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Reducing the Number of Laboratory Animals Used in Tissue Engineering Research by Restricting the Variety of Animal Models. Articular Cartilage Tissue Engineering as a Case Study

Rob B.M. de Vries, Ph.D.,[1] Pieter Buma, Ph.D.,[2] Marlies Leenaars, Ph.D.,[1] Merel Ritskes-Hoitinga, D.V.M., Ph.D.,[1] and Bert Gordijn, Ph.D.[3]

The use of laboratory animals in tissue engineering research is an important underexposed ethical issue. Several ethical questions may be raised about this use of animals. This article focuses on the possibilities of reducing the number of animals used. Given that there is considerable debate about the adequacy of the current animal models in tissue engineering research, we investigate whether it is possible to reduce the number of laboratory animals by selecting and using only those models that have greatest predictive value for future clinical application of the tissue engineered product. The field of articular cartilage tissue engineering is used as a case study. Based on a study of the scientific literature and interviews with leading experts in the field, an overview is provided of the animal models used and the advantages and disadvantages of each model, particularly in terms of extrapolation to the human situation. Starting from this overview, it is shown that, by skipping the small models and using only one large preclinical model, it is indeed possible to restrict the number of animal models, thereby reducing the number of laboratory animals used. Moreover, it is argued that the selection of animal models should become more evidence based and that researchers should seize more opportunities to choose or create characteristics in the animal models that increase their predictive value.

Introduction

Tissue engineering (TE) is a promising new medical technology. Similar to many other new technologies, however, it is not free of ethical challenges. As yet, the ethical debate about tissue engineering has strongly focused on the use of human embryonic stem cells and therapeutic cloning.[1] Moreover, some attention has been paid to the ethics of clinical trials with human subjects and the donation of cells for TE purposes.[2–4] The ethical aspects of the use of laboratory animals in tissue engineering research,[i] however, have largely been ignored.

Within TE research, laboratory animals are used both for studying the fundamental biological processes involved in tissue engineering and as models of human disease and injury.[ii] Particularly when animals are used as models of human degenerative diseases, they can experience significant discomfort during experiments.

From the perspective of animal ethics, the question may be raised whether the use of animals for these purposes is morally permitted at all. If at least some animal experiments are considered to be morally allowed, a second question is which experiments are so important for improving human health that their benefit to humans outweighs the disadvantages for the laboratory animals (compare with Ref.[5]).

[i]In addition, animals are sometimes used as donors of cells or materials for xenogeneic TE products (e.g. decellularized porcine heart valves).

[ii]Typical examples of animal experiments performed in the context of tissue engineering research are as follows: testing the osteogenic or chondrogenic potential of tissue engineering constructs containing human cells by subcutaneous implantation in nude mice or the testing of the regenerative capacity of a tissue engineering construct by creating a defect in an animal and treating the defect with the tissue engineering construct to see whether the injury heals.

[1]SYRCLE/3R Research Centre, Central Animal Laboratory (Post 231), Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.
[2]Orthopaedic Research Lab (Post 366), Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.
[3]Institute of Ethics, Dublin City University, Dublin, Republic of Ireland.

Although these questions are pertinent and important, we would like to address a third ethical issue, namely the implementation in tissue engineering research of the three Rs,[6] more particularly of the R of Reduction (compare with Ref.[7]).

There may be several ways in which the number of animals used in tissue engineering experiments could be reduced. As shown in an earlier literature review,[1] there is considerable debate about the adequacy of the current animal models in tissue engineering research, and many authors have stressed the need for animal models that more closely resemble human diseases than the existing models do (e.g., see Refs.[8,9]). Against this backdrop, we focus on the question of whether it is possible to reduce the number of laboratory animals used in tissue engineering research by selecting and using only those models that have the greatest predictive value.

