	Inventaris Wob-verzoek W16-10S								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS2015282								
1	Aanvraagformulier				x		x	x	
2	Projectvoorstel				x			x	
3	Niet-technische samenvatting	x		x					
4	Bijlage beschrijving dierproeven 1				x			x	
5	Bijlage beschrijving dierproeven 2			x					
6	Bijlage beschrijving dierproeven 3				x		x	x	
7	Bijlage beschrijving dierproeven 4				x			x	
8	Bijlage beschrijving dierproeven 5				x			x	
9	Appendix				x			x	
10	DEC-advies				x		x	x	
11	Ontvangstbevestiging				x		x	x	
12	Verzoek aanvulling aanvraag				x		x	x	
13	Reactie verzoek aanvulling				x		x	x	
14	Mail reactie DEC 10-11-2015				x		x	x	
15	Advies CCD		x						x
16	Beschikking en vergunning				x		x	x	

22 OKT. 2015

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.zbo-ccd.nl of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☑ Ja > *Vul uw deelnemernummer in* 10800 / 282

☐ Nee > *U kunt geen aanvraag doen*

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	Universiteit Utrecht
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	3 0 2 7 5 9 2 4

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

Straat en huisnummer	Instantie voor Dierenwelzijn Utrecht
Postbus	12007
Postcode en plaats	3501AA Utrecht
IBAN	NL27INGB0000425267
Tenaamstelling van het rekeningnummer	Universiteit Utrecht

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted] ☐ Dhr. ☑ Mw.
Functie	[redacted]
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted] ☐ Dhr. ☑ Mw.
Functie	[redacted]
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
1.7	Is er voor deze projectaanvraag een gemachtigde?	☐ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☑ Nee	

2 Over uw aanvraag

2.1	Wat voor aanvraag doet u?	☒ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.3
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	0 1 - 0 9 - 2 0 1 5
		Einddatum	3 0 - 0 8 - 2 0 2 0
3.2	Wat is de titel van het project?	Local controlled release of medication for the treatment of degenerative joint diseases	
3.3	Wat is de titel van de niet-technische samenvatting?	Medicijnen voor plaatselijke behandeling van rugpijn en artrose	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC Utrecht
		Postadres	Postbus 85500 3508 GA Utrecht
		E-mailadres	dec-utrecht@umcutrecht.nl

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

- [x] Nieuwe aanvraag Projectvergunning € 741,00 Lege
- [ ] Wijziging € Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

- [ ] Via een eenmalige incasso
- [x] Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

- [x] Projectvoorstel
- [x] Niet-technische samenvatting

Overige bijlagen, indien van toepassing

- [ ] Melding Machtiging
- [x] Project Proposal [redacted]UU_2015_(LSH ArIADNE_TargetCare)_Appendix_adjusted

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.6). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam	[redacted]
Functie	[redacted]
Plaats	Utrecht
Datum	15-10-2015
Handtekening	[redacted]

Format
Projectvoorstel dierproeven

- Dit format gebruikt u om uw projectvoorstel van de dierproeven te schrijven
- Bij dit format hoort de bijlage Beschrijving dierproeven. Per type dierproef moet u deze bijlage toevoegen.
- Meer informatie over het projectvoorstel vindt u op de website www.zbo-ccd.nl.
- Of neem telefonisch contact op. (0900-2800028).

1 Algemene gegevens

1.1	Vul uw deelnemernummer van de NVWA in.	10800
1.2	Vul de naam van de instelling of organisatie in.	Universiteit Utrecht
1.3	Vul de titel van het project in.	Local controlled release of medication for the treatment of degenerative joint diseases

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☒ Fundamenteel onderzoek
- ☒ Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid of het welzijn van mens of dier
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

3 Algemene projectbeschrijving

3.1 Achtergrond

Licht het project toe. Beschrijf de aanleiding, de achtergrond en de context. Besteed aandacht aan de bij vraag 2 aangekruiste categorieën.

- Geef in geval van 'wettelijk vereiste dierproeven' aan welke wettelijke eisen (in relatie tot beoogd gebruik en markttoelating) van toepassing zijn.
- Geef in geval van 'routinematige productie' aan welk(e) product(en) het betreft en voor welke toepassing(en).
- Geef in geval van 'hoger onderwijs of opleiding' aan waarom in dit project, in relatie tot het opleidingsprogramma en eindtermen, is gekozen voor dierproeven.

The project focuses on developing treatment strategies for osteoarthritis (OA), neck and back pain of canine and human patients in close collaboration with academia from the medical and bioengineering field. Neck and back pain are strongly related to degeneration of the intervertebral disc (IVD), the structure present between the vertebral bodies of our spinal column. It consist of a gel like core, the nucleus pulposus (NP) consisting mainly of proteoglycans attracting water and some collagen II, and is surrounded by a more fibrocartilage-like structure called the annulus fibrosus. During degeneration the NP loses its proteoglycans, its water binding capacity and thereby its function as shock absorbing and movement enabling structure. In osteoarthritis, the articular cartilage, also consisting of a proteoglycan- and collagen-rich matrix, the proteoglycans are lost, hence reducing shock absorption and frictionless movement of the joint.

Current treatment of these diseases is mostly palliative and consists generally of pain relief by oral administration of analgesics and anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs). Patients with late stage disease and refractory to pain medication can only be surgically treated. This is primarily done both in dogs and humans by arthroplasty in both OA and IVD disease. Arthroplasty is a successful treatment of OA, but requires revision of the device after around 15 years, which precludes its application in patients < 60 years. In IVD degeneration, arhroplasty is less successful and more dangerous due to the vicinity of the spinal cord. More often, the IVD is removed and the two adjacent vertebrae fused using autologous bone. All of these surgical treatments, independent of the disease, demand long term recovery, yield at best a suboptimal clinical outcome, and do not support a biological and hence functional repair of the diseased joint. For this reason, treatment strategies are being developed that either address the disease at earlier stages in order to prevent the need for heavy demanding surgery or are more effective at treating pain while using minimally invasive procedures.

Although the pathogenetic mechanisms are not yet fully elucidated, both OA and IVD degeneration share a clear association with inflammation, leading to loss of functional tissue components, through the induction of matrix degrading enzymes, and pain. The treatment strategies of OA and IVD concentrate on addressing the pain and inflammation with the aid of NSAIDs as well on improving the cartilage matrix quality by inducing an anabolic response with the aid of anabolic and/or anti-catabolic factors.

Although the widespread use of NSAID's such as [redacted] and corticosteroids in degenerative disease of joint cartilage and the IVD is justifiable, their application is accompanied by several drawbacks. First of all, the route of administration is usually systemic, which entails several side effects, in particular osteoporosis and reduced fracture healing (Seibel et al., 2013). Therefore, local administration is a logical approach. However, although in particular intra-articular administration of corticosteroids has shown to be efficacious, their effects are still limited to one month after injection due to rapid loss from the joint space, whereas multiple injections increase the risk of infection (Bellamy et al., 2009)'(Trampuz and Widmer, 2006). Also the local delivery of growth factors and peptides has been shown in in vivo models to enhance tissue regeneration, with even phase I clinical trials on the delivery of BMP-7 into the OA joint. We are already studying compounds previously identified to possess regenerative properties (Gawri et al., 2013). However, also for the application of regenerative factors multiple injections have been shown to be required (Johnson et al., 2012). A solution to this problem is provided by the use of biomaterial-based controlled release systems.

