should be left untreated for 1–2 months.[71,72] However, we note that the length of time between defect creation and construct implantation in practice varied considerably, from just 1[53] to 8 weeks.[73]

The success/failure rate of each study was analyzed, with success defined as a significant improvement in cartilage regeneration compared to the control group at study end-point. 43% of studies performed in acute models reported no improvement following treatment, whereas just 20% of studies in chronic models failed to regenerate the tissue. One study evaluated their treatment in both acute and chronic defects (with 6 weeks between defect creation and implantation surgery in the chronic model). Vukasovic et al.[74] compared the efficacy of a pre-seeded (in vitro) bilayer biomaterial containing horse derived type-I collagen (cartilage layer), and a mineralized blend of type-I collagen and magnesium-doped hydroxyapatite. This implant was either seeded with nasal chondrocytes or implanted as a cell-free device into OCDs over 12 months. Interestingly, the authors noted macroscopic differences between the acute and chronic models after 3 months, that is, the chronic defect was only partially filled, while the acute defect was almost completely healed. However, after 12 months, while there were no histological differences between treatment and control groups in the acute model, the cell-seeded group significantly outperformed the cell-free construct in the chronic defect model. This supports previous findings that pre-seeded biomaterial-based devices show promise in chronic defect models where the inflammatory signals make the migration of healthy host regenerative cells to the defect more challenging; this also corroborates the importance of acute versus chronic defect model selection in osteochondral studies, ultimately highlighting the importance of developing a standardized model for cartilage studies, facilitating comparable and more robust preclinical trials.

3.6. Study Duration

When designing an experiment, study time-points are critically important. Short studies (≤3 months) can be useful to provide information on biocompatibility, immune response, implant stability, or initial cellular responsiveness.[9] Longer studies are more expensive, but are needed to provide additional knowledge regarding device efficacy, newly formed tissue quality, long-term safety, implant integration, and interactions with the surrounding tissue including the opposing articular surface.[20] When evaluating the healing potential of biomaterial-based osteochondral or cartilage devices in large animals, assessment should ideally continue in excess of 6 months, since this is the time point at which hyaline cartilage formation and implant integration are first observed.[75]

In this review, considerable variability was noted in terms of study duration, ranging from 1 week to 2 years. Here, we specifically compare studies employing the same experimental set-up, but at different time-points. Overall we found that most studies of 6 months duration demonstrated significant improvements in tissue repair over time. Pei et al.[76] (who implanted a device containing βTCP seeded with autologous MSCs cultured in chondrogenic medium under stirring stimulation for 2 weeks prior to implantation within OCD), and Mumme et al.[77] (who compared the regenerative capacity of nasal versus articular chondrocytes seeded on a bi-layered type I/III collagen membrane, namely ChondroGide, in a cartilage defect) both observed significant improvements in histological scoring of their treatments groups at 24 weeks compared to earlier time-points in a goat model. Zhai et al.[55] compared sulfated GAG and collagen content and compressive moduli, 3 and 6 months post-surgery, reporting a positive correlation between the level of cartilage repair observed over time in both their cell-seeded tri-layered osteochondral scaffolds (details on scaffold composition and in vitro culture conditions found in Table S2, Supporting Information). Interestingly, the newly formed tissue in the biomaterial group presented features similar to the undamaged cartilage tissue. Likewise, Hao et al.[78] (who analyzed the OCD regenerative capacity of chitosan seeded with autologous chondrocytes for 24 h) and Fonseca et al.[79] (who performed a similar evaluation of OCD repair using PLGA porous scaffolds seeded with autologous chondrocytes for 24 h), noted significant improvements in histological scoring and GAG content over time across all treatment groups. However, when study length was extended beyond this—up to 12 months—further matrix reorganization and cell

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maturation was expected, but instead the results indicated implant failure.

