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Review
Preclinical animal models in single site cartilage defect testing: a systematic review

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Summary

Objective: Review the literature for single site cartilage defect research and evaluate the respective strengths and weaknesses of different preclinical animal models.

Method: A literature search for animal models evaluating single site cartilage defects was performed. Variables tabulated and analyzed included animal species, age and number, defect depth and diameter and study duration. Cluster analyses were then used to separate animals with only distal femoral defects into similar groups based on defect dimensions. Representative human studies were included allowing comparison of common clinical lesions to animal models. The suitability of each species for single site cartilage defect research and its relevance to clinical human practice is then discussed.

Results: One hundred thirteen studies relating to single site cartilage defects were reviewed. Cluster analysis included 101 studies and placed the murine, laprine, ovine, canine, porcine and caprine models in group 1. Group 2 contained ovine, canine, porcine, caprine and equine models. Group 3 contained only equine models and humans. Species in each group are similar with regard to defect dimensions. Some species occur in multiple groups reflecting utilization of a variety defect sizes. We report and discuss factors to be considered when selecting a preclinical animal model for single site cartilage defect research.

Discussion: Standardization of study design and outcome parameters would help to compare different studies evaluating various novel therapeutic concepts. Comparison to the human clinical counterpart during study design may help increase the predictive value of preclinical research using animal models and improve the process of developing efficacious therapies.

Key words: Cartilage, Osteoarthritis, Preclinical, Animal model, Literature review.

Introduction

Osteoarthritis (OA) has significant impact on the health care system. An estimated 9% of the United States population aged 30 and older have clinical OA[1]. Cartilage has limited healing capacity and as a result injury of the articular surface may lead to OA. Focal trauma causing defects in the cartilage surface is repaired with tissue that is commonly inferior to the original cartilage. The extent to which the new tissue resembles cartilage is determined by the species, age, size of the defect and its anatomic location. This inferior repair tissue may subsequently lead to OA. Various joint resurfacing treatments for focal traumatic events affecting the articular surface are available including debridement techniques, osteochondral transplants, or novel replacement devices[2–8]. Animal models in research are widely used to evaluate novel concepts for regenerative joint resurfacing.

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The average human defect is approximately 550 mm^3 in volume[9,10] and 95% of defects involve cartilage without affecting the subchondral bone[9]. A perfect animal model would precisely mimic the human. This has been difficult to produce[11]. Historically and indeed currently the role spontaneous regeneration of single site cartilage defects plays in the ultimate success of a study is a concern. Examples of spontaneously healing cartilage defects in ponies have been described by Convery *et al.* and in rabbits by Salter *et al.* in the late 1970s and early 1980s. Evidently consideration of the benefits and limitations of the available animal models given that no perfect preclinical animal model currently exists is important.

The purpose of this review was to evaluate preclinical animal models relating to single site cartilage defect research and to present comparisons between species. Furthermore to allow for an easy reference regarding commonly utilized species, animal numbers, defect location, defect dimensions and commonly considered critical sized defects. This could greatly improve the process of successfully bringing technologies from the bench to bedside and allow for more effective comparison between studies.

Methods

A search using PubMed and Web of Science for preclinical animal models evaluating single site cartilage defects was performed. The following keywords were used: murine-, laprine-, canine-, ovine-, caprine-, porcine-, and equine-model, human, OA, arthritis, osteochondral, chondral, subchondral, cartilage, resurfacing, repair, defect, animal model. The reference sections of the included studies were also screened. Original research utilizing animal models for single site chondral/osteochondral defect repair in diarthrodial joints of the appendicular skeleton from 1990 to 2007 was included. Information pertaining to the age, number and species of animals utilized, defect dimensions and location and study duration were tabulated. The age of skeletal maturity and the dimension of commonly recognized critical size defects for each species and the different mechanical loading environments were considered and discussed.

Studies involving the distal femora were isolated. Standard cartilage thickness for the medial femoral condyle was obtained from work by Frisbie *et al.*[12]. This is the only reference in the current literature reporting comparative values for cartilage thickness between species using a standardized technique. These values were used to calculate the chondral and subchondral components. Studies were then objectively assembled into substrata comprising 'groups' of 'similar' species, based upon cartilage thickness, defect depth and diameter. To achieve this we used cluster analyses following the methods outlined in Everitt *et al.*[13]. We employed *k* means clustering with from two to five clusters and, based on Milligan's study[14] we used Calinski's[15] stopping rule for selection of the optimal number of clusters, in this case three. Stata version 10.0 was used for all cluster analyses.