Of course, it is not feasible to answer this question for the entire field of tissue engineering in one article. In order to explore this question, we will, therefore, use the field of articular cartilage tissue engineering as a case study. We have selected this field, because it is a relatively old field of tissue engineering, which means that there is ample experience with a wide variety of animal models. In addition, some of the tissue engineering products developed in this field have already been studied in clinical trials, and meaningful comparisons can, therefore in principle, be made between the findings in animals and in humans. Various types of Autologous Chondrocyte Implantation (ACI) (e.g., see Refs.[10,11]) are currently applied in the clinic, and a commercially available system for cartilage repair has recently received European market approval (ChondroCelect™, EMEA/H/C/000878). Based on (reviews of) clinical studies carried out so far, a clear benefit of cell-based therapies over microfracture (MF) and osteochondral autografts (OATS) has not yet been demonstrated.[12–15] A comparison between the efficacy of ACI, on the one hand, and MF and OATS, on the other hand, is also difficult, as MF and OATS are used for relatively smaller defects ($<4\,cm^2$), and ACI can also be used for larger defects ($4–10\,cm^2$). The benefit of ACI is supposed to consist of a better quality of the repair tissue that would potentially slow down the progression to a more generalized osteoarthritis.[16]

Given that the suitability of an animal model directly depends on the specific research question asked—for example, one animal species may be suitable to study the efficacy of a treatment, whereas another species may be more suitable to study the safety of that same treatment—we focus solely on the animal experiments intended to provide information on the efficacy of the repair of local traumatic partial-thickness defects in human articular cartilage through tissue engineering techniques.[iii] This type of defect represents more than two thirds of the defects clinically encountered.[17,18]

We first provide an overview of the animal models that have so far been used for answering this type of research question and of the advantages and disadvantages of these models, particularly in terms of extrapolation to the human situation. Based on this comparison, we subsequently determine whether it is indeed possible to reduce the number of laboratory animals by selecting and using only those models that have the greatest predictive value for cartilage repair in humans.

Methods

In order to find articles that comment on the adequacy of the animal models[iv] used in cartilage tissue engineering research, the databases PubMed, EMBASE, and Web of Science were searched using combinations of the following groups of terms: (tissue engineering OR tissue engineered OR chondrocyte implantation OR chondrocyte transplantation) AND (articular cartilage OR hyaline cartilage OR cartilage defect OR cartilage repair OR cartilage regeneration) AND (animal model OR animal models OR preclinical).[v] MeSH terms (PubMed) and EMTREE terms (EMBASE) were used as much as possible. The search was limited by language (English) and date (published before 01-07-2011). This search yielded 160, 246, and 123 articles for PubMed, EMBASE, and Web of Science, respectively. Based on title and abstract, the articles not pertaining to articular cartilage were removed. The abstracts of the remaining articles were analyzed in order to determine whether the article in question primarily reported scientific findings about a particular tissue engineering construct or whether it also commented on (problems with) the animal model used. Articles of the latter type were studied in detail, and potentially relevant articles cited in these articles were also collected. This strategy resulted in 23 articles that were relevant for our aim.[19–41]

Moreover, six leading experts in the field of articular cartilage tissue engineering were interviewed regarding their views on and experiences with the various animal models used in the field. The reasons for adding interviews were (1) to acquire more detailed information about the models from scientists with relevant practical experience[vi] and (2) to obtain information about the difficulties with animal models not reported in the literature (given the possibility of publication bias in this regard). The initial group of experts was selected on the basis of advice by the management team of the Dutch Program Tissue Engineering; this group was extended by scientists identified as experts by the respondents of the (first) interviews. The interviews were semi-structured. The questionnaire[vii] used consisted of questions regarding the animal models that the respondents themselves use in their tissue engineering research (reasons for selecting the model and experiences with the model), their knowledge about other potentially suitable animal models, and their opinion on the possibilities of achieving reduction through the restriction of the number of animal models. In addition,

[iii] In other words, we set aside models for osteoarthritis and rheumatoid arthritis.

[iv] Strictly speaking, the phrase 'animal model' does not only refer to the species of the animals used, but also refers to the strain, the age, the sex, and, last but not least, to the way in which the defect is created (technique, location, size, etc.). Since it is common in the literature, however, to use the phrase rather loosely, we employed the term both in our literature search and in the description of the results in the former, broader sense of the phrase.

