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Review

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Cartilage Repair Using Hydrogels: A Critical Review of in Vivo Experimental Designs

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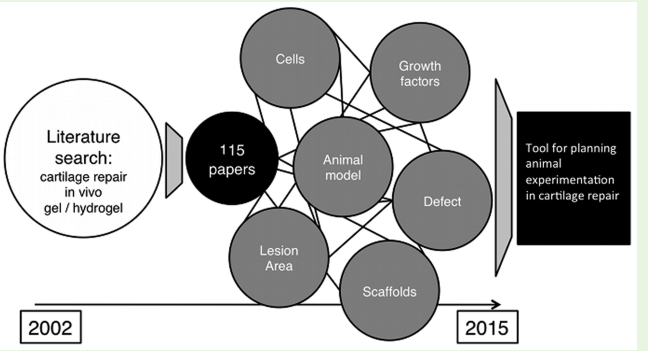
ABSTRACT: This review analyzes the outcomes and technical aspects of in vivo studies published in the past decade using gels and hydrogels for cartilage repair. Using PubMed search engine, original research publications during the period of 2002/01/01 to 2015/04/30 identified 115 published papers. Of these, 3 studies failed to find a statistically significant improvement of treatment group as compared to control and 18 studies did not clearly identify hyaline-like cartilage formation in the treated groups. The most frequent repaired lesion was the rabbit acute full thickness trochlear defect, using a scaffold combining a gel or hydrogel and other material. One third of the scaffolds were cell-free (35%) and the majority of the studies did not use growth factors (71%). The present review may constitute a useful tool in design of future studies, as limitations of study designs are pointed and results in terms of translation to human application is discussed.



KEYWORDS: *cartilage repair, hydrogels, gels, in vivo, animal, tissue engineering*

1. INTRODUCTION

Articular cartilage has limited intrinsic capacity for self-repair, because of the lack of vascular, neural, and lymphatic networks, as well as absence of progenitor cells.[1,2] According to Hjelle et al, cartilage lesions were found in 60% of patients submitted to knee arthroscopy.[3] Cartilage lesions commonly progress to osteoarthritis (OA), as a final state of disease evolution.[2,4] Presently, it is estimated that 10−15% of adults over 60 years old show some symptoms of disease and by 2050, 130 million people will suffer from osteoarthritis worldwide.[5] The clinical and economic impact is impressive, as the estimated direct and indirect costs related to OA has surpassed $65 billion annually.[6]

At earlier stages of cartilage damage, current therapies for cartilage repair of lesions are not satisfactory as they fail to restore a normal hyaline cartilage.[7−9] Surgical approaches can include microfracture, resurfacing techniques, and osteochondral grafting.[8−10] Autologous chondrocyte implantation (ACI) and matrix-assisted autologous chondrocyte implantation (MACI) are advanced approaches for regeneration of cartilage lesions.[9,10]

Microfracture and resurfacing techniques are easy to perform, cost competitive, widely adopted, and well-documented techniques that relieve symptomatic patients. However, regenerated tissue is composed mostly by fibrocartilage, thus providing short-term positive results in small cartilage lesions.[8,10]

Osteochondral grafting, the direct transplantation of an osteochondral autograft (mosaicplasty) or allograft, is the only technique available that satisfactorily restores hyaline cartilage.[4] However, donor site morbidity, risk of disease transmission, possible graft-versus-host immune response (in the case of allografts) and osteoarthritic exacerbation can occur due to lack of congruency between treated and untreated surfaces, thus limiting the use of those techniques.[4]

On the other hand, ACI and MACI are expensive techniques, which demand complex protocols and two different surgeries. Promising results have been reported,[4] but poor consistence of clinical outcomes with time, cells and/or cartilage fragment loosening, arthrofibrosis, osteophytes development, synovitis, infection and chondromalacea have been described.[4,10]

Many of the limitations of current available treatments justify the quest for more effective approaches and development of

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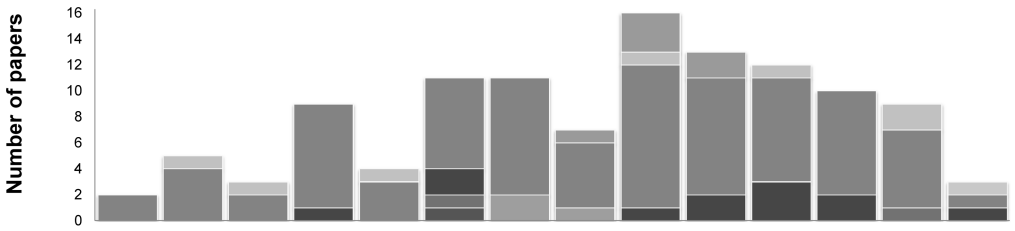

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Dog						1								
Goat							2	1						
Horse						1							1	
Mini pig				1		2			1	2	3	2		1
Monkey														1
Rabbit	2	4	2	8	3	7	9	5	11	9	8	8	6	1
Rat		1	1		1				1		1		2	
Sheep								1	3	2				

Figure 1. Number of original scientific publications per year published between 2002 and 2014 reporting in vivo experiments on cartilage repair according to animal model.

new biomaterials for cartilage repair. Interestingly, hydrogels have attracted great deal of attention because of its performance characteristics, i.e., are soft, of synthetic or natural origin, and can form three-dimensional networks that can be tuned for its biocompatibility, bioadhesiveness, and biodegradability.[11,12] Hydrogels present other advantageous features for tissue engineering applications,[11−14] such as extracellular matrix mimetic; swelling ability while maintaining shape; capability to undergo volume phase or sol−gel phase transitions in response to physical and/or chemical stimuli; tunable surface and bulk properties to modulate cells adhesion and thrombogenicity; support to high diffusion kinetics of nutrients and metabolic products within the construct.

There are several chemical or physical cross-linking techniques, photopolymerization, or even microfabrication technologies,[13,15,16] which can optimize hydrogel physicochemical characteristics and biological behavior,[13] and improve performance of hydrogels in cartilage tissue engineering strategies. Furthermore, hydrogels can be combined with other materials improving its properties.[17−19]

The application of hydrogels as volume fillers and cell carriers can contribute significantly to the development of more effective regeneration strategies[20] in irregular shape cartilage defects. Additionally, the opportunity to treat such lesions by a single step procedure using simpler surgical protocols, in which an injectable solution is delivered by a minimally invasive procedure, can minimize significantly treatment cost, improve patient safety and comfort, and support treatment in an outpatient setting.