Results

The literature review returned 113 published studies. Information pertaining to the number of studies performed, animal numbers utilized, total defect volume, proportional chondral and subchondral defect volumes are presented in Table I. This information is further presented in Figs. 1 and 2 comparing the animal data to human clinical data. Figure 1 differentiates the overall defect volume into the subchondral and chondral components. Figure 2 presents the relative proportions of the defects as a percentage of the total volume. A comparison between the cartilage volumes of commonly reported critical sized defect lesions is compared to the average reported defect cartilage component in Fig. 3.

CLUSTER ANALYSIS

Statistical cluster analysis was applied to all of the studies with respect to total defect volume, defect diameter and cartilage depth. Six studies reporting common human distal femoral defects were utilized allowing humans to be included in the analysis. A total of 101 studies involved were suitable for inclusion. Cluster analysis separated these studies into three groups. Group 1 consisted of a majority of the research studies and involved models with similar proportioned small defects (Table II). Group 2 consisted of mostly medium and large sized animals, predominantly goats and horses. Group 3 contained horses and humans only. The mean, standard deviation and 25, 50 and 75% confidence intervals for each cluster group are presented in Table III.

Rodent

The murine model is predominately used in the early stages of biomaterial testing. It is a common first *in vivo* experiment to provide proof-of-concept data. A majority of studies involve heterotopic chondrogenesis models implanting biomaterials sub-cutaneously in the back[1,3,16–25] or intra-muscularly[19,26]. However, articular defect models have been used in a limited number of cases[27–31].

Rodents are easy to handle and require limited specialty logistics rendering this model economically practical. On average 30 rodents have been studied to a 10 weeks endpoint (range 4–24). Rodents are difficult surgical models for cartilage defect testing as the growth plates do not close, they have very small joints and extremely thin cartilage (3–5 cells thick)[32]. This animal model is limited to very small defects (Table I and Fig. 1). Further, when used, a large proportion of the defect involves the subchondral bone (Fig. 2). It is extremely difficult to produce a surgical cartilage defect model in the rodent that is suitable for comparison to the human situation.

Rabbit

Many studies have utilized the laprine model for the evaluation of cartilage defect therapy. They are relatively inexpensive, require simple husbandry, reach early skeletal maturity at 9 months[4] and have a long track record of biomedical research. A 3 mm diameter has been considered the critical sized defect to prevent spontaneous healing[4,33,34]. This dimension is questionable due to reported spontaneous healing. Larger defects of 4 and 5 mm diameter are probably a more suitable sized defect[35,36]. The cartilage thickness in the medial femoral condyle of rabbits is approximately 0.3 mm thick[12]. The most common defect depth utilized is 3 mm[37–40]. Resultantly 90% of the defect volume involves the subchondral bone (Figs. 1 and 2).

A broad range of defect dimensions have been used resulting in a large standard deviation of defect volume (Table I). Whilst the cartilage component is similar within studies, the amount of subchondral bone defect volume is highly variable. This large difference in the exposure to the subchondral bone could be a source for variability in results. Thus comparison between studies is difficult.

Defects have been created in the femoral trochlea[16,33,38–52], the medial femoral condyle[37,53] and the lateral femoral condyle[53]. The femoral trochlea is a partial weight bearing location[32]. Due to the acute angulation of the laprine knee, their relatively light bodyweight (range 2–4.5 kg) and the use of partial weight bearing surfaces, the loading conditions are significantly less than in large animal models. As a result they are less stringent evaluations of cartilage repair.

The age of rabbits used ranges from 9 to 36 weeks. The age of skeletal maturity in rabbits is from 16 to 39 weeks of age[4,54]. Young and adolescent rabbits of up to 20 weeks have shown remarkable spontaneous cartilage regeneration[55,56] with normal hyaline cartilage present in control defects by 12 weeks post-surgery[37]. As a consequence in research utilizing young and adolescent rabbits the degree of regeneration attributable to intrinsic healing must be considered.

Researchers have followed the various laprine defect models for an average of 16 weeks (range 2–76). This short endpoint means that the long-term efficacy of a treatment is not assessed. The median number of rabbits used per study was 33 with a range of 6–210. The option to use large numbers of phenotypically similar subjects is a benefit of this animal model in comparison to other animal models[57].