[v] For the full search strategy, see Supplementary Data (Supplementary Data available online at www.liebertpub.com/teb).

[vi] Since the focus of most articles in the literature is on the performance of the tested tissue engineering construct, findings more specifically related to the models used will generally not be mentioned.

[vii] For the complete questionnaire, see Supplementary Data.

Table 1. Overview of Animal Models Cartilage Tissue Engineering

	Mouse/rat	*Rabbit*	*Dog*	*Pig*[a]	*Goat*	*Sheep*	*Horse*
Opportunity to study spontaneous defects	no	no	no	no	no	no	yes[R1,R4,R5,b]
Joint size[c]	<< [R1,R3]	<<	< [R2]	≈	≈ [R2,R4]	≈	>
Cartilage thickness[d] (mm)	<0.2 [R1,R3]	0.3–0.4	0.9–1.3 [R2]	1.5–2.0	1.1–1.4 [R1,R2,R5]	0.4–1.7 [R3]	1.75–2
Average defect volume studied (% of human defect volume)	0.4	9.6	14.9	19.4	45.6	65.1[e]	60.6
Nature of defect feasible	O	O	P	P	P	P	P
Loading conditions in joint	<<	<<	≈	> [f]	≈ [f]	≈ [f]	>> [fR4,R5]
Controlled rehabilitation feasible	no	no	yes	no	no	no	yes
Arthroscopy feasible	no	no	yes	yes	yes [R4]	yes[g]	yes
Availability of biochemical tests	high	high [R4]	low	low	low [R4]	low	high
Costs (buying and housing)	low	low [R3, R4, R5]	high [R2]	medium	medium [R2]	medium	very high [R1,R4,R6]
Ease of handling	high	high	high	low	high	high[h]	high
Companion animal status	no	no	yes [R2,R5]	no	no	no	yes [R4]
Other features		High intrinsic healing capacity [R2,R4]	A few skeletal mature individuals available	Rapid skeletal growth [R2]	Metabolic rate similar to humans [R4]		

[a]The data presented here apply to normal size pigs, not to minipigs. The latter are more easy to handle and have a lower growth rate; the average thickness of cartilage, however, is significantly lower (0.5–0.8 mm).

[b]Particularly in the case of horses, the main driving force behind the development of cell-based treatments is veterinary practice, notably the treatment of joint injuries in (race) horses, rather than human clinical practice.

[c]Optimal positioning and fixation of the tissue engineered construct feasible or not.

[d]On the medial femoral condyle. Note that exact cartilage thicknesses may vary not only between but also within species depending on age, breed, location, and joint (compare with Ref.[24]).

[e]High percentage mainly due to a study that used a very large defect (See Ref.[19]).

[f]Since animal models, unlike human patients, stand and walk on the operated limb immediately, the implants may be subjected to forces much higher than postoperative loading conditions in human patients. This problem might be mitigated by choosing another, less weight-bearing implantation site than the femoral condyle, such as the trochlea.

[g]But difficult due to a large fat pad (Ref.[19]).

[h]Though more adverse to human interactions than goats (Ref.[43]).

O, osteochondral; P, partial thickness; R1 (R2, R3, etc.), feature not only mentioned in literature but also by respondent 1 (2, 3, etc.); <<, much smaller/lower than in humans; <, smaller/lower than in humans; ≈, comparable to human situation; >, bigger/higher than in humans; >>, much bigger/higher than in humans; Human clinical situation: partial thickness defects, cartilage thickness 2,35 mm, defect volume 550 mm^3.

the respondents were asked for their suggestions for further literature; they mentioned three documents not yielded by the search strategy described in the previous paragraph.[42–44] Since the majority of the respondents wished to remain anonymous, they will be referred to as "respondent 1," "respondent 2," and so on.