Biomaterials as sustained delivery agents in cartilage regeneration. In addition to their role in supporting native or exogenously added cells, scaffolds, either solid or hydrogel-based, can be used for the delivery of cues required for regeneration. These cues may include regenerative factors such as growth factors or hormones, but also anti-oxidant and anti-inflammatory factors. Recent work by collaborators highlights the progress achieved in the field of joint cartilage repair with the aid of controlled delivery of biologics(Lam et al., 2015). Several biomaterials have been identified as potential vehicles in IVD degeneration as recently reviewed by Blanquer (Blanquer et al., 2012). Thus far only a limited number of publications on solid or hydrogel based-systems specifically developed for intra-discal controlled release is present. Nano- and microstructured injectable biomaterials have been used more often to achieve sustained release of regenerative factors, although they do not have a biomechanical nor intrinsic regenerative role in this type of application. Several bioactive substances have been incorporated into delivery systems for IVD regeneration employing primarily injectable natural hydrogels and solid synthetic microspheres. The loaded substances target different processes involved in cartilage degeneration, including mechanisms of cell senescence, matrix anabolism or catabolism, and inflammation. In this respect, [redacted] a systemic review on Biomaterials for intervertebral disc regeneration regarding their past performance and possible future strategies. This work has been resubmitted after minor revision and is under consideration at the journal *European Cells & Materials* (this manuscript is enclosed for your information. Based on this work it appears that the concept of employing biomaterials for the sustained delivery of agents in IVD regeneration is just emerging. However, the concept of local prolonged exposure to factors modulating regeneration and degeneration holds great promise by reduction of systemic side effects and increasing effectivity.

The project encompasses strategies for the treatment of OA and IVD degeneration that are at different levels in the chain of translation: those that are based on readily available FDA-approved compounds and are further developed in a preclinical setting before their translation in to "first-in-man" studies, and those that explore identified targets that have been shown at least in vitro and in vivo in small animal models to have a positive biologic effect. These strategies are fed with new compounds identified with the aid of fundamental research.

Note: the reference list has been included in the Appendix of the proposal

3.2 Doel

Beschrijf de algemene doelstelling en haalbaarheid van het project.

- In het geval het project gericht is op één of meer onderzoeksdoelen: op welke vra(a)g(en) dient dit project antwoord(en) te verschaffen?
- In geval het een ander dan een onderzoeksdoel betreft: in welke concrete behoefte voorziet dit project?

The overall aim is develop and validate injectable biomaterial-active compound combinations that effectuate controlled release of anti-inflammatory drugs and/or bioactive substances that induce an anabolic response for the treatment of IVD disease and OA. The deliverables of this project are

2.1 **Proof of concept of the applicability of slow release of small molecule anti-inflammatory drugs and regenerative factors for the long term inhibition of inflammation, tissue degeneration and pain.**

2.2 **Insight into the local side effects of high local and prolonged doses of AIAs and regenerative factors.**

2.3 **Detailed knowledge on the in vivo release kinetics of AIA and regenerative factors in relation to the implantation site and biomaterial degradation.**

Why are deliverables 2.1-3 achievable: The researchers in this project have gained experience in previous projects on the development and formulation of biomaterials for delivery of anti-inflammatory and regenerative therapeutics in OA and IVD degeneration. Also the previously identified regenerative factors are currently being incorporated in suitable biomaterial platforms by our industrial partners, experts in their field. In vivo, in particular promising preliminary results were obtained using an [redacted] based biomaterial platform (a refers to a biomaterial of a defined and fixed chemical composition that can be formed into different structures, including microspheres and hydrogels) for the generation of biomaterials in combination with anti-inflammatory drugs. The

biomaterial used is already used in several clinical trials for non-related diseases.

From a technological perspective, the research group has gained experience with several biomaterial platforms that effectuate sustained release of the loaded substance, including small molecules and peptides. These studies have been performed in the small and large animal models as implemented in the current project. Furthermore, we have active collaborations with companies developing these platforms within the context of other ongoing projects.

2.4 **Understanding of the role of inflammation in regeneration and demonstration of selected candidates as possible new regenerative and anti-inflammatory compounds for controlled local delivery.**

Why is this achievable: it is well known that every regenerative response is preceded by an inflammatory process. We have already identified some novel compounds that have been shown to possess regenerative and/or anti-inflammatory capacities in vitro. These will also be incorporated in suitable biomaterial platforms recently developed by our industrial partners, experts in their field. We have already achieved to bring to the veterinary clinic FDA approved anti-inflammatory compounds in several local controlled release biomaterials (intra-discal delivery).

3.3 Belang

Beschrijf het wetenschappelijk en/of maatschappelijk belang van de hierboven beschreven doelstelling(en).

Although seldom a cause of morbidity, diseases of the musculoskeletal system impose a substantial burden on Western societies that is in addition increasing with ageing of the population. Amongst the diseases with most impact are osteoarthritis (OA) and chronic low back pain. Low back pain has been identified as one out of seven high burden conditions by the 2013 Report Priority Medicines for Europe and the World, which identifies key areas of priority research for pharmaceutical innovation to meet public health needs. The prevalence of osteoarthritis was predicted to grow from 81.4 million in 2009 to nearly 95 million by 2020 across the seven major markets. Approximately 20% of the canine population will develop secondary OA due to developmental joint diseases. In a special barometer report exploring health in EU citizens, the most common type of pain restricting daily activity was low back pain (http://www.who.int/medicines/areas/priority_medicines/MasterDocJune28_FINAL_Web.pdf). It is one of the ailments associated with the largest loss of years lived without disability (Murray et al., 2012). In veterinary medicine IVD disease is a relatively common reason for euthanasia in dogs. Patients with late stage OA or disc disease and pain refractory to medication can only be surgically treated. Between 150 and 200 thousand spinal surgeries are performed annually in the EU. Surgical procedures such as disc excision and vertebral fusion lead to pain relief in the short-term, but they alter spine biomechanics, leading to further degeneration of surrounding tissue and adjacent discs (Higashino et al., 2010)'(Jacobs et al., 2013). Failure rate for lumbar fusions is 20% to 40% after five years. Surgical procedures that maintain spine biomechanics, i.e. arthroplasty, have limited longevity and sufficient long-term follow up to determine effects on adjacent structures, e.g. facet joints, is lacking (Thavaneswaran and Vandepeer, 2014). Furthermore, arthroplasty revisions are challenging and associated with high morbidity and costs (Hamilton et al., 2015).

Scientific relevance

The current project aims at treating osteoarthritis and chronic low back pain associated with intervertebral disc disease, by inhibition of tissue degeneration and enhancing intrinsic regeneration in human and canine patients. In addition, the incorporation of drugs into controlled release carriers for local application will provide for new formulations and treatments that circumvent systemic side effects. Even more so, it will deliver insight into the [redacted] and [redacted] release profile and degradation rate of the biomaterial platform. This basic knowledge can be further implemented in a faster translation from in vitro release profiles into the in vivo situation, taking into account the tissue specific properties that effect the behaviour of the biomaterial. Hereby, biomaterial platforms can be further fine-tuned in order to achieve optimal clinical results both in OA and IVD degeneration.

Based on the basic research part, the proposed project will also identify (new) mediators that are crucial in tissue matrix production and inflammation control and hence can be implemented to both degenerative joint and disc disease. Furthermore, it will give insight into the interplay of inflammation and regeneration, into the additive regenerative effect an advanced treatment strategy may engage, where these (new) mediators or chemotactic factors are used to attract endogenous MSCs.