Kon et al.[80] found no significant differences in the level of OCD repair at 6 versus 12 months using a cell-free aragonite-hyaluronate (Ar-HyA) scaffold. Similarly, Zhang et al.[81] reported no significant differences over time when analyzing MRI scoring, macroscopic/microscopic, and biomechanical or biochemical performance after 6 versus 9 months repair in chondral defects treated with human articular cartilage decellularized ECM scaffolds seeded with human umbilical cord Wharton's jelly MSCs (hWJMSCs). The authors did not offer explanation for this lack of improvement after the prolonged experimental period, however others have attributed this to the immature nature of newly formed tissue and its inability to withstand loading stresses leading to delamination, lack of implant fixation, superficial fibrillation, and loss of lateral integration once the "regenerative phase" has concluded.[75,82] Furthermore, McCarrel et al.[51] compared the effects of implanting cell-free bilayer porous scaffolds containing type-I collagen (cartilage layer) and 80% βTCP-20% polylactic acid (bone layer) into OCDs, against a MF control. They found that bone sclerosis peaked 4 months post-surgery, but returned to normal after 12 months; they also observed that the percentage of bone replaced in the defect only became significant 1 year after implantation. However, in both groups (more pronounced in the MF control), cartilage fissures were also observed after 12 months. Thus, while bone tissue analysis seemed to indicate improvement over time, cartilage integration worsened, suggesting different regeneration times within the osteochondral tissue and highlighting the importance of evaluating longer time points. Among the studies included in this systematic review, only Vukasovic et al.[74] (who investigated a bilayer scaffold containing equine type-I collagen (cartilage layer) and a mineralized blend of type-I collagen and magnesium-doped hydroxyapatite (bone layer), seeded with nasal chondrocytes) reported significantly improved cartilage histology scores after 12 months (versus 3 months), in chronic and acute ovine models. However no significant differences were observed when comparing against the cell-free group at 12 months. Given that cartilage regeneration peaks within the first 12 months,[83,84] it's possible that time-point selection may have played a positive role in their study.

3.7. Cell-Based versus Cell-Free Devices

Cell-based strategies comprise the most prevalent approaches for cartilage regeneration, although most require the use of biomaterial supports to enhance integration and mechanical properties.[85] Unfortunately, despite their initial promise, a number of cell-based products have been withdrawn from; or have not made it to the market -their success having been limited by high cost of development, validation, trialling, regulation, and production; or other manufacturing-related issues—for example when using allogenic cells, there are significant concerns regarding donor heterogeneity, and the prolonged culture periods required to yield sufficient cell quantities (which may lead to cell senescence before reaching the clinic).[86] Further, some products have been complicated by scientific problems, stemming from lack of standardization in terms of cell source, cell density, dosages, pre-incubation treatments, or differentiation methods.

On the other hand, cell-free strategies rely fully on biomaterials or other raw materials, e.g., decellularized cartilage or demineralized bone matrix, to mimic the native tissue structure and stimulate endogenous cells to regenerate the damaged tissue. The main advantage of this approach is the ability to avoid cell manipulation and associated regulatory issues. Some of these technologies have shown promising clinical potential, have been commercialized and are currently available on the market. However the long-term results to date are mixed.[87]

In this section, we compare the performance of cell-seeded and cell-free biomaterial-based devices in large animal models. Due to the wide variability of techniques and scoring systems used, we have focused on those that included direct comparisons between cell-seeded and cell-free devices (20 in total), as a means to determine the relative success of each approach (**Figure 5**). Overall, 55% of studies reported significant improvements in tissue regeneration using cell-based approaches compared to cell-free counterparts.

In total, 25% of studies used chondrocytes alone (80% derived from articular cartilage and 20% using nasal chondrocytes). 20% of studies used a combination of bone marrow derived mesenchymal stem/stromal cells (BM-MSCs) and chondrocytes. 5% compared the regenerative potential of bone marrow (BM)-derived mononuclear cells (BMNC) with that of chondrocytes, while another 5% compared BM-MSCs, adipose-derived stem cells (ASCs) and chondrocytes. 35% of studies used stem cells: 86% utilized BM-MSCs while the other 14% assessed the chondrogenic potential of ASCs. 5% evaluated the regenerative capacity of adipose-derived stromal vascular fraction compared to ASCs. Finally, 5% of studies assessed the chondrogenic potential of BM-aspirate.