Overview of Animal Models Articular Cartilage Tissue Engineering

Based on the scientific articles collected and the interviews held, we present in Table 1 an overview of the species of laboratory animals most often used for studying the repair of articular cartilage defects through tissue engineering,[viii] namely small rodents (mouse, rat), rabbit, dog, goat, sheep, pig, and horse.[ix] To prevent misunderstanding and as explained in the introduction, in this article and, therefore, in Table 1, we restrict ourselves to defect models. Particularly, mice are also used for other purposes, for example, either for studying the chondrogenic potential of a construct containing human cells by subcutaneous implantation in nude mice[x] [29,38] or for studying the mechanisms involved in cartilage repair through the creation of transgenic or knockout mice (compare with Ref.[23]).[xi] [45,46] Such uses fall outside the scope of this article. For each species, we indicate (1) what is known about the features considered relevant for extrapolation to the human situation and (2) (other) reasons for and against choosing the animal model in question.

[viii]The animal species that are generally speaking most closely related to humans are non-human primates. They are hardly used in cartilage tissue engineering research, however, most likely because of moral opposition from society (cartilage conditions are not life-threatening). But several respondents stressed that it is far from clear that in cartilage structure and function non-human primates are more similar to humans than the larger animal models used.

[ix]We discuss the models more or less in order of size (from the smallest to the largest model). The order chosen intends to reflect neither the number of animals used per species (from the least to the most often used model) nor the 'adequacy' of the model (from the least to the most predictive model).

[x]That cartilage formation in nude mice should not be used to predict the repair of a cartilage defect is confirmed by Habibovic et al. 2006, Reinholz et al. 2004, and by respondents 5 and 6.

[xi]Other purposes involve studying the developmental aspects of cartilage formation (e.g. Onyekwelu et al. 2009) or studying the possibilities of using embryonic stem cells for cartilage TE (e.g. Jukes et al. 2008).

For a correct interpretation of the data presented in Table 1, it is important to know that the average thickness of the cartilage layer on the medial femoral condyle in humans is 2.35 mm.[19,24] Moreover, in the human clinical situation, the average defect has an area of about 2.1 cm^2 [17] and a volume of approximately 550 mm^3.[19] Finally, the partial thickness defects, which we focus on in this article, by definition[xii] involve only the cartilage, without affecting the subchondral bone.[xiii] This underlying bone contains bone marrow and vasculature, as a consequence of which the defect may be exposed to different cells, growth factors, and other signaling substances; whereas the cartilage layer is immunologically privileged and does not contain blood vessels.[32]

Based on the data presented in Table 1, the following conclusions can be drawn regarding the similarities between the various animal models and the human clinical situation. With regard to the thickness of the articular cartilage and the nature and volume of the defects that can be studied, the horse would seem to come closest to humans (followed by the other large models, i.e., goat, sheep, and pig). As for joint size and loading conditions, goat and sheep appear to resemble the human situation more closely. Unlike the latter large models, however, the horse offers the opportunity to study spontaneous defects. Moreover, in humans, three different rehabilitation protocols are applied for defects on the lateral or medial femoral condyle (the most frequently occurring defect location), defects of the patella and trochlea, and combined defects of patella, trochlea, and femoral condyles. Such protocols cannot be applied in goat or sheep; the dog and horse are the only animal models in which a controlled rehabilitation program can be applied.

Restricting the Variety of Animal Models

Turning to the central question of our article, the data included in our overview suggest that there are possibilities for reducing the number of animal model species used in this area of articular cartilage tissue engineering, because

(1) some small animal models are being used, notably mice/rats and rabbit (and maybe dog), that are very probably less predictive of the human clinical situation than other, larger models (e.g., goat or horse);
(2) several large animal models are being used (goat, sheep, pig, horse, and maybe dog) that, based on existing data, do not seem to differ significantly in the extent to which the findings can be extrapolated to the human situation.