3.4 Onderzoeksstrategie

3.4.1 Geef een overzicht van de algemene opzet van het project (strategie).

The project encompasses strategies for the treatment of OA and IVD degeneration that are at different levels in the chain of translation:

(a) those that are based on readily available FDA-approved compounds and are further investigated and validated in preclinical studies for dose finding in small and safety and efficacy in large animals, in order to accelerate their translation in to "first-in-man/dog" studies, and

(b) those that explore targets shown in vitro and in vivo to have a positive biologic effect. Safety and dose finding is then done in rat models. The next step of translation is undertaken in large animal models (goat for OA and dog for IVD).

(c) those that are based on new compounds identified in fundamental research. Herein, we identify potential targets based on animal-free in vitro experiments. The promising bioactive substances are then further developed, incorporated in suitable controlled release biomaterial platforms. These will mainly be applied as [redacted], as these provide a more well controllable degradation (gels will easily fragment in a joint, which will hugely change their degradation and hence release behaviour) and as proteins are more protected from hydrolysis. Proof of principle studies will be done in small animals, followed by dose and safety studies, after which also large animal studies will be prepared for human and canine translation

The first bioactive substances to be studied will be the FDA-approved [redacted] inhibitor [redacted] and the corticosteroid [redacted] These substances are currently being used in the treatment of OA and chronic low back pain. They will be incorporated in microspheres and evaluated for their effect on inflammation, joint tissue integrity and pain in OA and IVD disease, in comparison to respective bolus injections. In addition to evaluating the effect on general joint integrity, special emphasis will be on the effects on subchondral bone by using µCT analysis. In terms of regenerative factors, Link N peptide is identified as a promising candidate for local delivery of regenerative factors (Gawri et al., 2013). In addition, BMP and stem cell chemo-attractants have shown been shown to be potent inducers of a regenerative response (Imai et al., 2007)(Pereira et al., 2014). Fundamental research has just recently started and hence has not yielded any possible targets.

3.4.2 Geef een overzicht op hoofdlijnen van de verschillende onderdelen van het project en de daarbij gebruikte type(n) dierproef of dierproeven.

Objective 1 - Dose finding and insight into local side effects

The effects of bioactive substances administered in controlled release biomaterials will be evaluated in in vivo in the rat OA model and the canine model of IVD degeneration.

a. ***Rat OA model:*** OA is induced in rats after transection of the anterior cruciate ligament and partial meniscectomy. Over the course of 4 weeks mild OA develops. This model is employed for both FDA-approved and the newly identified bioactive substances loaded on a biomaterial platform. The rat model is well established by one of our collaborators (dept Orthopedics, UMC Maastricht). Understanding of release kinetics and degradation of biomaterials by in vivo imaging is only technically feasible in a small animal model. Moreover, dosing studies can be done, which is not realistic in large animals. The rodent experiments will provide insight in possible side effects and effective doses, which can be used to make a rough estimate for the safe and effective dose in the large animals.

b. ***Rat model of induced IVD degeneration:*** IVD degeneration is induced in the caudal discs of rats via needle puncture. Over the course of 4 weeks moderate IVD degeneration develops. This model is employed for dose finding of the newly identified bioactive substances that have been shown to a have a positive biologic effect in *in vitro* studies. As such, the identified regenerative bioactive substances thus far include peptides and growth factors that are synthesized and hence expensive in their purchase. As such, dosing studies in large animal models are not realistic and the first steps are undertaken in this rodent model. The rodent experiments will provide insight in possible side effects and effective doses, which can be used to further test a smaller range of doses in spontaneous IVD degeneration in the experimental canine model, where several doses can be tested in one animal, to finally come to an estimate for the safe and effective dose in canine patients.

Objective 2 - Proof of concept in large animal models

The optimal doses found in objective 1 will be used in large animal models of OA and IVD degeneration to show proof of principle for long term inhibition of inflammation, pain and joint degeneration. Subsequently trials with canine and human patients can be initiated.

a. ***Goat [redacted] model:*** Optimal dosages in the rat model will be translated towards the goat [redacted] model of OA, to determine safety and efficacy before canine and human patients can be treated. The goat [redacted] model consists of [redacted], due to which a mild osteoarthritis develops over a period of 20 weeks. This model for OA closely resembles human and canine OA, as it does not involve ligament transection or injection of enzymes as done in small animal models, but is based on mechanical damage to the cartilage. The gross anatomy of the goat knee is fairly similar to both canines and humans, and in both canine and human patients the knee is the most frequently affected joint. Additional readout parameters to those used in small animal models will be cartilage tissue degeneration and synovial inflammation, in addition to biochemistry of ECM proteins and cytokines in synovial fluid.

Met opmaak: Engels (V.S.)

b. ***Canine model of IVD degeneration:*** Experimental Beagle dogs are employed on the basis that they consistently develop spontaneous IVD degeneration after ~1 year of age with similar clinical, radiographic, histological, and biochemical changes to humans. Hence studies with the FDA-approved bioactive substances in experimental dogs can be directly translated to canine and human patients with IVD degeneration.

As soon as safety and efficacy has been determined in the large animal models of OA and IVD a first translational step will be undertaken in canine patients treated treated at the Academic Veterinary Hospital of The Netherlands. Such studies will be performed within the 5-year period of the project. As a spin-off this project, a phase I clinical trial in humans will be feasible as soon as regulatory affairs and transfer of the newly developed treatment into a GMP-enviroment have been adressed.

Canine patients with OA Patients will be randomised to injection with loaded biomaterials or bolus. Readout parameters will be pain and quality of life (owner questionnaires), force plate analysis (on obective measurement of gait and lameness) and quantitative MRI imaging.

Canine patients with IVD disease will be randomised to injection with loaded biomaterials or bolus injections and followed up for a minimum of 6 months. Readout parameters will be pain as assessed by owner questionnaires, force plate analysis and MRI imaging, as here animals cannot be sacrificed to evaluate regeneration and inhibition of inflammation in a more detailed and exact manner. Force plate analysis gives a direct unbiased quantification of pain due to disease. Owners and examining veterinarian will be blinded to the treatment.

Objective 3 - [redacted] of release kinetics of biomaterials and association with biological in vitro systems

Rationale: Biomaterial platforms are chosen on the basis of the properties of the targets that will be released in a sustained way. However, their release is dependent on biomaterial degradation, which in itself will depend on the local environment where the biomaterial is implanted. Blood and tissue fluid flow will have a large impact, but also the presence of an inflammatory response, as this is commonly accompanied by enzyme activity. The ***rat OA and IVD degeneration model*** will be employed to determine the effect of the local environment on degradation and release *in vivo*. For this purpose, labelled and bioactive substance-loaded biomaterials will be injected [redacted] Loss of label intensity and release of the bioactive substances in the blood will be monitored longitudinally. These techniques will provide insight into the speed of degradation and release profiles and be also useful for new projects on local delivery systems. Insight into the effects of release kinetics on cell and tissue phenotype will be gained in cultures of cartilage and disc cells and tissues explants and in cartilage tissue co-cultured with synovial tissue.

Objective 4 - Control of inflammation in order to augment regeneration of cartilaginous tissues (OA&IVD degeneration); evaluate possible new regenerative and anti-inflammatory targets

Given the role of inflammation in regeneration, the interplay between inflammatory factors (including [redacted] and their metabolites) and growth factors/ chemo attractants will be studied in vitro extensively. In addition to further studying targets from literature, e.g. kartogenin, link-N, and caveolin-1 shown to be regenerative and play a role in inflammation(Gawri et al., 2013; Johnson et al., 2012)(Pavlides et al., 2014) new emerging targets will also be

evaluated in vitro. If these factors indeed show to have positive effects on regeneration in the *in vitro* (co-)culture models and show appropriate release kinetics with retention of activity in vitro, they will also be incorporated in the controlled release biomaterial platforms to test the regenerative and anti-degenerative potency of these new release systems in the ***rat OA and IVD degeneration models*** and subsequently in the ***large animal models***.