This review compiles in vivo studies reporting the use of hydrogels for repairing cartilage lesions and analyzes its performance in different animal models. A thorough analysis of experimental variables was further performed, constituting a useful tool for researchers when designing future in vivo studies for cartilage repair.

2. METHODS

2.1. Keyword-Based Search. Original research publications were identified by the use of PubMed search engine, during the period comprised between 2002/01/01 and 2015/04/30, and using the following keywords: "cartilage", "osteochondral", "cartilage repair", "tissue engineering", "scaffold", "cells", "gel", "gels", "hydrogel", and "hydrogels", using AND/OR Boolean operators. The terms such as "eye", "heart", "tooth", "skin", "root", "dermal", "dentin", "cardiovascular", "hepatic", "gastric", "gastrointestinal", and "biochemistry" were excluded.

2.2. Inclusion/Exclusion Selection. All abstracts were evaluated by four independent reviewers based on the following inclusion criteria: English language, and experimental protocol reporting in vivo use of hydrogels in repair of cartilage defects. The following exclusion were applied: Other language rather than English; in vitro studies; studies not involving use of hydrogels; studies reporting use of hydrogels in other application contexts or studies in which the hydrogel could not be considered as a scaffold. Whenever the abstract was unclear or insufficient for determination of its inclusion/exclusion, the Materials and Methods section and/or the complete publication were analyzed before a decision was made.

2.3. Evaluation and Final Selection. After selection of abstracts, a second evaluation was carried out, during which all publications were analyzed and discussed among the four reviewers in order to produce the final list of publications to be overviewed.

2.4. Full Text Review. All included articles were submitted to a full-text review. For each paper, the respective list of references was verified to identify possible relevant studies that might have been undetected through PubMed-based search.

3. RESULTS

3.1. Publication Selection and Review. Keyword based search identified a total of 14295 publications. After inclusion/exclusion selection, 902 papers related to study of articular cartilage repair have been identified. Then, evaluation and final selection of those papers, according to defined inclusion and exclusion criteria, identified a total of 93 papers. During the selection process, 809 studies have been excluded due to several reasons, such as in vitro experimental protocol, experiments not aimed at repairing cartilage defects, or papers reporting clinical investigations. For each paper, the list of references was verified, which allowed identification of 22 additional publications. Herein, the total number of published original articles identified, reviewed and included was 115.

3.2. Distribution of Publications Per Year. The distribution of publications per year is shown in Figure 1. Between 2002 and 2010, the number of publications reporting in vivo experiments concerning cartilage repair have increased every year. After 2010, the number of publications per year appears to have stabilized between 10 and 13 papers per year.

3.3. Animal Models. Upon analysis of the publications, several outcomes were obtained regarding animal models and

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respective experimental protocol (Figure 2). According to Figure 2A, the rabbit model was the most common for studying

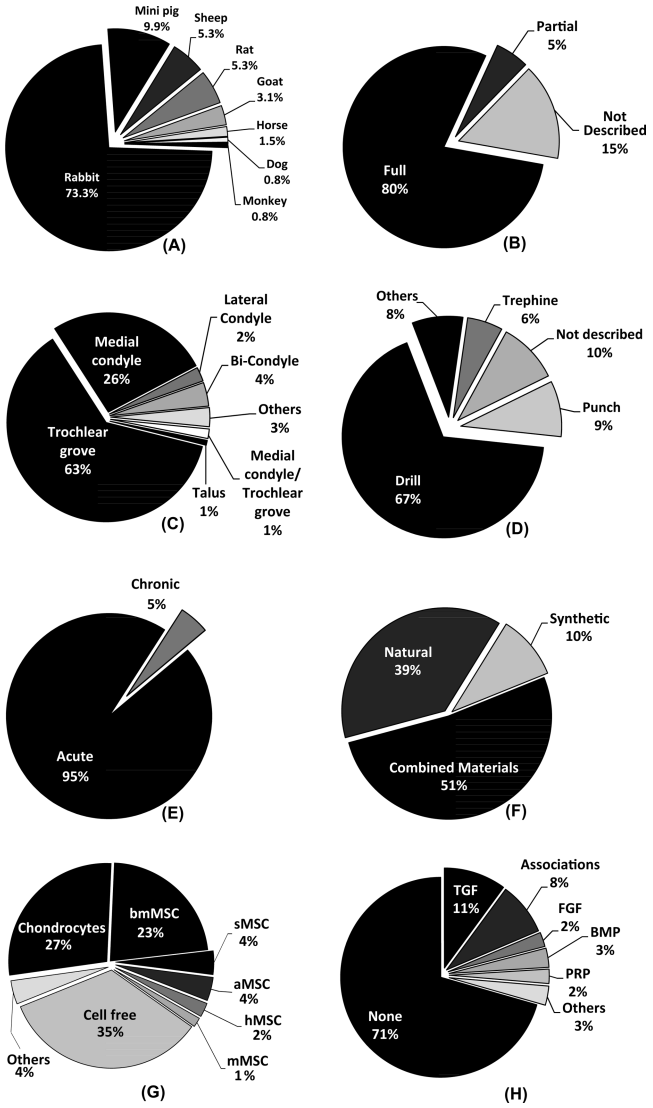

Figure 2. Distribution of animal models, characterization of the induced defect and lesion treatment and bioactive agents used: (A) animal model; (B) lesion type; (C) location of the lesion; (D) techniques for defect induction; (E) disease stage; (F) type of scaffold; (G) type of cells: adipose mesenchymal stem cells (aMSC), muscle mesenchymal stem cells (mMSC), synovium mesenchymal stem cells (sMSC), bone-marrow mesenchymal stem cells (bmMSC) and human mesenchymal stem cells (HMSC); (H) growth factors: transforming growth factor (TGF), bone morphogenetic protein (BMP), fibroblast growth factor (FGF), platelet-rich plasma (PRP).

cartilage repair by means of using hydrogels, comprising 73.3% of all studies. Noticeably, other animal models selected for evaluation of hydrogel performance included large animals, such as goat and sheep models, representing 3.1 and 5.3% of all studies, respectively (Figure 2A). Minipigs were the second animal more frequently used comprising 9.9% of all studies (Figure 2A).