Small models

In our discussion of the suitability of small models, we will focus on the rabbit, because what applies to the rabbit applies *a fortiori* to the mouse and rat. Many experiments studying the regeneration of articular cartilage use the rabbit as an animal model. However, the cartilage layer in the rabbit is much thinner than in humans, and the femoral condyle of rabbits has a large curvature,[xiv] [47] which makes the creation of partial thickness defects very difficult.[48] As a consequence, not only the volume of the defects that can be created but also the nature of these defects (osteochondral rather than partial-thickness defects) differs markedly from the human clinical situation. By contrast, the cartilage thickness in the large models comes much closer to the thickness of human cartilage, and, therefore, defects may be created that both in volume and in nature are much more comparable with the human situation.[24] Second, the load on the articular cartilage in rabbits is much lower than in humans.[19] Although exact data are not available, it might be assumed that the stresses on the joints of the larger models are more similar to human joints (compare with Ref.[49]). Finally, there are indications that the intrinsic healing potential in rabbits is greater than in larger mammals, including humans.[23,28,43] All in all, the risk of overestimating the efficacy of a tissue engineering treatment in humans is much bigger when the estimation is based on studies in rabbits rather than in large models.

Researchers, both in the literature and in our interviews, indicate that they are aware of these disadvantages of using rabbits. Nevertheless, many still carry out studies on rabbits. What arguments do they use to justify their use of rabbits? We found two types of arguments: (a) The rabbits are not used as direct models for cartilage regeneration in humans but have a different function, and (b) rabbits are, in many cases, so much cheaper, and results can be obtained so much faster, that these advantages outweigh the potential loss of predictability. We are not yet convinced by these arguments, as will be explained.

Ad (a). Many researchers stress that the small laboratory animals should not be used directly as a model for the human clinical situation, but rather for studying preliminary questions, for example, regarding biocompatibility, material formulations, and basic device design. That is to say, the experiments using these animals are directed more at studying certain features of the tissue engineering construct as such than at the translation of the findings to humans. Questions that researchers try to answer by means of the experiments are as follows: Is the tissue engineering construct capable of forming cartilage *in vivo*, and is it biocompatible? Is the construct capable of cartilage formation and of repair of a defect in a knee joint? Which biomaterial or which construct design (e.g., regarding pore size and distribution) leads to the greatest amount of cartilage formation? In other words, the small animal models are used for proof-of-concept studies and as a selection device to separate the promising from the less promising constructs.

With regard to the proof-of-concept studies, we recognize that in the early phases of the development of the field of

[xii] We adopt the following distinctions regarding the types of defect: partial thickness (defect involves only cartilage and covers part of the cartilage layer), full thickness (defect involves only cartilage and covers the whole cartilage layer up to but not into the subchondral bone), and osteochondral (defect covers both the cartilage layer and a part of the underlying bone).

[xiii] Human patients undergoing ACI have debridement of the cartilage defect before implantation, that is, the edges of the cartilage defect and the deeper layers are meticulously removed. During this process, the subchondral bone might be penetrated, resulting in bleeding from this underlying tissue. The extent to which mesenchymal stem cells, which might enter through the subchondral bone, contribute to defect repair in these patients is not known.

[xiv] This is one of the reasons why some researchers choose a less challenging model such as the trochlea (cf. Buma et al. 2003).

tissue engineering, it was necessary to show that regeneration through tissue engineering techniques was possible at all, and we understand why relatively favorable models such as rabbits were used. By now, however, it would seem to be unnecessary and, thus, undesirable that each and every new construct would be shown to be capable of cartilage formation in rabbits[xv] before being tested in a large model.

We wonder, moreover, whether the small models are really suitable as tools to select the constructs that are most promising regarding cartilage repair in humans. We would be inclined to say that a proper selection device would have to meet at least the following two conditions. First, the device should actually select, that is, a substantial part of the constructs being tested should fail the test, and, second, the device should select on the basis of the appropriate, desired features. We asked our respondents to give an estimate of the percentage of constructs that fail at the stage of the small animal models (because the construct tested turns out to be either unsuitable or not promising enough to be investigated in larger models). Their answers varied greatly: Some estimated that between 25% and 50% of the constructs do not survive the first *in vivo* testing phase. Others believed that more than 75% fail at this stage. However, due to the small cartilage thickness, the osteochondral nature of the defects, and the relatively large innate healing potential of rabbits, we would expect that many constructs will pass the test in rabbits. It is true that if a construct fails to form (sufficient) cartilage in a small animal model, then it will very probably also fail to work in a large model and in humans. However, if, given the rather favorable conditions in rabbits, only a few constructs fail, the question is whether you should not start with larger models straightaway.