3.7.1. Mesenchymal Stem Cells

The majority of cell-laden biomaterial-based approaches for cartilage regeneration use MSCs, specifically BM-MSCs. MSCs possess great expansion and chondrogenic potential. They have anti-inflammatory properties[88] and are relatively easy to extract and expand in vitro. Drawbacks include their tendency to differentiate towards hypertrophic phenotypes when exposed to long-term chondrogenic stimuli, resulting in osteochondral ossification in vivo.[89,90] From the pool of articles reviewed, similar numbers of papers reported both significant improvement and no difference with MSC-seeded constructs compared to cell-free alternatives. This lack of consistency raises some questions: what other factors need to be considered when using MSCs for regenerative purposes? Considering that at least half of the studies did not significantly improve repair compared to cell-free counterparts, are MSCs the best cell population to use for enhancing cartilage regeneration? Also of note, there is still no consensus as to the right cell markers to define MSC populations.[91] Less than 15% of all papers reviewed using MSCs, BMNCs, or BM-aspirate completed a full characterization of cell surface markers prior to implantation,[54] which makes it more difficult to develop standardized protocols.

MSCs are highly responsive to biochemical and biomechanical cues from their surroundings,[32] thus controlling or manipulating their environment could play a key role in optimizing

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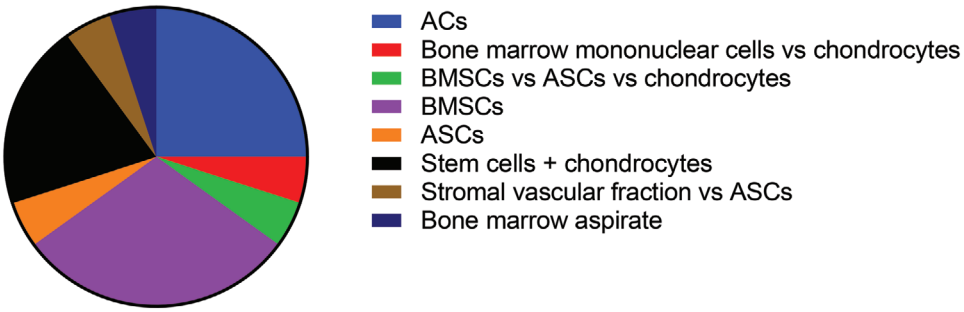

Figure 5. Analysis of cell type used in cell-based TE approaches. Percentage of studies relative to the total number of studies comparing cell-seeded to cell-free approaches.

MSC-based therapies. One example demonstrating how differences in biomechanical cues can influence results was evident when comparing the negative results from Jagodzinski et al.[92] (who designed an osteochondral device comprising a xenogenic decellularized bone cylinder seeded with BM-derived cell concentrate, combined with a collagen I/III mesh cartilage layer) with the positive outcome observed by Lim et al.[93] (who seeded BM-MSCs on a PEG-fumarate hydrogel for OCD repair). Both studies utilized MSCs, however only Lim et al.[93] observed higher percentages of newly formed tissue (including hyaline cartilage) in comparison to their cell-free control scaffold. Thus, the differences in outcome between these two studies highlight the role of the specific biomaterial's biophysical and biochemical properties in enhancing the stem cell's regenerative potential.

Additionally, the stem cell differentiation protocol employed during the pre-implantation phase will likely influence the success of a biomaterial-based device. Current differentiation protocols are not fully standardized, leading to inconsistencies including variable risks of MSC differentiation to hypertrophic cartilage which results in poor quality tissue formation in vivo.[94,95] Preliminary work, associated with the use of pre-differentiation protocols, has evidenced outcomes ranging from: i) improved tissue regeneration; ii) no difference; or iii) worsening tissue quality.[95–97] Here, 5 out of 6 studies using MSCs applied a differentiation protocol prior to implantation,[93–98] employing distinct culture media formulations, and either TGFβ1 or TGFβ3 to induce differentiation. Four of these studies subsequently reported significantly improved cartilage regeneration compared to cell-free controls. Zscharnack et al.,[98] in one chondral repair study, used type-I collagen hydrogels implanted cell-free, loaded with MSCs cultured with chondrogenic medium for 2 weeks, or with undifferentiated MSCs as a control. Their choice of 2 weeks was determined after they observed that 14 days of in vitro differentiation was optimal to maximize proteoglycan expression, while longer incubation periods led to apoptosis, compromising graft viability. After 14 days in vitro they noted significant differences in stiffness between hydrogels that had been incubated in differentiation media, compared to those incubated in expansion media. This alone may explain the superior performance of the pre-differentiated constructs, offering the cells more protection from surface strain and mechanical forces compared to undifferentiated controls.