Cluster analysis placed all laprine models in group 1. Care must be taken during study design using this model and in interpretation of results due to potential spontaneous healing and unique weight bearing conditions. However, with due consideration the rabbit is a model which is useful in evaluation of early phases of a therapy.

Table I

Overview of literature with reference to study cohort size, defect volume (mm^3) and cartilage thickness (mm). Cartilage thickness of the medial femoral condyle from Frisbie et al.[12]. *Defect volumes were calculated for cylindrical shaped defects using* $V = \pi r^2 h$, *for cube shaped defects using* $V = lwh$ *(*r = *radius,* h = *depth,* l = *length and* w = *width of the cartilage defect)*

Species	Medial femoral condyle cartilage thickness (mm)	Studies performed		Animal number utilized	Total volume (mm^3)	Cartilage volume (mm^3)	Subchondral volume (mm^3)
Murine	0.1	5	Mean	30	2.17	0.12	2.05
			SD	15.88	2.85	0.06	2.81
			Mode	30	5.3	0.18	0
Laprine	0.3	39	Mean	18.86	53	7.15	45.86
			SD	11.57	54.64	13.35	52.78
			Mode	16	21.21	2.12	19.09
Ovine	0.45	13	Mean	23.69	359.54	18.03	341.51
			SD	15.74	683.35	19.97	663.79
			Mode	20	n/a	12.5	0
Canine	0.95	16	Mean	34.82	82.39	18.43	63.86
			SD	46.85	197.94	17.4	181.9
			Mode	8	11.94	11.94	0
Porcine	1.5	10	Mean	9.56	107.43	43.76	63.67
			SD	2.35	87.87	24.05	78.9
			Mode	10	183.22	34.35	0
Caprine	1.1	13	Mean	30.55	251.65	45.71	63.67
			SD	20.39	448.46	35.1	78.9
			Mode	50	31.1	17.49	0
Equine	1.75	17	Mean	9	334.73	192.67	142.06
			SD	2.03	237.87	94.21	213.08
			Mode	8	137.44	137.44	0
Human	2.35	n/a	Mean	n/a	552.25	552.25	0

Dog

Studies involving the dog are subjected to intense scrutiny because of their companion animal status. With other animal models available, cartilage defect research in the dog has been limited as a result. The cartilage thickness of the medial femoral condyle is 0.95[12] to 1.3 mm in thickness[4,32]. Defect diameters have ranged from 2 to 10 mm with 4 mm being the most common[58–66]. The cartilage thickness allows for surgical defects involving the articular cartilage without the subchondral bone. However, a majority of studies still utilize an osteochondral defect.

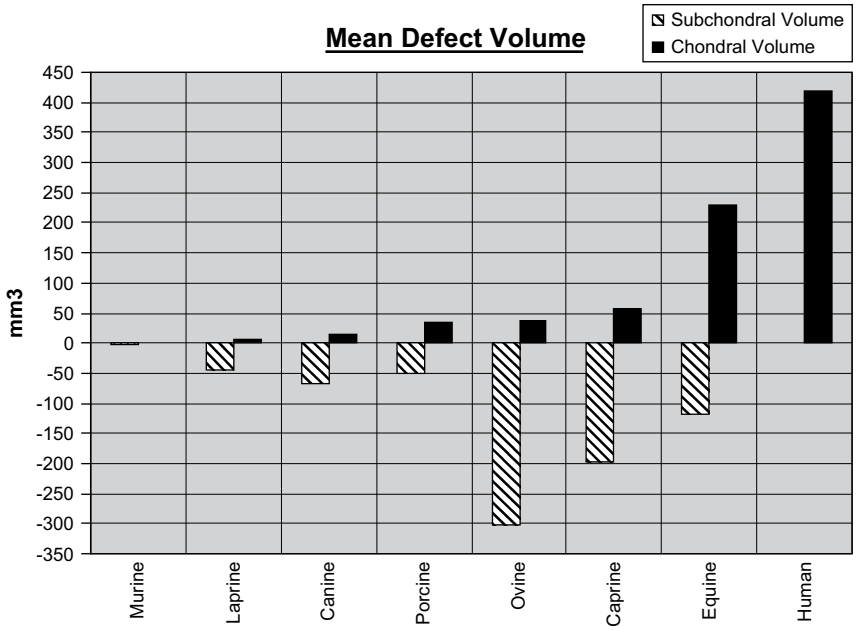

Fig. 1. Mean chondral and subchondral defect volumes of species analyzed compared to human clinical defects. Subchondral bone plate is zero.