3.4.3 Beschrijf en benoem de logische samenhang van deze verschillende onderdelen en de eventuele fasering in de uitvoering. Vermeld eventuele mijlpalen en keuzemomenten.

This project pursues biological repair of the diseased joint and IVD by combing both translational and basic research. The coherence between the different components and the different steps of the projects is illustrated in the figure included in the Appendix of the proposal.

By controlling inflammation we aim at reversing degeneration and augmenting regeneration of the degenerated cartilaginous tissue. In order to assist fast translation to the clinical situation (human and veterinary patients), the optimal dose of FDA-approved controlled release bioactive substances, for example [redacted] and [redacted] that efficiently control inflammation and degeneration without local side effects is identified in the rat OA model and the canine model of IVD degeneration. The efficacy of the treatment is further explored in a large animal model of OA (goat; [redacted] model). In order to be able to extrapolate these results to canine and human patients with OA/IVD degeneration and improve the strategy treatment the release kinetics of the bioactive substances is further studied dependent on the location of application and the tissue state (healthy/degenerated). As soon as safety and efficacy has been determined in the large animal models of OA and IVD, and release kinetics are comprehended, a first translational step will be undertaken in canine patients with OA or IVD disease within the 5-year period of the project. Likewise, a phase I clinical trial in humans will be feasible shortly after completion of the project, as soon as regulatory affairs and transfer of the newly developed treatment into a GMP-environment have been adressed.
Furthermore, we study in depth the interplay of inflammation and chondrogenic signalling pathways in order to understand the mechanisms that drive regeneration of the cartilaginous tissue. Based on this fundamental work, newly identified anti-inflammatory and regenerative targets are studied in terms of their biologic effect on degenerated cartilaginous tissues. If these newly identified targets appear to be effective, follow up *in vivo* studies will employ rat models of OA and IVD degeneration for dose in finding.

3.4.4 Benoem de typen dierproeven. Vul per type dierproef een bijlage Beschrijving dierproeven in.	
Volgnummer	Type dierproef
1	Rat model for mild osteoarthritis
2	Rat model of induced IVD degeneration
3	[redacted] model for the induction of mild osteoarthritis (goat)
4	Canine model of IVD degeneration
5	Degradation of biomaterial and release kinetics
6	
7	
8	
9	
10	

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10800
1.2	Provide the name of the licenced establishment.	Universiteit Utrecht
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Rat model for mild osteoarthritis

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The animal models is employed in order to address questions raised under key objective 1 (Dose finding and insight into local side effects). To induce osteoarthritis in rat knees, the anterior cruciate ligament will be transected and a partial meniscectomy will be carried out. This model has been used in many publications before to induce OA and to study the effect of anti-inflammatory and regenerative treatments. Unilateral OA is induced and common groups

included in such an experiment are: placebo injected with the unloaded biomaterial (sham) and treatment groups injected with biomaterials loaded with different dosages of the bioactive substance. The major readout parameter is cartilage quality by histological scoring.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Prior to the experiment, the rats will undergo a clinical examination, including assessment of the stability of the knee joint. Severity procedure = minor

Also, pressure plate measurements will be performed to obtain starting values. Severity procedure = minor

Unilateral osteoarthritis is induced in rats under anaesthesia and proper peri-operative and post-operative analgesia by transection of the anterior cruciate ligament and partial medial meniscectomy. In total the surgical procedure will take about 30-45 minutes. Severity procedure = moderate

To assess the effect of bioactive compounds on osteoarthritic joints, 4-6 weeks after osteoarthritis is induced, these formulations will be injected directly into the knee joint. This injection will be performed on rats under anesthesia and proper pre-injection analgesia. Severity procedure = minor

The follow up period is 12-30 weeks post-injection, as this has been used in previous related experiments carried out in the scope of another project. A termination of the experiment, rats will be euthanized. Severity procedure = minor

per bioactive substance to be studied the following groups are studied:
- 1. unilateral OA, treated with placebo (biomaterial alone)
- 2. unilateral OA, treated with bioactive substance dose A + biomaterial
- 3. unilateral OA, treated with bioactive substance dose B + biomaterial
- 4. unilateral OA, treated with bioactive substance dose C + biomaterial

As soon as the safe dose and most efficacious dose has been established in a follow up study with a group of rats will be studied were unilateral OA is treated with a single injection of the bioactive compound with the same experimental set up and procedures. In this way we can minimize the animals employed, given that we with the current set up we will only study ONE dose of the bioactive substance with single injection and not all three dosages (A, B, and C)

Number of relevant comparisons = 6 / number of rats =6 as explained in the statistical methods below
Where possible studies for bioactive substances loaded on the same biomaterial will be studied in parallel sothat the control group is shared between the two studie, minimizing the number of animals by six.

Read out parameters:

In vivo:
- The rats will be assessed clinically: joint effusion will be measured, lameness will be scored and knee stability will be tested.
- Pressure plate measurements will be performed on a regular basis to assess the amount of functional disability caused by the osteoarthritis that has been induced and its possible relief by the bioactives.
- To measure systemic distribution and clearance of the injected actives, blood samples will be collected on predetermined timepoints.

Post mortem:
- microCT will be performed to assess radiologic changes in the knee joints at the end of the experiment. Knee joints that have received a specific treatment

will be compared to knee joints that have received a sham treatment.
- The knee joints will be collected for histopathological and biochemical analyses: histopathological assessment will be performed to study the severity of osteoarthritis present at the end point of the study. Also, additional analyses (stainings, immunohistochemistry) will be carried out, dependent on the research question. A part of the tissue can be collected for biochemical analyses: i.e. to measure if there is remaining biomaterial left.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Power analysis is performed with the aid of the freely available software G*power 3.1.9.2. Power analysis is performed on the primary read out parameter: the histological Mankin Osteoarthritis Score (as also used by others, for example Naveen et al 2013). Based on a statistical power of 85%, an alpha error level of confidence corrected for the number of relevant comparisons between groups set at 0.85% (alpha=0.008), and assuming standard deviations as observed in previous studies:

The Mankin OA score has a range from 0 to 14. We aim at an average Mankin score of 6 after OA induction, with a standard error of 1,5. We hypothesize that the injection of bioactive compounds as a treatment for OA will reduce the Mankin score with 30-40%. A total of six animals per experimental group has been calculated. Although we do not expect loss of animals during the experiment, we will included 1 extra animals per experimental group to prevent loss of power due to unforeseen circumstances. All in vivo studies are approached in this manner.

Reference:
Naveen SV, Ahmad RE, Hui WJ, Suhaeb AM, Murali MR, Shanmugam R, Kamarul T. Histology, glycosaminoglycan level and cartilage stiffness in monoiodoacetate-induced osteoarthritis: comparative analysis with anterior cruciate ligament transection in rat model and human osteoarthritis. Int J Med Sci. 2013 Dec 21;11(1):97-105.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

We prefer to use female rats since male rats can increase a lot in size and weight during the experiment. During this long term follow up, the female rat knees will fit in the microCT scanner, while this will not be the case for the male rats. We use rats from the age of 12-14 weeks, because then they are large enough for intra-articular injections and blood collection. Furthermore, skeletal growth does not influence IVD development any more at this age and both the OA and IVD model can be combined in one animal in this age range. Furthermore, female rats are preferred for this kind of experiment, since they are easy to keep and easy to handle. No effects on cartilage regeneration have been shown of fluctuating hormone levels, although in humans the frequency of OA in females is higher than males. However, as joint anatomy is also different in human females, it is not clear whether this is due to direct effects on cartilage metabolism or to the indirect effects of e.g. suboptimal alignment. Moreover, although ovariectomy was shown to have a clear effect on the development in various models of OA in female rodents, the administration of estrogens (=artificial fluctuation) to healthy reproductive female mice showed no clear effects, thereby suggesting that a total lack of estradiol rather than fluctuation affects disease progress (Sniekers YH, Weinans H, Bierma-Zeinstra SM, van Leeuwen JPTM, van Osch GJVM. Animal models for osteoarthritis: the effect of ovariectomy and estrogen treatment—a systematic approach. Osteoarthritis and Cartilage. 2008;16(5):533–541) Thus far there have been no reports on gender differences in IVD degeneration.