3.3.1. Age and Weight of Animals. Table 1 summarizes the data regarding age and weight of animals for all the studies analyzed. The absolute ranges depend very much on the animal model. The animal model with the wider age interval was the sheep, with a relative interval of 40−260 weeks. As concerns weight, the animal models with wider weight intervals were sheep and horse, with a relative interval of 22.5 to 80 kg and 307 to 439 kg, respectively.

3.3.2. Number of Animals Per Study. Table 2 presents the number of animals used for each study and the time interval for the time points according to each animal model. The most common number of animals used per study was 12, as this was the mode obtained for mini-pig, rabbit, and sheep models. As for the duration of the studies, 12 weeks was the mode obtained for the most used animal models, rabbit and mini-pig, yet ranging from 4 and 8 to 52 weeks, respectively.

3.4. Experimental Protocol. *3.4.1. Type and Geometry of Defects.* From Figure 2, it is possible to state that the most frequently induced cartilage defect was a full thickness lesion (80%, Figure 2B), done in the trochlea (63%, Figure 2C) by drilling (67%, Figure 2D) and treated at an acute stage (95%, Figure 2E).

Cartilage defect dimensions were also thoroughly analyzed, including area, depth, and volume (Table 3). Most defects had a circular shape, yet 8 articles reported a rectangular or square shape.[21−28] Therefore, for comparison purposes, it was adopted the defect area to characterize surface dimension. Dimensions varied according to the animal model employed. In general, dimensions of induced cartilage defect were proportional to the size of the animal. For rat, the minimum area of the lesion was 0.6 mm^2 and for horse the maximum area was 176.7 mm^2. For rabbit, the most frequently adopted animal model, the mode of the lesion area was 7.1 mm^2, whereas the lesion area varied between 1.8 and 200 mm^2. The very large variation in defect area results from one study where the defect included the complete excision of tibial plateau.[29]

3.4.2. Type of Scaffold. From Figure 2F, it is evident that "combined materials" prevail as the most frequent type of scaffold (51%). These are composed by two or more materials of either of natural or of synthetic origin. The other types of scaffold that has been mostly investigated were natural derived scaffolds (39%) and synthetic scaffolds (10%).

When analyzing use of scaffolds with cells, about 65% of all studies analyzed involved the use of cells, in a so-called combination repair strategy. Nevertheless, about 35% of cartilage lesions where treated with hydrogels alone (Figure 3A).

3.4.3. Type of Cells. For combination approaches where scaffolds are combined with cells, 27% of studies used chondrocytes, whereas 38% used mesenchymal stem cells (Figure 2G). A thorough description of cell types and concentrations used in the analyzed studies are displayed in Table 4. Chondrocytes were the most widely used cell type in a concentration range between 5.00×10^4 and 5.00×10^7 cells/mL, followed by mesenchymal stem cells (MSC) that have been used in a range between 1.50×10^5 and 7.20×10^5 cells/mL. We noticed that 35% of the scaffolds were cell-free.

3.4.4. Bioactive Agents. Besides the use of cells with the hydrogels in combination strategies to repair cartilage lesions, growth factors have been also explored to improve quality of regenerated tissue. According to Figure 2H, published papers used at least one growth factor (29%) for repair of cartilage lesions. Transforming growth factor (TGF) was the most frequent choice, accounting for 11% of studies, whereas bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) were each used in 3 and 2% of all studies, respectively. Insulin growth factor (IGF), growth differentiation factor

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Table 1. Maximum, Minimum, Average, and Mode of Age and Weight of Animals Used for in Vivo Experiments on Cartilage Repair According to Animal Model As Reported in Analyzed Publications[a]

	Age (Weeks)				Weight (Kg)			
Animal	Max	Min	Avg	Mode	Max	Min	Avg	Mode
dog[b]			104.0				9.4	
goat					47.6	23.9	33.8	
horse	182	156	169.0		439.0	307.0	373.0	
mini pig	156	16	43.2	24/32/44	42.0	11.0	26.2	36.5
monkey[b]			312.0				8.0	
rabbit	96	8	20.8	24	4.7	1.8	3.1	2.3/3.3
rat	12	5	10.7	12	0.4	0.3	0.3	
sheep	260	40	152.4	117	80.0	22.5	64.9	68.0/80.0

[a]Abbreviations: max, maximum; min, minimum; avg, average. [b]Only 1 study with this animal model.

Table 2. − Maximum, Minimum, Average, and Mode of the Number of Animals and Duration of Study Adopted for in Vivo Experiments on Cartilage Repair According to Animal Model As Reported in Analyzed Publications[a]

	no. of animals/paper				duration (weeks)			
animal	max	min	avg	mode	max	min	avg	mode
dog[b]			9.0				10.0	
goat	20	4	12.5		24	12	19.0	24
horse	6	6	6.0		32	24	28.0	
mini pig	27	6	14.8	12/16/18/20	52	8	20.0	1
monkey[b]			16.0				24.0	
rabbit	81	3	27.3	12	52	4	13.9	12
rat	121	9	40.2		12	4	7.0	
sheep	24	3	11.4	10/12	52	3	27.0	16/52

[a]Abbreviations: max, maximum; min, minimum; avg, average. [b]Only 1 study with this animal model.