One may also wonder whether the experiments in small models actually select on the basis of the right properties. If the conditions in osteochondral defects indeed differ so much from those in partial-thickness defects, we would say that a direct correlation would need to be established between the findings in both types of defects before the former can be used as a selection device for the latter. However, as far as we were able to determine, no studies have been carried out that (were intended to) show a correlation between the findings in small models with osteochondral defects and large models with partial-thickness defects; for example, by testing a number of different constructs both in a small and a large model and demonstrating that the results in both types of models and defects are comparable. In other words, for the time being, it is an assumption rather than an established fact that the small models select on the basis of the appropriate, desired features.

Ad (b) The strength of the argument that the advantages of using small animal models—cheaper purchase/husbandry and faster results—outweigh the loss of translatability largely depends on the validity of the considerations discussed in the previous paragraphs. If the small models are not selective enough, and, therefore, nearly all constructs still have to be tested in large animals, then it is questionable whether you really save that much money. Similarly, if your selection in small models is fast but you select on the basis of the wrong properties, then you may in the end (after unsuccessful clinical studies and because of new animal experiments) lose rather than win time and money by starting with small models.

In the light of the considerations just presented, we would suggest that, unless and until a direct correlation between the findings in small models with osteochondral defects and large models with partial-thickness defects is established, researchers skip the small models and start with large models right away. To prevent misunderstandings, our proposal to skip the small models applies, as indicated previously, only to the use of mice, rats, and rabbits as defect models. More particularly, we do not intend to object to the use of nude mice for studying the chondrogenic potential of a tissue engineering construct containing human cells or to the use of genetically modified strains of mice for studying the mechanisms behind cartilage repair. After all, such experiments cannot yet be carried out in the larger animal models. Furthermore, in order to avoid the waste of large amounts of animals and money because of biocompatibility problems or flaws in the design of the construct, we suggest that researchers start with small pilot studies in large animals, rather than with large, full-blown studies in small animals.

Large models

As indicated at the beginning of this section, based on current knowledge, the various large animal models do not seem to differ significantly in the extent to which results found in them can be extrapolated to the human situation. Although the cartilage thickness of horses comes closest to the thickness of human articular cartilage,[24] the loading conditions are so divergent that they seem to detract from the overall translatability. However, if the differences between the large models are indeed not significant, then we would argue that the use be restricted to one model. Preferably, the selection of this model should be based on feedback from the clinic.[xvi]

We are well aware that the choice for a particular animal model is not solely determined by the extent to which the results may be extrapolated to the human situation, but that other reasons may play a role as well. More particularly, there might be moral reasons for selecting an animal model that is less predictive of the human situation but has a lower moral status[xvii] [50] rather than using a model with a high

[xv] One might argue that, in order to be able to compare the results of studies using new techniques/constructs with the results of previously performed studies, one should keep performing studies in rabbits. We would object, however, that, even though a comparison with results from previous studies is important, a translation to the human situation is more important. Given that there are strong arguments that the larger models are more predictive than the smaller models, a comparison to previous literature cannot be a decisive reason for continuing to use rabbits.

[xvi] It might be that one large model corresponds with the human clinical situation in one respect, whereas another large model corresponds with it in another respect. In that case, the aspect on which the clinical trial will be focused determines which model should be used in the preclinical studies.

[xvii] We are aware that some animal ethicists contest the assumption that the concept of moral status admits of degrees, particularly ethicists who defend a single criterion of moral status, for example, the capacity to feel pain, that is either present or not (such as Peter Singer). Most animal ethicists today, however, defend a multi-criterial approach in which the moral status of a being is determined by a combination of criteria, some of which are intrinsic properties, some of which are relational (e.g. being a part of a larger community), and some of which may admit of degrees (cf. Warren 1997).