3.7.2. Chondrocytes

Chondrocytes were the first cell type to be clinically utilized for cartilage regeneration purposes, through ACI. ACI is particularly successful in young patients with isolated injuries, however long-term outcomes are yet to be determined.[99] Chondrocytes are only present in low numbers in native cartilage, meaning expansion in vitro is necessary to obtain enough cells to treat a defect. During expansion, chondrocytes typically undergo de-differentiation, losing their morphology, function, and phenotype before switching towards hypertrophic or fibrous phenotypes. Currently, chondrocytes are the second most-used cell type in preclinical studies for cartilage TE. As with MSCs, their success rate is variable when compared to cell-free approaches. Out of 6 studies in this review comparing chondrocyte-seeded constructs with cell-free controls (Table S2, Supporting Information), 3 showed no significant differences between the cell-seeded treatment versus control,[52,53,79] though 3 reported significantly better results using chondrocyte-based approaches.[74,78,100]

Despite lack of consistency in experimental design across these last three reports, it's important to note some key elements in their strategies that may have led to their favorable outcome. In the case of Vukasovic et al.[74] as previously mentioned, higher regenerative potential was observed when using their type-I collagen and mineralized blend of type-I collagen and magnesium-doped hydroxyapatite seeded with nasal chondrocytes—in this case, the authors concluded that the cell-seeded approach was advantageous in their chronic osteochondral damage model. Similarly, Zuo et al.[100] utilized a combination of mosaicplasty with biomaterial-cell-seeded devices as a therapeutic strategy in their chronic osteochondral model. In particular, they reported significant osteochondral repair in defects treated with mosaic-like osteochondral plugs in combination with either autologous chondrocytes seeded on PLGA scaffolds coated with fibrin and cultured with TGFβ for 4 weeks prior to implantation, or with autologous BMNCs seeded in PLGA scaffolds for 1 h prior implantation. The authors did not find any significant differences when directly comparing the chondrocytes with the BMNCs, pointing to the advantages of using cell-seeded biomaterials in chronic defect models regardless of pre-culture conditions. On the other hand, Hao et al.[78] used an acute osteochondral model and attributed their success to the combination of the chondrogenic

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properties of their chitosan hydrogel, together with 24 h pre-seeding of chondrocytes. Despite the positive results achieved, the authors also highlighted the need to improve the mechanical properties of the chitosan in order to better withstand relevant load forces.

When directly comparing MSC and chondrocyte efficacy, results were varied. Guillen-Garcia et al.[101] discovered fibrous tissue in defects treated with BM-MSCs, with no improvements observed in comparison to an empty defect. Gene expression analyses measured high levels of collagen type-I, with low collagen type-II and aggrecan expression, suggesting the MSCs did not differentiate towards the chondrogenic lineage. However, chondrocyte-seeded collagen membranes outperformed the control in terms of cartilage repair after 3 months. Interestingly, Caminal et al.[102] reported no significant differences in histological or MRI scoring when comparing the regenerative potential of BM-MSCs versus chondrocyte-seeded PLGA scaffolds in an osteochondral defect. There were however, significant improvements observed when comparing to an untreated control after 6 months. There were a number of differences between the experimental set-up of both these studies that may have influenced the outcomes including study duration (3 versus 6 months), cartilage versus osteochondral defect, scaffold type, and acute versus chronic defects. It's important to highlight that chondrocytes were particularly successful in the full thickness cartilage defect, while MSCs can potentially undergo osteogenic and chondrogenic differentiation, simultaneously regenerating both bone and cartilage—most useful in osteochondral lesions.