Table 3. Maximum, Minimum, Average, and Mode of the Number of Lesions, Lesion Area, Lesion Depth, and Lesion Volume Adopted for in Vivo Experiments on Cartilage Repair According to Animal Model As Reported in Analyzed Publications[a]

	no. of lesions				lesion area (mm^2)				lesion depth (mm)				lesion volume (mm^3)			
animal	max	min	avg	mode	max	min	avg	mode	max	min	avg	mode	max	min	avg	mode
dog[b]			2.0				19.6				4.5				88.4	
goat	2	2	2.0	2	28.3	19.6	24.0		4.0	3.0	3.3	3.0	113.1	58.9	78.9	58.9
horse	2	2	2.0		176.7	176.7	176.7		2.8	2.8	2.8		486.0	486.0	486.0	
mini pig	8	1	2.8	2	56.7	12.6	32.3	28.3	6.0	1.0	2.6	1.0/3.0	169.6	28.3	87.1	28.3
monkey[b]			2.0				7.1				5.0				35.3	
rabbit	6	1	2.1	2	200.0	1.8	15.4	7.1	15.0	0.5	3.7	3.0	217.8	3.5	51.0	21.2
rat	2	1	1.5	1	3.1	0.6	1.9	1.8	2.0	1.0	1.5		6.3	1.8	3.3	
sheep	12	1	5.0	4	50.3	8.0	32.4	28.3/38.5	12.0	2.0	6.7	12.0	423.1	32.2	205.5	77.0/339.3

[a]Abbreviations: max, maximum; min, minimum; avg, average. [b]Only 1 study with this animal model.

(GDF), connective tissue growth factor (CTGF), and Nel-like molecule-1 (NELL-1) account for a total of 3% of studies (Figure 2H). Noticeably, platelet-rich plasma (PRP) has also considerable expression in this context, accounting for 2% of all studies (Figure 2H).

3.4.5. Time Points and Study Groups. Table 5 presents number of time points, number of study groups, and number of lesions per study group for the analyzed publications. Concerning the number of time points, the majority of studies included at least 2 time points, yet the number ranged from 1 time point up to 7 time points. Three study groups was the most common among all animal models, yet the number of lesions per study group averaged 12−15.4, for the mini-pig and rabbit models, respectively.

For all animal models, the number of study groups was between 2 and 9. Concerning the number of lesions by study group (N), this index was calculated according the equation:

$$N = \frac{\text{no. of animals} \times \text{no. of lesions by animal}}{\text{no. of study groups}}$$

According to Table 5, the number of lesions per study groups was between 3 and 74.0.

3.4.6. Characterization of Cartilage Repair. Several techniques have been used to evaluate regeneration of cartilage tissue within the induced lesions. Histological staining of cartilage explants were done in all studies, including hematoxylin and eosin staining in most of the studies, complemented with at least one of the following: alcian blue, toluidine blue, safranin O, and/or Masson's trichrome staining. Immunohistochemistry staining, commonly used for identification of collagen type II and/or collagen type I, were done in 71 reports. For histological evaluation several histological scores were chosen: O'Driscoll, Pineda, ICRS, Mankin, Moran, Wakitani, Wayne, Selleŕs, Caplan, and Susante. In 15 papers, two of the previous scores were used simultaneously. O'Driscoll

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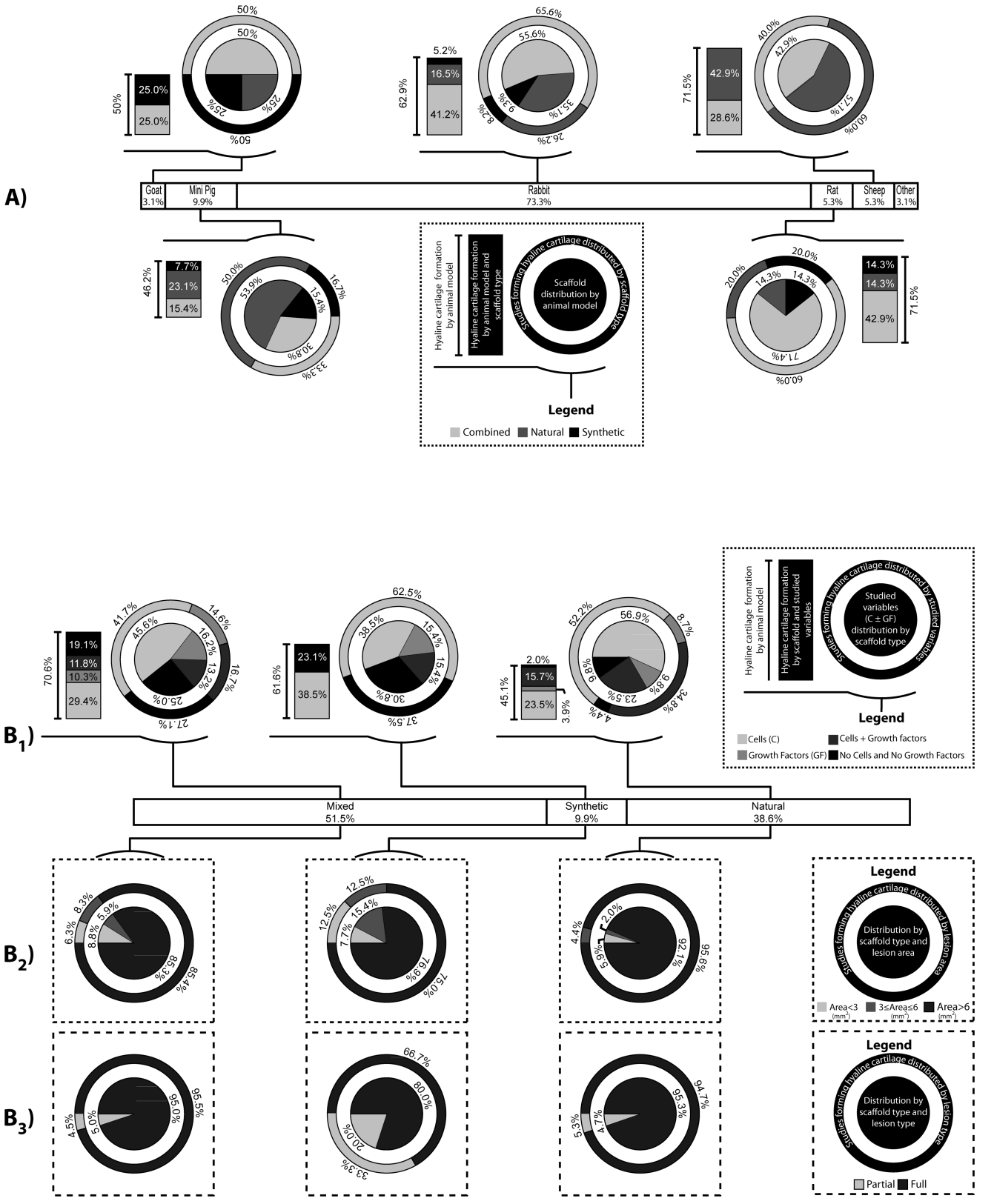