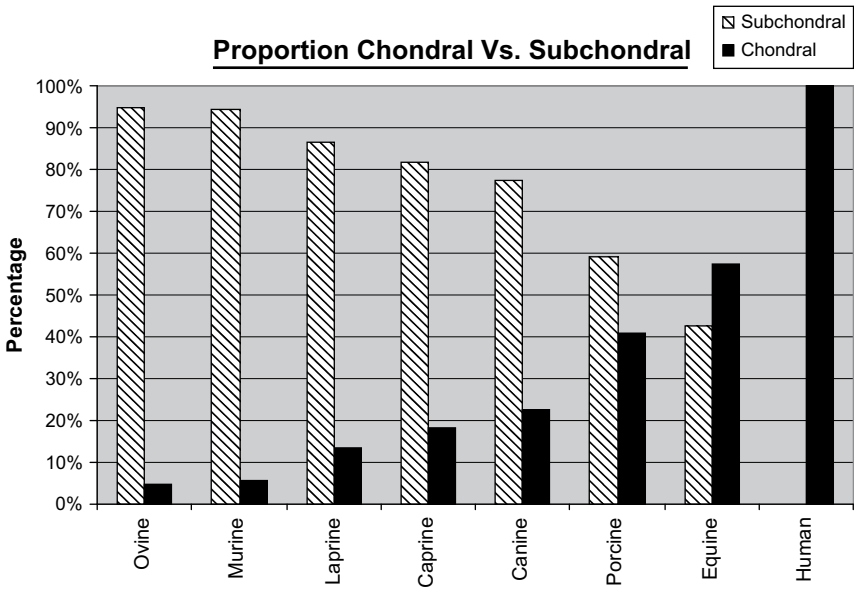

Fig. 2. Chondral and subchondral components of the preclinical animal model defects as a percentage of the total defect compared to the human patient.

The average cartilage volume of these defects is considerably less than reported human defects[3,10] (Fig. 1). An extremely large defect of 10 mm diameter and 10 mm depth used by van Dyk in 1998, produced a cartilage defect volume of 55 mm^3 considerably smaller than the average human lesion[10]. Furthermore, a large proportion of defects involve the subchondral bone which is another important difference (Fig. 2).

A benefit is that arthroscopic evaluation of the knee joint is feasible. Allowing macroscopic visualization and biopsy of defects during the course of research without requiring necropsy[63].

Defects have been located in the femoral trochlea[58,59,62–65], the medial femoral condyle[61] and both condyles concurrently[60,66].

A difficulty with the canine model is obtaining consistent skeletally mature subjects. This is probably because by the age of skeletal maturity at 12–24 months of age[4] most human–animal bonds are very strong. The median age was 41 months (range 18–72). A median of 29 dogs has been operated (range 25–30). The average endpoint was 16 weeks (range 2–78).

Cluster analysis placed all but the van Dyk canine study in group 1. This demonstrates that unless extremely large

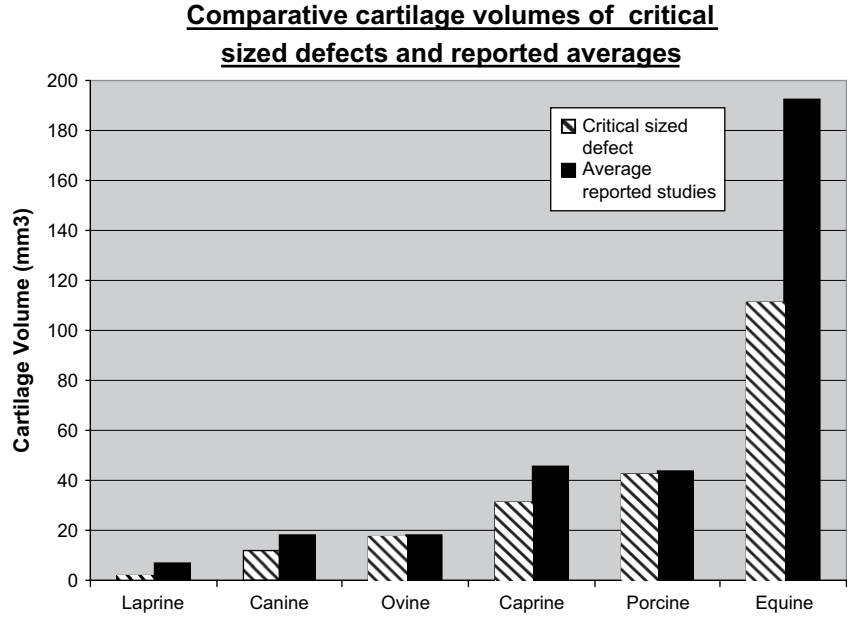

Fig. 3. A comparison of the cartilage volume of critical sized defects to the average reported cartilage defect volume assuming both are full thickness cartilage defects.