3.7.3. Cell Combinations

In terms of efficacy, cell-seeded biomaterials out-performed their cell-free counterparts most significantly when different cell types were combined.[54,55,81,103] The studies reviewed combined MSCs with articular or growth-plate chondrocytes (further details regarding experimental design for each study can be found in Table S2, Supporting Information), a co-culture that is widely accepted as a means of enhancing the chondrogenic potential of both cell types.[104] The mechanism underlying this phenomenon remains elusive but one study has reported that the presence of MSCs on a chondrocyte-loaded hydrogel stimulates higher collagen and GAG production in vitro.[105] Others describe the secretion of chondrocyte paracrine factors including TGFβ-1, insulin-like growth factor 1 (IGF-1), and bone morphogenic protein type 2 (BMP-2), that initiate and support more stable BMSC chondrogenesis in an ectopic in vivo model.[106] Increasing evidence suggests paracrine effects, strongly dependent on close cell proximity, are driven by extracellular vesicles.[107–109] Although, the exact mechanism by which the co-culture of BMCS and chondrocytes enhance chondrogenesis in vivo is not fully stablished, it is clear that the combination of these cell populations play an important role in the regenerative outcome of biomaterial-based devices for osteochondral repair.

3.7.4. Cell Density

Cell seeding density is another important element which influences the efficacy of cell-seeded devices. It's known that cells respond to their environment in terms of biomechanical properties; similarly, cells respond to nearby cell concentrations.[110,111] During cartilage development in vivo, condensation of MSCs and chondrocytes facilitates cell–cell surface interactions driving differentiation.[112] Guillen-García et al.[101] looked at increasing seeding densities of autologous articular chondrocytes in a full-thickness ovine defect using a matrix-induced-ACI approach. They showed that higher chondrocyte densities ($5 \times 10^6/cm^2$ versus $1 \times 10^6/cm^2$) significantly enhanced the expression of pro-chondrogenic markers such as aggrecan and collagen type-II, and improved histological scoring. Studies in vitro have corroborated these findings whereby increased articular chondrocyte density resulted in more point sources for matrix production (assuming there is a sufficient supply of nutrients).[113,114] Although this single study does not support a strong conclusion on the use of a determined cell density, we consider it useful to highlight the importance of considering the optimization of the cell density on cell-laden biomaterials prior implantation in order to maximize the chances of success of the implant.

3.7.5. Pre-Implantation Cell Culture Period

Another important consideration when using cells for tissue regeneration is the matter of differentiation and/or pre-incubation of biomaterial-based devices prior to implantation. To date, the literature has described mixed degrees of success in this area.

Based on our analysis, biomaterials that were seeded and cultured between 2–5 weeks in vitro prior to implantation consistently demonstrated superior in vivo performance compared to cell-free devices within the same study, regardless of differentiation or expansion protocols. Zscharnack et al.[98] found that in vitro culture for 14 days in expansion medium significantly improved the chondrogenic potential of BMSCs seeded on type-I collagen hydrogels implanted in ovine femoral condyle defects for 6 months. Zhang et al.[81] showed that a combination of hWJMSCs and chondrocytes in an ECM-based scaffold incubated for 3 weeks in expansion medium, produced new tissue with similar gross morphology and histological staining to native undamaged cartilage, and with significantly higher mechanical strength than cell-free scaffolds. They also showed that hWJMSCs where still present in the biomaterial-based device after 9 months in vivo. Wei et al.[54] demonstrated significantly better histological scores and improved bone formation combining articular chondrocytes and BMSCs in a bilayer device incubated for 3 weeks in expansion medium, compared to a cell-free construct. Zhai et al.[55] found that cell-seeded scaffolds in an acute goat OCD resulted in significantly enhanced tissue regeneration compared to cell-free counterparts. Here, they used a tri-layered device with a chitosan/gelatin chondral layer, a chitosan/gelatin double freeze-dried intermediate layer, and a βTCP bone layer. The cartilage layer was seeded with articular chondrocytes cultured for 7 days in a bioreactor with expansion medium followed by 28 days of chondrogenic medium to ensure chondrogenic phenotype was maintained, while the subchondral layer was seeded with BM-MSCs during surgery. Vukasovic et al.[74] reported faster healing using their cell-seeded bilayered scaffold in both chronic and acute ovine defects, following 21 days incubation in a perfusion bioreactor with proliferation medium.