predictability but a correspondingly high moral status (a clear example would be using a nonprimate mammal, rather than a more representative primate, for research into a non-life-threatening disease in humans). The two features on which such a difference in moral status might be based that are most often discussed in the literature are as follows: (1) differences in the neural/psychological complexity [distinction between self-conscious, conscious, and nonconscious animals (e.g., see Ref.[51])] and (2) differences in the intensity of the relationship with humans [companion animals vs. e.g., farm or laboratory animals (e.g., see Ref.[50])]. We agree that differences in psychological complexity may be used to justify differences in moral status (because higher cognitive capacities imply more and different interests that might be thwarted[xviii 51–53]). Self-conscious mammals, notably non-human primates, are not used in cartilage tissue engineering research, however, and the large animal species that are used do not seem to differ in psychological complexity; they all seem to belong to the second category, that of conscious animals (compare with Ref.[54]). With regard to the intensity of the relationship with humans, we recognize that a bond between an animal and humans can lead to special duties. For example, arguably people have a greater moral responsibility for the animals they care for than for those not under their care.[55] The basis for this responsibility, however, lies in the actual relationship between, for example, individual companion animals and their owners,[56] and the special duties involved, therefore, do not extend to other members of the same species that are not in such a relationship. In other words, a closer bond with humans does not imply a higher moral status, in the sense that using laboratory animals not generally regarded as companion animals (goat, sheep, and pig) would in itself be morally preferable to using companion animals (such as dog or horse). We, therefore, reject the claim, made in some of the articles included (e.g., see Ref.[19]), and by some of our respondents, that the fact that dogs and horses are companion animals entails that there are more moral problems. Accordingly, we would argue that the choice for the large model to be used should not be influenced by issues such as psychological complexity or companion animal status, but be solely determined by the extent to which the results are expected to be predictive of the outcome in humans.

Reducing the Number of Animals

In the previous paragraphs, we aimed at showing that and how the number of laboratory animals used in the field of articular cartilage tissue engineering could be reduced by restricting the use to those models which are considered most predictive. One might object, however, that although we have made plausible that reducing the number of *models* is feasible, reducing the number of models does not necessarily lead to a reduction in the *number* of individual animals used. After all, if the experiments currently performed in different animal species were all performed in the same species, then the number of animals would seem to remain the same.

For several reasons, however, we believe that a reduction in the number of models will also lead to a reduction in the number of laboratory animals used. First, currently, similar experiments are carried out in different animal models, particularly experiments that are first performed in rabbits and, subsequently, in a larger model. Second, the results of these different experiments cannot be easily compared. A direct comparison of different constructs would require extra experiments, because now the experiments often differ in both construct and animal model species used. By restricting the number of models, 'duplicating' experiments in different species could be prevented more easily, and the experiments actually carried out would be far more informative for other and new experiments. Moreover, new experiments might require fewer animals, because more studies using the same animal model will be available, enabling a more accurate assessment of the variability in outcome measures.

Further Moral Implications

We would like to stress that the view that the larger models are more predictive of the human situation than the smaller models is largely based on (plausible) hypotheses about the relevance of the anatomy and thickness of the cartilage layer rather than on actual feedback from clinical findings in humans. So, strictly speaking, the (lack of) clinical adequacy of the findings in different laboratory animal species has not yet been proved; or, as one of the respondents put it, the problem is not so much that we know the models currently used are not predictive and that we need to consider how to deal with this lack of predictiveness. The problem is rather that we do not know for certain how predictive the various models actually are.[xix 27] This lack of knowledge about the actual predictiveness is, on the one hand, understandable given that tissue engineering is a new field, having entered the clinic only recently. On the other hand, however, the opportunities for feedback on the adequacy of the animal models that were offered by the clinical studies performed have hardly been used. As far as we could ascertain, as yet, no studies in the field of articular cartilage tissue engineering have been published that explicitly link the design and results of clinical studies to the design and results of the underlying preclinical animal studies.[xx 57] We would, therefore, urge the researchers in the field to design future clinical studies in such a way that the studies cannot only be used to determine the safety and/or efficacy of a tissue engineering construct in human patients but can also be used to assess the actual adequacy of the animal models used.