Sprague-Dawley are purchased from the Charles River laboratories on the basis that previous experiments are on the same strain.
Taking into consideration all the objectives described in this project we estimate to use 63 rats for objective 1 (2 bioactive substances loaded on the same biomaterials, control group with boimaterial alone is shared between the two bioactive substances) and 69 rats for objective 4 (similar study design for 2 bioacitve substances, + 6 animals extra in case the control group cannot be shared) in the period of 2015-2020. Altogether, 132 rats.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement: The joint is a complex organ, consisting of different tissue- and cell types. Moreover, the homeostasis of the tissue is being regulated by biomechanical forces, both internally and externally. In the case of OA, changes occur in multiple areas and also the biomechanical loading can change, all influencing the disease progress. Blood flow determines the speed at which injected drugs disapper from the joint after intra-articular injection. Up to now, it is not possible to mimic a whole joint in vitro, therefore animal experiments remain necessary in this phase of the development of drugs. However, a lot of tests regarding possible toxicity and release profiles have been performed in vitro, prior to the start of animal experiments.

Reduction: a power analysis has been performed to calculate the minimum amount of animals needed to see a significant effect. In order to further minimize the number of animals used, where application we adress the questions of the project in joint in vivo experiment. A typical example is Objective 3, in which we study the [redacted] and [redacted] degradation of biomaterials. In a single in vivo study, we employ for the unloaded biomaterials the same rats to study in parallel the degradation rate after [redacted]. Only when the biomaterials are loaded with the bioactive substance rats are employed for the study of a single location, in this case the joint, and investigate the release profile in the blood circulation and the effects locally. The latter is done on the basis that controlled release of the bioactive substance into the circulation and hence may influence all parts of the body.

Refinement: In order to decrease the stress levels of the rats, they will be trained to get used to handling, blood collection and pressure plate measurements. Moreover, the rats receive proper analgesia after induction of OA, to inhibit acute pain.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals are allowed to accommodate to their new enviroment for at least 1 week, they are housed in groups. They will be trained prior to the experiment to get used to handling, fixation for blood collection (in a so-called blood collection 'sleeve') and pressure plate measurements. The first week after OA induction, the rats will be checked daily and if necessary, extra analgesia will be given.

Furthermore, animals are given cage enrichment and the animals are often examined and weighed by the researcher, which does not cause any pain but is seen as enrichment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

complicated wound healing causing minor discomfort

Explain why these effects may emerge.

occasionally rats tend to chew on their sutures, in less than 10% of the animals

Indicate which measures will be adopted to prevent occurrence or minimise severity.

We use thin biodegradable sutures (size 4-0) that maintain their strength during the healing period of the skin; proper placement of the sutures prevets swelling of the skin and thereby prevents irritation to the animal. Surgery is performed by trained veterinarians for this procedure. In case of wound dehiscence, the skin is sutured again and animals do not chew on their sutures any more (based on past experience)

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

General clinical signs will be frequently observed. If clinical signs indicate severe discomfort (e.g. body weight loss 15% in 2 days) and animals cannot be treated adequately, animals are euthanized. Furthemore if an animal is unable to stand or walk in spite of treatment with proper analgesia for at least one day, the humane endpoint is reached.

Indicate the likely incidence.

We already have performed similar experiments in the past with n=21 rats and have not experienced any incidents that required the implementation of humane endpoints. Incidence is expected to be < 5%.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

moderate

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

It is necessary to sacrifice the rats at the end of the experiment, to gain insight in the histopathological and biochemical processes in the joint. This can only be performed post mortem.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10800
1.2	Provide the name of the licenced establishment.	Universiteit Utrecht
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Rat model of induced IVD degeneration

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

This animal model is employed in order to address questions within key objective no 1 (Dose finding and insight into local side effects of readily available bioactive substances) and key objective 4 (Evaluate possible new regenerative and anti-inflammatory targets). All strategies employed within this project rely on the employment of minimally invasive injectable treatments guided by fluoroscopy to ensure correct placement of the needle, in which an imaging marker

or a bioactive substance, alone or loaded on a biomaterial that effectuates sustained release of the bioactive substance is injected once. The size of needle employed and the volume injected in each IVD will not induce IVD degeneration and hence will not disturb the balans of the IVD (Mao et al., 2011). Major readout parameters will be increase in disc height (measured on plain radiographs or on the basis of microCT scan) and the production of tissue extracellular matrix as determined by histology.

Reference
Mao HJ1, Chen QX, Han B, Li FC, Feng J, Shi ZL, Lin M, Wang J. The effect of injection volume on disc degeneration in a rat tail model.Spine (Phila Pa 1976). 2011 Jul 15;36(16):E1062-9.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Rats are allowed to acclimatize for at least 1 week and thereafter the experiments are initiated. IVD degeneration is induced in caudal IVDs (tail) of rats ander general anesthesia and proper pre-operative analgesia. In total the procedure will take about 30-45 minutes. Degeneration of the caudal IVDs occurs after a single puncture. Procedure with minor severity.

Over the course of 4 weeks degeneration progresses and is confirmed by means of radiography 4 weeks after induction (Zhang et al. 2011). At T=0 (start of the treatment), treatment groups will be injected into the rat caudal IVDs on the side contralateral to the annular puncture. All injections will be done under anesthesia and fluoroscopic guidance to confirm correct position of the needle tip prior to injection. Procedure with minor severity

At T=3 months after injection of the treatment groups, the in vivo experiment will be terminated and rats will be euthanized. Procedure with minor severity.

A typical example of experiment investigating (a) the additive effect of a controlled release system over a single injection of the bioactive substance alone and (b) the differential effect of loading dose of the bioactive substance is:
- 1. degenerated IVD, untreated
- 2. degenerated IVD, single injection of bioactive substance (dose 1)
- 3. degenerated IVD, biomaterial alone
- 4. degenerated IVD, biomaterial + bioactive substance (dose 1)
- 5. degenerated IVD, biomaterial + bioactive substance (dose 2)

relevant comparisons: 1 vs 2, 1 vs 4, 1 vs 5, 3 vs 4, 3 vs 5, 2 vs 4 (a total of 6)

Read out parameters

In vivo:
- the release profile of the bioactive substance into the blood circulation is determined in plasma sampled at predetermined time points. Procedure with minor severity.
- Potential regenerative effects will be studied by obtaining conventional radiographic images at T=-4 weeks prior to induction of degeneration (under sedation), at T=0 (time of treatment, under sedation), at T=3 months (directly post-mortem radiographs and microCT images). The primary read out parameter is the percentage of change of the Disc Height Index (DHI) for each IVD measured on radiographs and on microCT scan images. Procedure with minor severity.