Figure 3. Correlation of data variables. (A) Inner circle: distribution of scaffold type used in each animal model; outer circle: efficacy of each scaffold type in forming hyaline cartilage. Lateral column displays overall efficacy of the animal model in yielding hyaline cartilage outcomes, further discriminated by scaffold type. (B1) Inner circle: distribution of cell ± growth factors used in each scaffold type; outer circle: efficacy of each cell ± growth factor combination in forming hyaline cartilage. Lateral column displays overall efficacy of scaffold type in yielding hyaline cartilage outcomes, further discriminated by cell ± growth factor combination. (B2) Inner circle: distribution of lesion area used in each scaffold type; outer circle: efficacy of lesion area in yielding hyaline cartilage outcomes. (B3) Inner circle: distribution of lesion type used in each scaffold type; outer circle: efficacy of lesion type in yielding hyaline cartilage outcomes.

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Table 4. Maximum, Minimum, and Average Number of Cells Used for in Vivo Experiments on Cartilage Repair According to Cell Type and Animal Model As Reported in Analyzed Publications[a]

		animal							
cell type	cell concentration (cells/mL)	dog[b]	goat	horse	mini pig	monkey[b]	rabbit	rat	sheep
aMsc	max						1.50×10^{07}	1.00×10^{06}	
	min						1.00×10^{06}	1.00×10^{06}	
	avg						9.00×10^{06}	1.00×10^{06}	
bMsc	max		5.00×10^{07}		7.00×10^{06}	1.00×10^{06}	5.00×10^{07}		7.20×10^{05}
	min		5.00×10^{07}		1.00×10^{05}	1.00×10^{06}	1.50×10^{05}		4.00×10^{05}
	avg	1.00×10^{07}	5.00×10^{07}		2.28×10^{06}	1.00×10^{06}	1.28×10^{07}		5.07×10^{05}
BNC	max				1.00×10^{05}				
	min				1.00×10^{05}				
	avg				1.00×10^{05}				
chondrocytes	max		5.00×10^{06}		5.00×10^{07}		5.00×10^{07}	5.00×10^{04}	4.00×10^{07}
	min		1.00×10^{06}		2.00×10^{05}		7.50×10^{04}	5.00×10^{04}	1.00×10^{06}
	avg	-	3.00×10^{06}	1.20×10^{07}	1.59×10^{07}		5.76×10^{06}	5.00×10^{04}	1.63×10^{07}
HMsc	max						1.00×10^{06}	2.00×10^{07}	
	min						1.00×10^{06}	2.00×10^{07}	
	avg						1.00×10^{06}	2.00×10^{07}	
mMsc	max						2.00×10^{06}		
	min						1.00×10^{06}		
	avg						1.50×10^{06}		
PBC	max						2.36×10^{07}		
	min						2.36×10^{07}		
	avg						2.36×10^{07}		
periostal cells	max								8.00×10^{06}
	min								8.00×10^{06}
	avg								8.00×10^{06}
sMsc	max						1.00×10^{08}		
	min						1.00×10^{06}		
	avg						3.82×10^{07}		

[a]aMSC: adipose mesenchymal stem cells; bmMSC: bone-marrow mesenchymal stem cells; bmNC: bone-marrow nucleated cells; HMSC: human mesenchymal stem cells; mMSC: muscle mesenchymal stem cells; PBC: peripheral blood mononuclear cells; sMSC: synovium mesenchymal stem cells; max, maximum; min, minimum; avg, average. [b]Only 1 study with this animal model.

Table 5. Maximum, Minimum, Average, and Mode of the Number of Time Points, Number of Study Groups, and Number of Lesions Per Study Group Adopted for in Vivo Experiments on Cartilage Repair According to Animal Model As Reported in Analyzed Publications

	no. of time points				no. of study groups				no. of lesions/study group		
animal	max	min	avg	mode	max	min	avg	mode	max	min	avg
dog[a]			2.0				2.0		9	9	9.0
goat	3	1	2.0	2	4	2	3.0	3	12	3	8.3
horse	2	1	1.5		2	2	2.0		6	6	6.0
mini pig	3	1	1.5	1	5	2	3.1	3	24	5	12.0
monkey[a]			3.0				2.0				16.0
rabbit	7	1	2.3	2	9	2	3.3	3	74	3	15.4
rat	3	1	1.8	1	7	3	3.9	3	20	13	16.8
sheep	3	1	1.7	1/2	6	2	3.9	4	20	6	10.8

[a]Only 1 study with this animal model.

scoring was used, alone or combined with another score, in 29 publications, followed by Wakitani scoring in 20 studies and by the ICRS scoring in 19 papers. In 32 studies, no histological score was used to evaluate the quality of cartilage regeneration. As outcome of histological evaluation, most studies have obtained statistically significant improvement in cartilage regeneration for treated groups as compared to control groups. In 3 papers,[30−32] no significant histological improvement was observed between treated and untreated groups, and in 4 papers,[33−36] no histological differences were found between study groups. Most studies reported on the development of hyaline-like cartilage, while 8 studies[37−44] reported no cartilage like tissue or a mixture of fibrous cartilage and hyaline-like cartilage in the repaired tissue. In 14 studies,[23,30−32,45−51] the repair tissue was not classified as hyaline-like cartilage. In 5 papers,[21,52−54] a tendency for deterioration of cartilage tissue along time was reported.

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Quantitatively, gene expression was evaluated in 25 studies (21,7%). Characterization of mechanical performance of regenerated tissue was highly uncommon, as it was performed in 8 studies. Imaging evaluation including magnetic resonance imaging (MRI), microcomputed tomography (μ-CT), laser scanning arthroscopy, and optical coherence tomography (OCT) was performed in 19 studies.