More generally, the extent to which findings were believed to be translatable to humans did not play as large a role in the choice for a particular laboratory animal species as we expected. Our interviews showed that the choice to use a particular animal model is only partly determined by the anticipated predictiveness of the model and is just as much

[xviii] For further justification of this position, see e.g. VanDeVeer 1979, DeGrazia 1996, 2007.

[xix] This might also imply that a construct which does not work in a particular large model actually does work in humans (cf. Giannoni et al. 2005).

[xx] Saris et al. (2009) show a relation between cartilage repair in humans using Characterized Chondrocyte Implantation and the results of preceding animal experiments. These experiments, however, consisted of tests conducted on nude mice in order to determine the cartilage-forming potential of the human chondrocytes to be implanted rather than the studies of cartilage repair in an animal model.

based on considerations of costs, ease of handling, and the like. Furthermore, in most cases, this choice is not the result of an explicit and extensive comparison between different potential models. As a matter of fact, our respondents turned out to have little knowledge about other models than the ones they used themselves. They mentioned the following three reasons behind researchers in practice paying relatively little attention to the selection of animal models (and feedback from the clinic): Developing animal models does not yield much scientific status; comparing different models is generally considered to be boring or scientifically uninteresting; and it is hard to acquire resources for these activities. We believe, however, that there are good scientific and moral reasons to pay more attention to selecting and developing animal models. Making the choice for a particular model more 'evidence-based' will bring out the model that comes closest to the human situation and will, therefore, make it more likely that results found in the laboratory animals can be translated to the clinic. Moreover, only those experiments will be carried out that will yield the most relevant results. This prevents laboratory animals that are less adequate models from being subjected to scientifically less relevant experiments. The method we would suggest to make the selection of animal models more evidence based is the use of systematic reviews of animal experiments[xxi].

To prevent misunderstanding, we do not hold the tissue engineering researchers accountable for the lack of correspondence between the various potential animal models and the human situation. That lack of correspondence is a given and can hardly be changed. However, these researchers can be called to account for using animal models that are likely to be less predictive of the human situation than other models available. This does not only apply to species of laboratory animals used, but also applies to other characteristics of the model, particularly when these characteristics can easily be influenced by the researchers (compare with Ref.[35]). For instance, most laboratory animals used are quite young (e.g., rabbits that are younger than 20 weeks). These young animals, however, may not be skeletally mature and may have an increased spontaneous repair capacity that may override any repair strategy under evaluation.[19,25,29,37,41,47] Moreover, most human patients treated are not that young. Similarly, in most studies, freshly created defects in animals with normal articulations are immediately treated with the tissue engineering construct ([39] being a notable exception). The majority of patients, on the other hand, have a longer history of complaints (chronic defects) before they are treated for their joint damage,[38,39] and this has an impact on the repair capacity of the defect.[58] Finally, in many cases in which large animal models are used that allow for the creation of partial-thickness defects, researchers still create and study osteochondral defects.[19] Thus, we would urge the researchers to use or develop models that are more comparable to the human situation, at least in the respects that they can influence.

[xxi]Although one of the studies we used to collect data about the various animal models (Ahern et al. 2009) described itself as a systematic review, this study did not meet the standards of a high-quality systematic review. The search strategy and the selection criteria were not described in sufficient detail. Moreover, in our view, the review did not focus enough on the properties potentially relevant for translation to humans.

Conclusion

We do not believe that our findings—notably the use of smaller but less predictive animal models, the use of several more or less equally predictive large animal models, and the lack of a systematic feedback between preclinical and clinical studies—are typical of the field of articular cartilage tissue engineering. For example, there are indications that similar questions are at issue in the field of bone tissue engineering (compare with Ref.[29]). However, in order to draw more general conclusions regarding the adequacy of the animal models used in tissue engineering, more detailed studies of other fields of tissue engineering would be required. We recommend carrying out these studies.

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No competing financial interests exist for any of the authors.

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Address correspondence to:
Rob B.M. de Vries, Ph.D.
SYRCLE/3R Research Centre
Central Animal Laboratory (Post 231)
Radboud University Nijmegen Medical Centre
PO Box 9101
6500 HB Nijmegen
The Netherlands

E-mail: r.devries@cdl.umcn.nl

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