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Cartilage maturation may also influence successful integration of the TE device with the surrounding tissue, which is critical to construct success[115]—lack of integration can result in device dislodgement and poor regeneration. While immature constructs present higher proliferation and adhesion activities,[116–118] mature devices offer improved cell retention and mechanical properties, while in contrast there is a risk of poor integration.[119,120] Depending on the biomaterial used, maturation in vitro prior to implantation may further enhance mechanical properties and ECM deposition, ultimately yielding a device with a composition more similar to native cartilage, favoring in vivo integration.[115,121] Notably, most studies reviewed here not only pre-incubated their constructs, but also used an MSC/chondrocyte co-culture system which we have previously identified as a means to promote chondrogenesis; therefore, it's likely that both co-culture and in vitro maturation processes played a role in the success of these devices.

4. Perspective

This review aimed to provide an in-depth analysis of parameters affecting the outcome of preclinical studies investigating biomaterial-based devices for osteochondral and cartilage repair in large animal models. We used a RoB approach to select studies with robust experimental design according to a series of previously established parameters.[19] We found that the choice of animal species, comparator group, defect location and nature (acute versus chronic), cell type and study duration were highly variable amongst studies, and in many cases these differences might have influenced the outcome of the study. Within the articles assessed in this review, minipig was the most commonly used model, followed by sheep, goat, and horse in that order. As discussed in deep by Moran et al.[20] the common selection of minipig might be associated to the small size of the animals, their relatively easy manipulation and housing, and more importantly the cartilage thickness which is the closest to the human cartilage thickness. We identified a strong relationship between the animal's skeletal maturity and the success of cell-based treatments, with significantly better regeneration observed in immature animals. However, immature animal models are not the best representative model of the clinical situation. The age-dependent reduction in regenerative capacity is well known to affect most mammals including humans.[32,122] Importantly, the average age of patients with articular joint damage is estimated between 21–45 years.[123] By this age, most human patients will have reached skeletal maturity,[124] as such, the use of immature animals is a considerable limitation in terms of clinical translatability.

Regarding comparator group selection, the majority of the studies reviewed utilized an empty defect as their primary control, providing useful estimates of intrinsic healing capacity. In studies looking at more than one anatomical site, empty defects should be evaluated in the same location as the treatment group. Internal controls (in each animal) are especially important in order to minimize intra-species variability. While we are aware that this necessitates use of a bilateral model (treatment and control groups in the same location on opposite limbs), which itself is limited by increased weight-bearing in the defect site and potential ethical issues,[9] overall the inclusion of such a control facilitates the fairest comparison possible between control and treatment groups. Alternatively, we found that the analysis of undamaged tissue as a positive control (ideally obtained from the same treated animal, and typically from the contralateral limb), was extremely valuable in evaluating both repair of the defect, but also in further characterizing the quality of the newly formed tissue by comparing it against an internal control.

The femoral condyle and trochlea were the most common sites of assessment in all models. Although biomechanical stress and healing capacity are strongly influenced by defect location, many studies compared groups in different anatomical locations under the premise of possessing the same healing capabilities. We acknowledge experimental design or animal availability may not always allow this standard of comparison, however considering the significantly superior loadbearing compressive forces and healing capabilities of the condyles, and the clear differences between locations in terms of biomechanical and anatomical features, we again emphasize how valuable it is to compare treatments in matching sites, to maximize clinically-relevant results. Moreover, when testing a biomaterial-based device, it is of paramount importance to choose a location that matches the intended application of the biomaterial, that is, if the biomaterial is optimized to withstand high loadbearing compressive forces, then the best test location would be the condyle. It's also important to highlight a lack of studies directly comparing large animals to humans in terms of anatomy, joint forces, and healing capacities. A thorough comparative analysis would not only inform the selection of optimal models and experimental design, but might also improve the likelihood of clinical translation.