3.4.7. Side Effects. Several side effects have been reported in the studies analyzed such as inflammation, degeneration, tissue hypertrophy, among others. No information was given related to this issue in 26 studies. Inflammatory response was reported in 9 studies,[24,31,52,55−60] including, synovitis, fibrosis, and fissures. By its turn, 13 papers reported degenerative or pathological changes like osteophytes, cyst formation, or bone hypertrophy. In these studies, one of the following were used: a periostal flap in a chondrocyte cell-laden scaffold,[21] PRPs,[61] a growth factor (TGFβ,[62,63] BMP-2,[31] FGF[52]), or cells (ASC,[64] BMSC,[65] MSC,[25,54] chondrocytes[66] chondrocytes/periostal cells[67]). In another study,[68] the control group developed a degenerative change.

4. DISCUSSION

The present systematic review revealed that hydrogels used for cartilage repair include those composed by single natural or synthetic biomaterials, or by combination of these, designated as "combined materials" (Figure 2F). Advantages/disadvantages of natural and synthetic biomaterials for cartilage repair are detailed elsewhere.[69,70] Among the literature revised, 39% of studies proposed natural materials including collagen,[21,22,25,26,28,32,39,47,50,52,58,63,64,71−76] alginate,[37,38,40,48,62,77−80] fibrin,[29,33,81,82] platelet-rich plasma,[61,83] hyaluronic acid,[27,31,57,84,85] gellan gum,[86] chitosan,[42,87] and sugar cane biopolymer.[88] For 10% of studies, synthetic materials included oligo(poly(ethylene glycol) fumarate) (OPF),[43,46,89] poly(*N*-isopropylacrylamide-*co*-acrylic acid) (poly(NiPAAm-*co*-AAc)),[90] poly(L-lactide-*co*-3-caprolactone) (PLCL),[91,92] Si-HPMC,[36] polypeptides,[35,65,93] and α-CD-EG 4400.[94] The scaffolds using combined materials were composed by two or more natural materials, representing 51% of the studies,[17−19,49,60,66−68,95−100] by association of two or more synthetic polymers,[101−109] by the association of natural materials with a synthetic polymer,[20,23,44,45,51,54−56,59,76,110−128] 128 by association of others materials.[24,30,34,41,53,129−132] When analyzing Table 6, it is clear that most biomaterials succeed (to a higher or less extent) on regenerating hyaline matrix, while delivering bioactive agents such as cells and/or growth factors, as well as fulfilling fundamental requirements for translation into human scenario. Major limitations of these gels/hydrogels relate to unsatisfactory mechanical properties, capable to immediately withstand load after treatment, as well as a mismatch of biomaterial degradation rate as compared to tissue regeneration (either too fast or to slow). The combination of the hydrogel with a rigid scaffold has been tested (for example PLCL,[23] PLA/PLGA,[92] aiming to improve mechanical properties, whereas the downside relates to loss of injectability, and consequently, adequacy of the system to be delivered by a minimally invasive approach. Cross-linking mechanisms differ among the biomaterial types, yet can be used, to a certain extent, to fine-tune mechanical properties as well as degradation rate of the hydrogels. Not less important in the cartilage repair equation, is the capacity to mimic the complex layered structure of articular cartilage tissue. Although current gels and hydrogels are still limited in this regard, future developments in the

Table 6. Characteristics of the Biomaterials Used As Gels/Hydrogels for Cartilage Repair[a]

		injectability	delivery of bioactive agents	adverse immune response	cellular recognition	pathogen transmition	cross-linking	mechanical properties	degradation	integration with native tissue	hyaline cartilage	ref
NATURAL	collagen	+	+	−	+	−	+	−	−	+−	+−	21,22,25,26,28,32,39,47,50,52,58,63,64,69−74
	alginate	+	+	+	−	+	+	−	−	+−	+−	37,38,40,48,62,75−78
	fibrin	+	+	+	+	+	+	−	+	+	+	29,33,79,80
	platelet-rich plasma	+	−	+	+	+	+	−	−	+	+	61,81
	hyaluronic acid	+	+	+	+	+	+	−	−	+−	+−	27,31,57,82,83
	gellan gum	+	+	+	−	+	+	+	+	+	+	84
	chitosan	+	+	+	+	+	+	+	+	+−	+	42,85
SYNTHETIC	oligo(poly(ethylene glycol) fumarate)	+	+	+	+	+	+	+	+	+	+−	43,46,87
	poly(*N*-isopropylacrylamide-*co*-acrylic acid)	+	+	+	+	+	+	+	/	+	+	88
	silanized hydroxypropyl methylcellulose	+	+	+	+	+	+	+	/	/	+	36
	polypeptides	+	+	+	+	+	+	−	−	+	+	35,65,91

[a]Label: + : positive for cartilage repair; −: negative for cartilage repair; /: lack of information.

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biomaterials field might pursue this target, by providing more sophisticated, smart, and multifunctional materials for improved cartilage regeneration.[133,134]

Regarding the animal model used, the rabbit was the preferred, comprising 73.3% of all studies. Rabbits gather several features that make it an attractive model for cartilage regeneration research. It is of easy handling, caging. and care, has a good cost effectiveness and enough dimensions of the trochlear groove and condyles for the induction of a 3−4 mm diameter cartilage defects.[135] However, the relatively thin cartilage thickness (approximately 0.4 ± 0.1 mm in the trochlear groove),[136] has limited the volume size of the cartilage defect.[135] Another limitation of this animal model is the high degree of the rabbit knee flexion, creating a partial weight-bearing condition when the trochlear groove is chosen as location for cartilage defect induction/repair.[137] The present review revealed that the majority of studies used were immature rabbits younger than 8 months, the minimum age considered for maturity of rabbits.[138] The above-mentioned disadvantages and the high potential of the rabbit model for spontaneous healing,[84,139] especially in immature animals, are important limitations to address when the rabbit is used as a translational model to human knee cartilage. Herein, it was noticed a progressive use of larger and more weighted animal models, allowing bigger cartilage defects that reproduce better the size, depth, and conditions of human cartilage lesions.[135] Furthermore, some of these models, as opposed to rabbit, have a low spontaneous cartilage repair ability,[140−142] and similr to humans, suffer from osteochondritis dissecans and osteoarthritis pathologies.[135]