Table II
Cluster analysis utilized to group studies with relation to similarities in cartilage thickness, defect diameter and volume

Species	Group			Total
	1	2	3	
Murine	5			5
Laprine	27			27
Ovine	9	1		10
Canine	14	1		15
Porcine	6	2		8
Caprine	9	4		13
Equine	1	10	6	17
Human			6	6

defects are created the canine model is mostly a small defect model.

The dog is a reasonable model for preclinical testing with regard to its ability to have defects involving only the cartilage, the option of second look arthroscopy, similar anatomy and weight bearing conditions. In addition, dogs are easily trainable on treadmills and therefore the canine model is suitable for exercising studies. However, due to relatively small defect volumes and ethical reasons it is not a widely utilized species.

Sheep

The sheep is a commonly utilized animal model as they are readily available, easy to handle and are relatively inexpensive. In addition the anatomy of the knee is similar to humans. "Second look" arthroscopy is possible which is beneficial. However, due to a large fat pad and the degree of flexion required to visualize the femoral condyles this procedure requires a skilled arthroscopist.

Sheep have articular cartilage that is of variable thickness. Lu states that the cartilage ranges from 0.4 to 1 mm[67], Frisbie reports 0.45 mm and reference texts suggest 1.68 mm as an average thickness for the medial femoral condyle[4,12,32,67]. This variability makes the defect volume involving the cartilage and the subchondral bone likely to be different between individual subjects. This could produce variable results within a study. Defects have varied widely in the volume of cartilage and subchondral bone involvement[67–74]. Therefore, the standard deviation between published studies is large. This limits the ability to draw comparisons between ovine studies.

Table III
Cluster analysis groups demonstrating means, standard deviations and p25, 50, 75 confidence intervals for thickness, diameter and volume between the 3 groups

Group		Mean	p25	p50	p75	SD
1	Thickness	0.66	0.30	0.45	0.95	0.44
	Diameter	4.30	3.00	4.00	5.40	1.59
	Volume	12.37	2.89	7.16	17.50	12.50
2	Thickness	1.46	1.10	1.75	1.75	0.39
	Diameter	10.11	10.00	10.00	10.00	1.64
	Volume	114.31	79.52	130.93	137.44	35.49
3	Thickness	2.05	1.75	2.05	2.35	0.31
	Diameter	16.79	15.00	15.68	17.55	2.85
	Volume	496.15	309.25	401.38	586.25	254.50
Total	Thickness	0.97	0.03	0.95	1.50	0.66
	Diameter	6.82	4.00	5.00	10.00	4.64
	Volume	88.02	3.77	15.11	79.52	177.83

Pearce used a very large defect of 15 mm diameter in 2001[75]. A defect of this diameter can produce a cartilage defect volume of 170 mm^3, which is a similar volume to the lower range of human defects[9,10]. The large cartilage defects created in the sheep are associated with large total defect volumes (Fig. 1). Additionally, these defects produce a large proportion (>90%) in the subchondral bone (Fig. 2). Another disadvantage is the very dense and hard subchondral bone. This often prevents reproducible bone defect using trephine and fracture techniques without requiring drilling of the defect. This limits the choices of experimental design, especially for therapies requiring a healthy bleeding subchondral bone bed.

Cluster analysis placed all except one study in group 1 despite sheep having relatively large total defect volume because the cartilage volume was still comparatively low.

The average cartilage defect used in distal ovine femora has been 7.4 mm in diameter (range 2–15). The critical size defect has been reported as 7 mm[4]. The location of the cartilage defects in the ovine model has involved the medial femoral condyle[68,72,75–77], both femoral condyles[70,71,78] and the femoral trochlea[69]. On average 18 (range 4–40) sheep have been enrolled with an endpoint of 21 weeks (range 2–78).

Skeletal maturity is at 2–3 years of age. In the literature, sheep are commonly referred to as mature without an age being stated.