Post-mortem:
Radiography and micro-computed tomography to evaluate the presence of extradiscal mineralization, one of the possible complications of intradiscal treatment with bioactive factors. Thereafter, rat tails will be harvested and IVDs will be accordingly processed for biochemical analyses of the tissues and/or after macroscopic evaluation will be fixed, decalcified, and thereafter subjected to histopathological evaluation (Boos scoring and immunohistochemical stainings).

Reference
Zhang H1, Yang S, Wang L, Park P, La Marca F, Hollister SJ, Lin CY. Time course investigation of intervertebral disc degeneration produced by needle-stab injury of the rat caudal spine: laboratory investigation. J Neurosurg Spine. 2011 Oct;15(4):404-13.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Power analysis is performed with the aid of the freely available software G*power 3.1.9.2. Power analysis is performed on the primary read out parameter: disc height index. Based on a statistical power of 85%, an alpha error level of confidence corrected for the number of relevant comparisons between groups set at 0.008 (alpha=0.05 / 6, in case of 5 treatment groups to be studied as described in the example above), and assuming standard deviations as observed in previous studies: in the rat degenerative disc model in which variation of 15% was observed after induction of IVD degeneration and a variation of 15-20% was observed after injection of a bioactive substance with a significant biologic effect, 5 IVDs should be sufficient in each group to observe significant differences. In order to minimize the number of animal employed per study and to minimize the "RAT" effect as random effect, we employ a block design of the study. Hence, based on power analysis 10 rats would be assigned to address the specific aims by means of longitudinal fluoroscopy and post-mortem biochemical (n=5 rats), and histological analysis (n=5 rats). When biomolecular analysis in needed for functional analysis, additional rats (n=5) will be included in the study, given that biomolecular analysis will employ the whole IVD.
All in vivo studies are approached in this manner and dependent on the expected effect and the documented variation, we select the appropriate number of animals. When estimation of the size effect is not possible based on the available information, a pilot study with n=3 IVDs per treatment group in 3 rats will be conducted. Based on these findings, proper power analysis will determine the minimum number of IVDs/rats needed. In this project combinations of bioactive substances are not foreseen.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The rat model of stab-induced IVD degeneration is a well described model that has been used previously for showing the effect of regenerative factors. Rats older than 3 months (age range 12-14 weeks) are being employed in the study so that they have reached skeletal maturity and the problems of IVD remodeling due to skeletal growth can be eliminated. Animals will be obtained from registered breeding companies such as Harlan or Charles river.

We prefer to use female rats for three reasons:
(1) in order to reduce the number of animals employed we will, where possible, combine the current animal procedure with animal procedure no. 1 (rat OA model) where female mice are employed sothat they fit into the micro-CT scanner for analysis knee-bone pathology.
(2) male rats can increase in size and gain a lot of weight during the longer term experiments with 6 months follow up.
(3) in order to reduce variability and make comparisons between experiments possible we will need to concentrate on female rats within this project proposal.

Gender is expected not to influence the study. No effects have been described of progesterone on regeneration of tissue by NP cells and only two studies mention that estradiol can protect agains induced cell death. As this is very limited and restricted to artificially induced cell death, and in human nor canine patients a connection between gender and disc degeneration was found, also at menopausal age, it is very unlikely that any effect of cycle will be evident.

Taking into consideration all the objectives described in this project we estimate to study two anti-inflammatory and four regenerative bioactive substances. Pilot studies will help determine power of the study or predefine the range of the dose of the bioactive substance to be studied (n=40). For each bioactive substance we expect to use 15 rats, i.e. a total of 90 rats for six bioactive substances. Altogether we expect to use 110 rats for this purpose in the period of 2015-2020, including n=20 for additional animals in case degeneration has not reached the expected level.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement: These studies cannot be replaced with simple in vitro models or explant tissue cultures given that we need to translate the treatment strategies not only in a tissue-context but also a disease dependent-context where multiple signalling pathways are deranged. Within this project we employ the rat model of induced IVD degeneration as a preclinical/screening platform for new bioactive substances where dose finding and safety needs to be determined prior to taking the step towards a large animal model like the dog. The rat model is on the overall less severe as experimental animal procedure compared to the dog animal model described under Appendix no 4 (both moderate severity but in rats 5 levels and in dog 8 levels). In experimental rats the use of several levels of IVDs with a randomized block design strengthen the power of the studies (Reduction). Up to 5 caudal IVDs can be treated within in one experimental rat, indicating that up to 5 treatment groups can be included.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals are allowed to accommodate to their new environment for at least 1 week, they are housed in groups, and only housed separately for one day post-induction of disc degeneration and post-treatment in order to prevent possible wound healing complications. Furthermore, animals are given cage enrichment and the animals are often examined and weighed by the researcher, which does not cause any pain but is seen as enrichment. In order to decrease the stress levels of the rats, they will be trained to get used to handling and blood collection. Moreover, the rats receive proper analgesia after induction of IVD degeneration, to inhibit acute pain. If necessary, analgesia will be continued. Supervision of surgeries and postoperative care, anesthesia, postoperative analgesia, and imaging are performed by European board-certified veterinary specialists in surgery (ECVS), anesthesia (ECVA), and diagnostic imaging (ECVDI). The body weight of the rats will be recorded weekly to obtain an impression of the overall health and wellbeing of the animal.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Occasionally wound healing may be delayed or wounds may become superficially infected; in that case the rat may have to be housed solitary for a short period of time. There are no other specific signs to be expected except for general clinical signs related to the controlled release of the bioactive substances in the circulation that may affect organ function. Incidence is estimated to be less than 5%. As the IVDs in the tail are used, no neurological signs are expected other than possibly a bend in the tail, which however was not described in literature based on this model.

Explain why these effects may emerge.

Occasionally rats tend to chew on each other's wounds when housed in groups. Given that the induction of IVD degeneration is done in a minimally invasive way whereby the skin and IVD is punctured with a 21G needle, there may be a small puncture hole that will have to heal secondarily.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Frequent (at least daily) observation immediately after application of the injectable treatments will monitor general clinical signs of the animals. If clinical signs indicate severe discomfort and animals cannot be treated adequatly, animals will be euthanized.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

General clinical signs are observed frequently. If clinical signs indicate severe discomfort and animals cannot be treated adequately, animals are euthanized. One possible complication may be extensive infection of the tail.

Indicate the likely incidence.

literature does not report such complications and contact with researchers employing the model did not indicate that this is likely to happen.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

moderate for all animals of the study given that in each animal 5 caudal IVD will be used to induce IVD degeneration - if single IVD degeneration is induced the procedure would be considered mild.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

in order to collect post-mortem information that is very important in order to define the effect of the regenerative treatment, and understand the underlying mechanism of action

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10800
1.2	Provide the name of the licenced establishment.	Universiteit Utrecht
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: [redacted] for the induction of mild osteoarthritis (goat)

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

After determining the optimal dose in small animal model (OA induction in the rat, appendix no 4), the effect of the bioactive molecules in their biomaterial based controlled release systems will be studied in a goat model where OA is induced by scratching the cartilage of the femural condyles. Mild OA and inflammation of the synovial lining will develop [redacted] as measured by histological scoring and biochemical analysis of extracellular matrix

compounds. All treatment strategies employed within this project rely on the employment of minimally invasive injectable treatments, in which the bioactive substance alone, or loaded on a biomaterial that effectuates sustained release of the bioactive substance is injected once. In specific objectives where the advantages of controlled release have not been shown yet, the treatment may be repeated after 3 months. In studies of safety, duration of the treatment is limited to 3 months, while in studies where also efficacy is studied, ideally the duration of the study is 6 months post injections. Regenerative effects should be robust and lasting and therefore long term efficacy studies aim at a follow up of 6 months.