An articular cartilage defect is classified as full or partial-thickness defect according to the penetration into the subchondral bone.[116,143] Considering the known cartilage thickness of the different animal models,[135,144] the majority of the defects overviewed in this review are deeper than the expected cartilage thickness for those models, therefore, these were classified as full-thickness defects or as osteochondral defect. This is a very important drawback regarding the relevance of the models used for evaluation of cartilage repair performance, given that in humans superficial cartilage lesions are the most common, and only 5% are osteochondral defects.[3]

Most of the studies have reported the treatment of cartilage defects at an acute stage. From the total publications analyzed, only 6 were related to chronic stages of the cartilage defect.[34,63,64,75,111,113] It is recognized that a chronic cartilage lesion is a diverse condition as compared to an acute cartilage lesion.[26,34,145] This fact highlights the importance of addressing the correct stage of lesion progression in animal models when translating to human treatment.

As for tissue characterization, the majority of the studies included immunohistochemical evaluation of the neo-cartilage by evaluating the presence of collagen type I and type II, whereas expression of type X was determined in only 3 studies.[19,115,132] It is important to identify the expression of collagen type X, in order to exclude the possibility of hypertrophic tissue development or a transient cartilage.[64]

Determination of gene expression was performed in 24 studies. In these, an increase in cartilage-related gene expression was found in the regenerated tissue. Nevertheless, given the mismatch of information regarding gene expression, it is not possible to perform a full comparison between studies.

Assessment of mechanical performance of the new tissue is a relevant dimension when evaluating quality of the cartilage repair. Yet, its implementation is difficult, as it depends on anatomical location, measurement methodology and specific conditions of the joint.[66] The mechanical properties of the repaired tissue were evaluated in only 8 studies. In most of these, properties of the new tissue were similar or close to native cartilage.[24,32,34,77,114] Some authors found inconsistent results[75] and repaired tissue showed a higher stiffness as compared to normal cartilage.[24,94] As expected, similar mechanical properties between repaired tissue and normal cartilage was correlated with regenerated hyaline-like cartilage, except for the study by Pulkkinen et al.,[32] which despite mechanical properties being similar to native cartilage, the repaired tissue did not correspond histologically to hyaline-like cartilage. From these studies, two main issues can be highlighted: (i) large variety of reported methodologies among studies for determining mechanical performance of regenerated cartilage; (ii) adopted methodologies that do not reflect natural physiological condition.[66] These issues pose additional challenges when assessing quality of the regenerated cartilage in animal models using new biomaterial/therapeutic candidates, and when translating such performance during proof-of-concept or preclinical setting, to human performance in clinical setting.

The majority of the studies did not compare the treatment groups with reference treatments, adopted as clinical standard, such as microfracture or osteochondral grafting, which would be of high value to infer the relative efficacy of the new biomaterial/therapeutic candidates. For full-thickness defects (the most frequent defect type studied), the nontreated control group acts in a similar way to microfracture as there is exposure to bone marrow. Yet, for partial-thickness defect, only 1 study compared the outcome with microfracture treatment.[86] Concerning osteochondral grafting, only 2 studies compared the results of between scaffold treated groups with osteochondral grafting.[34,48]

Regarding the use of cells, most studies used chondrocytes, although mesenchymal stem cells (MSC) have been also highly explored.[39] Adipose mesenchymal stem cells (aMSC), muscle mesenchymal stem cells (mMSC), synovium mesenchymal stem cells (sMSC), and bone-marrow mesenchymal stem cells (bmMSC) were used, which avoid donor site morbidity in the cartilage tissue. Among the different stem cell sources, it was stated that sMSC and bmMSC show a greater chondrogenic potential as compared to aMSC or mMSC, while one study reported, in addition, greater proliferation potential of sMSC.[28] Many researchers have reported an improvement in bone and cartilage formation[39,59] when MSC were implanted. These improvements were promising, with a superior cartilage bonding to adjacent native cartilage, when compared with articular chondrocytes.[64] However, some authors[44,50,54,121] did not find better results in cartilage regeneration when MSCs were used.

Regarding the the use of growth factors, a relationship between use of growth factors and inflammatory response or pathological changes, was not found. However, reported responses were identified in only 5 experiments that have used growth factors.[24,31,62,63,65] For 1 case using BMP-2, extensive ectopic bone formation was observed.[31]

TGF−β seems to be dose-dependent and lower concentrations are more effective in repairing cartilage defects and decrease osteophyte formation.[62] TGF-β1 has been suggested to have a pro-inflammatory response, but no study using TGF-β1 reported an inflammatory response. TGF−β1 promoted

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trabecular bone subchondral appearance but did not improve cartilage cell morphology or glycosaminoglycan (GAG) expression,[44] while TGF-β3 was suggested to have a chemotactic cue for cell homing.[114] The combination of BMP-7 and TGF-β1 was found to induce chondrogenic differentiation.[115]

To be considered mature hyaline cartilage, the repaired tissue must exhibit normal morphology of chondrocytes and normal safranin O staining and possess an adequate structural organization with vertical columnar alignment of chondrocytes. When the last condition is not attained, the repair tissue is classified as immature. If the tissue is composed of dense spindle-shaped fibroblasts, the tissue is graded as fibrous tissue. When the repair tissue contains cells beginning to differentiate toward chondrocytes, the tissue is called as undifferentiated mesenchymal tissue.[48,146] Another important aspect is that 3 studies did not obtain statistically significant improvement in treated groups when compared with untreated group.[30−32] Although the majority of studies reported improvement of cartilage regeneration in treated groups, 22 studies did not recognize formation of hyaline-like cartilage at the repaired defect site. Therefore, better scoring of repaired tissue does not mean necessarily hyaline-like cartilage formation. Further discussion might focus on reliability and adequacy of scores used to evaluate regenerated cartilage tissue. Bonasia et al, tested the inter- and intraobserver reliability of 10 scores and concluded that, for evaluation of cartilage repair in animal models, the ICRS II, O'Driscoll and modified O'Driscoll scores are preferential given their high reliability, and the fact that the whole joint is available for histological assessment.[147] On the other hand, for evaluation of human cartilage biopsies, the ICRS I or II or Oswestry score are preferable given the limited tissue available.[147]