In conclusion, the sheep is a readily accessible model for cartilage defect testing. However, owing to it's variability in cartilage thickness and an often large subchondral defect component and relatively late skeletal maturity it is a model with some limitations.

Goat

The goat is a commonly utilized model. The femoro-tibial joint allows for "second look" arthroscopic examination[79], has thick cartilage, and a joint anatomy similar to humans. The cartilage thickness has been reported as 0.8, 1.1, 1.2 and 2 mm thick for the medial femoral condyle[79]. This variability likely results in variations of the volume of cartilage and subchondral bone defects within studies. A benefit of the cartilage thickness in this species is the allowance for partial and complete thickness defects as desired. This option is not possible in smaller animal models. Published studies have mostly created osteochondral defects. As previously mentioned, 95% of human cartilage defects do not involve the subchondral bone, as a result the ability to produce partial thickness defects is of importance as it more closely resembles the human[9,50]. The subchondral bone is softer when compared to sheep and common surgical techniques to create osteochondral defects can be readily and successfully applied.

The proportions of cartilage and subchondral bone involvement in goats are closer to the human situation than previously mentioned models (Fig. 2). Cartilage defects of 150 mm^3 can be produced by a 12 mm diameter defect. Despite this still being in the lower range of common human cartilage defects, these defects may allow cautious correlation between caprine trial conclusions and possible human expectations. As a result the goat is suitable to model small sized cartilage defects. On the other hand a limitation of published studies is the utilization of defects with large subchondral components which is considerably different to the human (Fig. 1).

Cartilage defect diameters range from 4.5 to 12 mm. The most frequently reported defect diameter is 6 mm[20,56,79–88]. Cartilage defects of 3 mm diameter have been reported to heal spontaneously[81]. A critical defect size of 6 mm does not heal after 6 months[86].

Cartilage defects have involved the lateral[56,86] and medial femoral condyles[80–85,87–90], and trochlear groove[79,80,82,85]. The average number of animals used was 14 (range 6–32) and an average age of 35 months (range 18–72). Goats have commonly been followed to an endpoint of 26 weeks (range 2–104).

Skeletal maturity is similar to sheep at 2–3 years of age and husbandry requirements and cost are also comparable. The goat is a relatively robust and flexible animal model commonly used for small cartilage defect trials.

Pig

The pig is a not a commonly used model for cartilage defect research. This is due to their large size, handling difficulties and involved logistical requirements in housing pigs. These problems can be slightly ameliorated by the selection of minipigs which are commonly utilized. A potential benefit of using pigs is their cartilage thickness. Chiang and Frisbie have reported the cartilage to be 1.5 mm and Hembry 2.0 mm thick in the medial femoral condyle[12,73,91]. This allows for the production of partial or full thickness defects as required. This feature has been utilized to study partial thickness cartilage defect therapies[92]. Despite the large cartilage thickness of pigs, historically, the total defect volume has not been as large as some other animal models (Fig. 1). It is important to note however that defects created often have a large portion of the defect involve only the cartilage. As a result the proportions of these defects are closer to the human defect dimensions when compared to previously discussed models (Fig. 2).

An average of 24 pigs were utilized (range 11–57)[73,91,92] and followed to an endpoint of 20 weeks (range 1–52) at an average age of 57 weeks (range 12–234 weeks). The FDA states that minipigs reach skeletal maturity by 42–52 weeks. Research by Vasara has reported that immature pigs spontaneously repair 6 mm cartilage defects[92]. In adult pigs a critical sized defect of 6 mm is supported from work by Harman *et al.* in 2006[93]. It is important that defect models in pigs be undertaken in adult animals to minimize spontaneous cartilage healing. Lesions have been created in the trochlear groove[91,92,94,95], medial femoral condyle[91] and both femoral condyles[73,96,97]. The pig has a large cartilage thickness for experimentation but researchers must contend with animal housing, size and handling difficulties which are less of a problem with other animal models.

Horse

The horse is the largest model available. The horse is a companion animal and as a result ethical issues are a factor. The horse requires large facilities for both housing and surgery, greater technical skills and equipment are also required that are not commonly available. Horses are typically not bred for biomedical research use and it is therefore difficult to obtain large numbers for a homogenous study cohort without considerable expense. Horses retiring from various athletic careers are often available but require screening for pre-existing musculoskeletal disease. On the other hand, because the horse is a long lived and athletic animal, it makes an appealing model to evaluate resurfacing technologies in chronic defects. Additionally the availability of post-operative exercise allows for evaluation of repair under rigorous loading conditions.