Reference:
[redacted]

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

OA tends to develop insidiously, sometimes without evidence of prior injury or a clear inflammatory component and is primarily driven by focal cartilage damage which increases in severity by biomechanical loading. This is best simulated in the [redacted] model. This model for OA closely resembles human and canine OA, as it does not involve ligament transection or injection of enzymes as done in small animal models, but is based on mechanical damage to the cartilage.

After initial adjustment of the animals to their environment, [redacted] intact. Procedure severity: moderate
After OA has developed at T=0 under sedation, the bioactive substance, either or not in local delivery biomaterial or the biomaterial alone will be injected into the stifle joint. Procedure severity: minor

Treatment groups of a safety study: 2 groups of goats, in both groups unilateral OA is induced. In one group the biomaterial alone is injected in the OA stifle and in the other group the biomaterial + bioactive substance. Each treatment is compared to the contralateral healthy stifle and to each other (4 comparisons)
Treatment groups of an efficacy study where the additive effect of controlled release of the bioactive substance is compared to a single injection of the bioactive substance alone: 2 groups of goats; in both groups unilateral OA is induced. In one group the bioactive substance alone is injected in the OA stifle, while in the other group the bioactive substance + biomaterial is injected in the OA stifle. Each treatment is compared to the contralateral healthy leg and to each other.

At T=3 (safety study) or 6 months (efficacy study), the in vivo experiment will be terminated and goats will be euthanized. Procedure severity = minor.

Read out parameters

In vivo: Potential regenerative and anti-inflammatory effects will be studied by obtaining conventional T2-weighted MR images and quantitative MR images in a longitudinal manner and determination of the cytokine and cell profile of synovial fluid. Procedure severity = minor
This is done under anesthesia at T=0 weeks (baseline, after induction of OA) and post-mortem after termination of the experiment.

Post-mortem: Radiography and computed tomography to evaluate the presence of extra-articular osteophytes and the trabecular structure of the subchondral bone. Thereafter, the joint is collected and processed accordingly for biochemical and histopathological examination. Histological scoring of cartilage

degeneration will be the major readout parameter.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Power analysis is performed with the aid of the freely available software G*power 3.1.9.2. Power analysis is performed on the primary read out parameter of each experiment. The primary read out parameter is histological score of the cartilage. Based on a statistical power of 85%, an alpha error level of confidence corrected for the number of relevant comparisons between groups set at 0.125% (alpha=0.0125, when 4 relevant comparisons), and assuming standard deviations as observed in previous studies: 15%-30% was observed after injection of anti-inflammatory bioactive substance, four to six goats should be sufficient in each group to observe significant differences. All in vivo studies are approached in this manner and dependent on the expected effect and the documented variation, we select the appropriate number of animals.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The [redacted] model for goats has been set up and validated (manuscript submitted) by the department of Rheumatology and department of Orthopaedics at the UMCU. The gross anatomy of the goat knee is fairly similar to both canines and humans, and in both canine and human patients the knee is the most frequently affected joint. Also in terms of function and biomechanics the goat knee joint resembles the human and canine knee joint more than rats. In horses, an accepted animal for research on regeneration of osteochondral defects, the metacarpophalangeal joint is studied, which is analogous to the human wrist with two layers of carpal bones. However, developing a [redacted] model of the metacarpophalangeal joint in horses would be hampered by the limited access to the dorsal aspect of the metacarpophalangeal joint and the narrowed space between phalanx 1 (P1) and the metacarpus (MC3) and in addition, this is not the joint most affected in either canine or human patients. Although the goat is ruminant and hence not ideal for studying enteral therapies, this project concentrates on local delivery of medication and hence this is not an issue. Goat models have been used as cartilage regeneration models before by the research group. Due to the size, the joint of goats is easily accessible for surgery and multiple read out parameters can be obtained from one animal. Female animals will be used as they are easier to handle and house together. Goats older than 1 year, and hence skeletally mature, will be purchased from a registered Dutch breeder in order not to have issues with skeletal remodelling seen during growth. There is only limited evidence regarding the role of gender hormones in OA in large animal models.

During this project we want to study two anti-inflammatory and two regenerative bioactive substances loaded on controlled release biomaterials and hence expect to employ max. 48 goats (4 studies).

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

In vivo studies with goats are done after extensive in vitro laboratory investigation and dose-finding in small animals. Only the safe and promosing bioactive substances loaded on controlled release biomaterials are investigated further. As such, a translational step in a large animal model cannot be replaced by in vitro testing, given that OA is a complex disease affecting the whole joint as an organ. Translational studies can only be done in vivo. In order to minimize animals, where the same biomaterial platform is employed for two studied bioactive substances, the placebo treated joints (i.e. with unloaded biomaterial) will serve as a control for both treatments. As such experiment for the two bioactive substances will be run in parallel in order to share the control between the two studies.
The UMCU has developed a preclinical platform employing the goat as an experimental animal for osteoarhritis. They will be envolved in the in vivo studies that will be performed to ensure that all technical aspects are met and thereby to ensure reduction of the animals employed in the study. Further reduction of the animals employed in the study is achieved by the use of imaging techniques (1.5T Magnetic Resonance Imaging) that can follow cartilage quality in a longitudinal manner, and by refined post mortem analysis which achieves multiple read out parameters from each joint studied (for example, histology, biomolecular, and biochemical analysis)

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals are allowed to accommodate to their new enviroment for at least 2 weeks, they are housed in groups, freely walking in pens of approximately 20 square meters. There will be no dietary restrictions and the goats will have access to water ad libitum. They are only housed separately immediately after induction of the OA in order to prevent possible wound healing complications. Furthermore, animals are given toys as enrichment, including licking stones, car tires, and balls. Animals are often examined by the researcher, which does not cause any pain but is seen as enrichment. All injections will be done by a veterinarian diplomate of the European College of Veterinary Surgeons with expertise in orthopedics.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

In the goat, unilateral OA is induced and hence moderate unilateral lameness is expected to occur. Immediately after induction of OA, animals will receive pijnmedication over the course of the 1st week for pain relief. Other adverse effects that may occur is delayed or complicated wound healing.

Explain why these effects may emerge.

Lameness is related to the model, during the induction of OA goats are treated with oral pain medication (1st week after surgery). 6 weeks post-induction of OA, mild OA has developed and the animals are inherently mildly lame but do not receive pain medication, as the efficacy is studied of local delivery of anti-inflammatory and regenerative bioactive substances with an inhibitory effect on pain directly by inhibiting inflammation and/or indirectly by regenerating the tissue. Delayed wound healing occasionally occurs and is related to sutures that fail on the basis that the animals chew on them.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

During induction of OA, regardless of the treatment, proper pain medication can be given to the animals to minimize pain. After induction of OA, when anti-inflammatory oral pain medication is not feasible due to the aim of the study, opoids will be employed.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

- General clinical signs will be observed frequently. If the animals show severe discomfort and cannot be treated properly, they will be euthanized. Humane endpoints may include: jaw-grinding, 'staring' into space, reluctance to move, guarding of affected areas, limping or carrying a limb), vocalisations

Indicate the likely incidence.