The studies analyzed herein evaluated repaired tissue mostly by the O'Driscoll score, followed by the Wakitani. One of the limitations of these scores relate to the lack of validation by biochemical analysis.[148] Only the Bern score has undergone such validation, yet has been considered more adequate for analysis of tissue-engineered constructs[148] instead of repair of cartilage in animal models. Accordingly, it was not used in any of the revised studies. For the O' Driscoll score, safranin O staining grading is not reflected in the final score and a limited difference was observed between a "moderate" and a "poor" quality of regenerated cartilage.[148] Although the O' Driscoll score includes evaluation of repaired tissue structure, it does not consider other parameters such as mineral degeneration, vascularity, subchondral bone, viability cell population, inflammation, and cartilage plug quality.[148] As previously reported,[149] evaluation of cartilage repair should make use of more than one score, complemented by biochemical, automated histomorphometry, and biomechanical correlation.

Data Correlation. Given the above-mentioned compilation of data, one would be tempted to understand which combination of factors would seem the most promising in yielding regeneration of cartilage tissue. Despite the high number of variables and possible combinations, an excel VBA application was developed in order to correlate data. Studies were characterized as "hyaline" or "no hyaline" based on the studies' author classification of repaired tissue. Subsequently, studies were selected based on the use or no use of cells (C) and/or growth factors (GF), by animal model or lesion size, ultimately correlated by type of scaffold (natural, synthetic, or combined materials). Outcomes are displayed in Figure 3.

Regarding the animal model (Figure 3A), despite the rabbit not being recommended as a model to evaluate cartilage repair due to small cartilage thickness, and high spontaneous regeneration,[150,151] when analyzing Figure 3A, it is evident that among all animal models, the rabbit has been the most widely used, comprising 73.3% of all studies. Of these, 62.9% claimed to have generated hyaline-like cartilage tissue. Apparently, combined scaffolds were responsible for such outcome, comprising 41.2% of the hyaline repaired tissue. Nevertheless, for bigger animal models (goat, sheep, and mini pig), the natural origin scaffolds seem to result in superior hyaline-like cartilage regeneration, as compared to those using combined or synthetic hydrogels.

When analyzing from another perspective, it was possible to determine that 55.6% of all rabbit studies used combined scaffolds, and these generated 65.6% of all hyaline-positive outcomes. This trend is maintained for all animal models, whereas the synthetic scaffolds seem to yield inferior outcomes.

Figure 3B1 displays an analysis of the combination of cells (C) and/or growth factors (GF) with the different types of hydrogels, and their synergistic effect on cartilage repair. In fact, of all studies analyzed, 51.5% used hydrogels composed of combined materials and resulted in a 70.6% success rate on generating hyaline-like cartilage. Those using scaffolds of natural origin (38.6%) seem less successful, where only 45.1% generated hyaline regeneration. Nevertheless, it does seem that the presence of cells generally improve probability of successful regeneration of tissue, as major percentage of successful outcomes where achieved through the use of cells in combination with the scaffold, while the positive effect of the presence of growth factors is not so evident (Figure 3B1).

An additional correlation factor was lesion size, where type of scaffold (natural, synthetic or combined), was related to the lesion area (<3 mm^2, 3−6 mm^2, or >6 mm^2) and relative percentage of incidence on generating hyaline cartilage was analyzed (Figure 3B2). Overall, hydrogels of combined materials seem to perform better than natural or synthetic hydrogels, in nearly all dimension ranges, yet it seems that for larger lesions, natural origin hydrogels provided better outcomes.

On what regards deepness of lesion (Figure 3B3), full-thickness was the most used and the most successful in obtaining hyaline cartilage, according to their authors. However, interpretation of this outcome is limited to the reduced number of studies that have tested repair of partial lesions (only 5% of all studies).

Study Limitations Acknowledged by Authors. Some authors pointed several limitations in their studies: the dimension of the sample[27,30,34,39,67,103,43,108] and specific problems with design of the study.[44,65,96,101,102] In addition, several limitations have been pointed out, such as lack of biomechanical evaluation,[28,67,99,104−106,112,129,131] short follow-up,[27,43,62,77,101,103,105,106,109,113,122,130,131] animal immaturity, and type of animal model,[77,104,105,110,117] poor representativeness of human pathology,[93] origin-cell identification not possible in the majority of the studies,[25,26,67,131] absence of a specific rehabilitation program,[113] and experimentation under no load bearing conditions.[34,106] The International Cartilage Repair Society (ICRS)[150] and the American Society for Testing and Materials (ASTM)[151] have published guidelines and recommendations for preclinical studies aiming cartilage repair, that could be considered by researchers in order to generate valuable and comparable data, ultimately contributing to

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stronger advancement of knowledge in the field of cartilage repair.

5. CONCLUSIONS

In summary, hydrogel biomaterials seem to be promising candidates for cartilage repair, given that hyaline-like cartilage development was proved in a considerable number of studies. A potential advantage of using hydrogels for cartilage repair is its suitability for arthroscopic delivery, yet, in many studies, hydrogel properties did not seem compatible with this minimally invasive approach. Overall, further development on surgical technique will be required.

The majority of the published papers addressed small, acute and a full-thickness cartilage defect in a nonweight bearing area. These conditions are very different from those found in human patients which is a concerning limitation considering translation of experimental learnings toward human treatment. The need for animal models and experimental designs that consider those aspects is obvious and must be considered in future animal experimentation studies.

In addition, anticipation of potential therapeutic efficacy in human demands a more conclusive mechanical evaluation of the regenerated tissue, as well as long-term studies. Not less important is the need of standardization of the evaluation procedures, especially on what concerns histology in order to enable comparison among different studies. The use of uniform guidelines for the definition of the general conditions and techniques to be used in cartilage repair experiments is mandatory to ensure comparability of studies.

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Notes

The authors declare no competing financial interest.

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ACS Biomater. Sci. Eng. XXXX, XXX, XXX−XXX