The cartilage thickness of the horse is approximately 1.75–2 mm for the medial femoral condyle[12,32]. This cartilage thickness is similar to a human cartilage thickness of 2.2 mm[32,98]. Because the horse is an athlete presenting clinically with a wide gamut of joint degeneration consistent with OA, it has spawned a vast amount of basic and clinical research addressing joint health. This is significant when considering assessment of outcome parameters. Most state of the art *in vitro* biochemical, molecular, gene therapeutic and immunohistochemical assays have been described for the various equine joint tissues and fluid[99–115].

The large size of the horse allows for "second look" arthroscopic examination, and the cartilage thickness allows for the production of partial or full thickness defects. This flexibility is not commonly available in other species. Defects can be produced that are of a size and proportion that most closely reflect the human situation (Figs. 1 and 2)[101–105,107–111,113–118]. Furthermore equine studies have historically involved cartilage defects that are considerably higher than what is considered the critical sized defect (Fig. 3). Cartilage defects in excess of 350 mm^3 with no subchondral bone involvement can be produced. Of the discussed animal models, this is most similar, to human cartilage defects (Fig. 2). As a result the horse was the only species to be placed in group 3 with humans by cluster analysis (Table II). This being the case the horse is the only animal model in which defect dimensions relevant to human clinical practice can be produced. Defects have ranged from 8 to 15 mm in diameter. The critical sized defect has been reported as 9 mm[4].

The horse is a large animal with an average weight of 400–500 kg. This weight places defects under stringent loading conditions during and following anesthetic recovery. While the horse provides a model in which defect dimensions can correlate to the human clinical scenario, the loading environment is of significant concerns. Despite stall confinement, the static continuous loading of weight bearing portions in the joints cannot be minimized as for a human patient. Anatomical selection of the defect site is therefore critical and it is important to have realistic expectations for results from a device subjected to such considerable loads.

The lateral trochlea of the femur is the most common location for cartilage defects[101–105,107]. Defects have also been created in the lateral condyle of the metacarpophalangeal joint[117] and the middle carpal bones[108]. A median of 8 horses has been used in each study with a range of 6–12 and followed to an endpoint of 19 weeks (range 2–52). The average age was 46 months with a range of 12–72.

While the horse is certainly the most appealing animal when it comes to size of joint anatomy and cartilage morphology, the rigorous loading environment must be carefully considered when designing a study.

Discussion

There are many animal models that are used in cartilage defect research. Large animal models such as the goat or the horse may more closely resemble the human compared to smaller animal models such as rodents or rabbits. But, it is usually not fiscally feasible or practical to conduct initial experiments in larger species. Therefore, it is generally well accepted to choose a small animal model for initial lines of investigation. However, final preclinical evaluation of an articular cartilage reconstruction technique may require confirmation in a large animal model.

The statistical comparison of defects involving the distal femora performed in this study analyzed defects involving both femoral condyles and the trochlear groove with respect to volume and the relative proportions between species. Calculation of the proportional cartilage to subchondral volume was based on work by Frisbie *et al.*[12]. These values were utilized as they are the only evaluation of cartilage thickness between species that uses the same methodology. Differences between the cartilage thickness between the femoral condyles and the trochlear groove were not considered in the statistical analysis as a majority of the defects involved the medial femoral condyle and due to no published data using consistent technique between sites and or species.

Studies involving small cartilage defects can be adequately performed on canine, ovine, porcine or caprine models as are supported by our comparative analyses. The most stringent test of a single site defect therapy is the equine model. This is a reflection of the horse's larger joint anatomy and morphology allowing for a larger defect of proportions that is more reflective to the clinical human. The equine is historically the least commonly utilized and only recently is gaining broader interest.

The further understanding of the biomechanical properties of normal articular cartilage and the functional requirements for repaired articular cartilage will be critical for the advancement of tissue engineering of articular cartilage. In order to achieve these goals, new techniques to measure the biomechanical properties of normal, degenerated and engineered cartilage, including minimally invasive and non-invasive techniques for *in vivo* measurement will have to be developed. The standardization of these techniques and the selection of the appropriate animal model will be critical for a meaningful comparative evaluation of tissue-engineering techniques.

Conflict of interest

None of the authors has anything to declare for this review manuscript.

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