Considering that chronic defects are more relevant to human pathologies,[61] it was interesting to identify the correlations between chronic defect models and the success of cell-seeded biomaterial treatments. While acute studies were more common, we found that studies using chronic defects produced more favorable results in terms of repair capacity versus untreated controls. This might be partially attributed to the inflammatory conditions associated with chronic models, that is, the inflammatory environment makes the infiltration of host cells into the defect site and subsequent regeneration of the tissue more challenging, thereby resulting in greater differences between the empty control and the biomaterial-based treatment in these models. These findings highlight the importance of establishing and validating chronic models as standard preclinical tools for biomaterial-based device evaluation.

In terms of study duration, shorter experiments were found to provide useful information on biocompatibility and immune response. However, accurate information regarding device efficacy and regeneration ideally requires at least 12 months investigation. Initial cartilage regeneration has been reported within 6 months of device implantation, with significant improvement reported at that time-point in most studies (Table S2, Supporting Information). However, reduced resistance to mechanical stress over time triggers accelerated degeneration and defect aggravation. Therefore longer experiments are essential to fully evaluate device efficacy. Additionally, differences in healing time between bone and cartilage should be considered, since it has been established that healthy bone is a precursor for cartilage regeneration, providing mechanical and structural support.[125]

Cell culture onto a biomaterial prior to implantation is somewhat limited by certain challenges including cost of cell

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acquisition, potential immunogenic reactions, and regulatory hurdles imposed on biological devices.[8] Thus, cell-seeded devices should demonstrate significantly improved performance over cell-free counterparts to justify their use. Most studies reviewed here employed cell-based approaches, and generally these scaffolds outperformed their cell-free counterparts. Pre-seeding biomaterials with a combination of BMSCs and chondrocytes stimulated with chondrogenic factors for at least 2 weeks prior to implantation seems to provide the best overall response in terms of osteochondral regeneration and quality of the newly formed tissue when compared to native undamaged tissue. However, it is important to highlight that specific cell type is not so critical in determining device success—for example, little difference (and indeed significant variation in outcomes) is observed when directly comparing the efficacy of MSCs versus chondrocytes, the two most commonly used cell populations in cartilage/osteochondral TE. Further, Pot et al. completed a meta-analysis of studies specifically looking at cartilage regeneration in cellular versus acellular implants, and found that cell-based implants improved cartilage regeneration by 18.6%, irrespective of cell-type(126). Conversely, animal age and the process of in vitro pre-culture play more important roles as described previously.

We appreciate that this review is somewhat limited by a lack of quantitative meta-analyses of the studies reviewed. This is due to the huge variability of outputs reported, as well as the lack of consistency in units or scoring systems employed. The International Cartilage Repair Society (ICRS) has drafted various guidelines aiming to provide direction for preclinical and clinical studies.[10,126] They emphasize histological evaluation as a robust output to assess cartilage repair,[127] with a wide variety of scoring systems available to the investigator, focused on different applications such as level of regeneration, cartilage quality etc. However, not all of these systems have been properly validated and more importantly, the different scoring parameters are not interchangeable with other scoring systems, thus making them impossible to compare directly.[128] In addition, in most cases, even when using the same scoring system, authors often incorporate their own modifications to the system, tailoring it to their specific application, and making it very difficult to compare scores between studies.

In conclusion, this systematic review on large animal models of joint repair has enabled us to identify a series of key factors in experimental design that influence the regenerative outcome of biomaterial-based device evaluation in the treatment of cartilage or osteochondral defects. We have provided an objective analysis on how the selection of adequate comparators (empty defect as negative control and undamaged tissue as positive control) significantly adds statistical power to the analysis of regenerative potential of the biomaterial-based treatment. Interestingly, our analysis enabled us to identify the femoral condyle as the best location for testing the regenerative potential of mechanically strong biomaterials (when using the appropriate comparators). In addition we determined that the pre-implantation culture period, is a more critical factor than cell type when utilizing a cell-seeded biomaterial approach. Finally, we want to emphasize how the experimental design influences the final outcome of the biomaterial-based device, and more importantly the necessity for standardizing protocols in order to facilitate an objective comparison across biomaterial-based devices for joint repair.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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