The dept of Rheumatology, UMC Utrecht has performed similar experiments in the past with at least n=16 goats and have not experienced any incidents that required the implementation of humane endpoints

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

moderate

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

In order to collect post-mortem information, including the major readout parameter histological scoring, important in order to define the effect of the regenerative treatment and also understand the underlying mechanism of action

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10800
1.2	Provide the name of the licenced establishment.	Universiteit Utrecht
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 4 Type of animal procedure: Canine model of IVD degeneration

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Dependent on the question and the treatment strategy investigated we will study the effect of the regenerative and anti-inflammatory strategies in mild and severe IVD degeneration. Beagle dogs older than 1 year have in all IVDs mild degeneration (Bergknut et al. 2012). Severe degeneration of the IVD with evident loss of disc height is induced by puncture of the IVDs (Serigano et al. 2010). All strategies employed within this project rely on the employment of

minimally invasive injectable treatments guided by fluoroscopy to ensure correct placement of the needle, in which the bioactive substance alone, or loaded in a biomaterial that effectuates sustained release of the bioactive substance is injected once. Major readout parameters include clinical symptoms, disc height loss and inflammation, which are only present in animals with severely degenerated IVDs, and restoration of IVD tissue, as determined by histology and biochemical analysis of the content of tissue extracellular matrix molecules. Regeneration can be observed both in mildy and severely degenerated discs.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

At the initiation of the in vivo study, all experimental dogs will undergo general clinical and orthopedic examination and will be subjected to MRI to determine the grade of IVD degeneration by means of Pfirrmann scoring. MRI will be performed with a 1.5 Tesla scanner. Six weeks before the start of treatment (T=-6 weeks), 5 lumbar IVDs per dog will undergo nuclectomy as follows: under fluoroscopic guidance a needle will be inserted to center of the disc and the NP will be aspirated. Equal volumes of nucleus pulposus (NP) tissue will be removed from each IVD and confirmed by weighing the nuclectomized tissue on a micro-balance. To confirm that degenerated Pfirrmann grade 4-5 discs are indeed created by this procedure, an MRI will be performed at T=0 months, i.e. 6 weeks after induction of IVD degeneration.

At T=0 (start of the treatment), the bioactive substances either or not in controlled release biomaterials will be injected into the canine IVDs on the side contralateral to the annular puncture, if applicable. All injections will be done under fluoroscopic guidance to confirm correct position of the needle tip prior to injection. At T=3 months, the in vivo experiment will be terminated and dogs will be euthanized

Read out parameters

In vivo:

Potential regenerative and anti-inflammatory effects will be studied by obtaining conventional T2-weighted images and quantitative T2 maps in a longitudinal manner. At all time points, i.e. at T=-6 weeks (baseline, at induction of severe disc degeneration), at T=0 (time of treatment), and at T=3 or 6 months, the following read out parameters will be determined:
- Pfirrmann grade of mid-sagittal slices of T2-weighted MR images (Bergknut et al. 2011).
- Disc height index (DHI) will be calculated on T2W images for each IVD (Willems et al. 2015).
- For analysis of quantitative MR images (for example T2 values), an oval shaped region of interest (ROI) will be manually segmented on mid-sagittal sections, to select NP tissue in each IVD using the free open-source DICOM viewer Osirix (Pixmeo, Geneva, Switzerland). Imaging values will be computed by calculating the mean signal intensity in each ROI (Willems et al. 2015).
Post-mortem:

Radiography and computed tomography to evaluate the presence of extradiscal mineralization, one of the possible complications of intradiscal treatment with bioactive factors. Thereafter, tissues will be collected for histological, biomolecular, and biochemical analysis. Where indicated, after fixation tissue samples will be subjected to micro-CT evaluation.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Power analysis is performed with the aid of the freely available software G*power 3.1.9.2. Power analysis is performed on the primary read out parameter of each experiment. Where regeneration is the focus, the primary read out parameter is disc height index and glycosaminoglycan content by biochemistry, while where inflammation is the focus, the primary read out parameter are (anti)inflammatory cytokines extracted from the treated IVDs. Based on a statistical power of 85%, an alpha error level of confidence corrected for the number of relevant comparisons between groups set at 0.8% (alpha=0.008, when 6 relevant comparison), and assuming standard deviations as observed in previous studies: 15%-30% was observed after injection of anti-inflammatory

bioactive substance, four to seven discs should be sufficient in each group to observe significant differences. In order to minimize the number of animal employed per study and to minimize the dog effect as random effect, we will inject several IVDs per animal, employing a block design of the study. Up to eight different treatment groups can be studied in each dog, including the untreated and placebo treated IVDs.

As an example in the study where the effect of a bioactive substance incorporated in a controlled biomaterial platform is studied in comparison to a single injection of the bioactive substance alone the following groups will be studied
- 1. untreated mild IVD degeneration
- 2. untreated severe IVD degeneration (induced by puncture as described in the experimental approach)
- 3. biomaterial alone in mild IVD degeneration
- 4. biomaterial alone in severe IVD degeneration
- 5. bioactive substance in mild IVD degeneration
- 6. bioactive substance in severe degeneration
- 7. biomaterial + bioactive substance in mild IVD degeneration
- 8. biomaterial + bioactive substance in severe IVD

Relevant comparisons- for mild IVD degeneration: 1 vs 3, 1 vs 5, 1 vs 7, 3 vs 7, and 5 vs 7
Relevant comparisons- for revere IVd degeneration: 2 vs 1, 2 vs 4, 2 vs 6, 2 vs 8, 4 vs 8, and 6 vs 8
All these treatment are applied in a block design based on a latin square in each animal, and hence each treatment is present as n=1 per experimental animal. Hence, max 7 beagles would be assigned to address the specific aims by means of longitudinal quantitative MR imaging and post-mortem biomolecular, biochemical, and histological analysis. All in vivo studies are approached in this manner and dependend on the expected effect and the documented variation, we select the appropriate number of animals.

References:
* Bergknut N, Rutges JP, Kranenburg HJ, Smolders LA, Hagman R, Smidt HJ, Lagerstedt AS, Penning LC, Voorhout G, Hazewinkel HA, Grinwis GC, Creemers LB, Meij BP, Dhert WJ. The dog as an animal model for intervertebral disc degeneration? Spine (Phila Pa 1976). 2012 Mar 1;37(5):351-8
* Bergknut N, Auriemma E, Wijsman S, Voorhout G, Hagman R, Lagerstedt AS, Hazewinkel HA, Meij BP. Evaluation of intervertebral disk degeneration in chondrodystrophic and nonchondrodystrophic dogs by use of Pfirrmann grading of images obtained with low-field magnetic resonance imaging. Am J Vet Res. 2011 Jul;72(7):893-8.
* Serigano K, Sakai D, Hiyama A, Tamura F, Tanaka M, Mochida J. Effect of cell number on mesenchymal stem cell transplantation in a canine disc degeneration model. J Orthop Res. 2010 Oct;28(10):1267-75.
* Willems N, Bach FC, Plomp SG, van Rijen MH, Wolfswinkel J, Grinwis GC, Bos C, Strijkers GJ, Dhert WJ, Meij BP, Creemers LB, Tryfonidou MA. Intradiscal application of rhBMP-7 does not induce regeneration in a canine model of spontaneous intervertebral disc degeneration. Arthritis Res Ther. 2015 May 27;17:137.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The canine species is considered to be a suitable model to study the process of IVD degeneration: like humans, dogs suffer from spontaneous IVD degeneration, and the degenerative process involves similar clinical, macroscopic, histopathological, and biochemical changes as humans. The Utrecht referral animal university clinic frequently encounters canine patients with back pain and neurologic deficits due to IVD disease. Hence the dog serves as a preclinical model for translation towards the “first in man” studies, while it serves also the veterinary patient.

Each in vivo study is performed on the lumbar IVDs of adult Beagle dogs older than 1 year in order to have early degenerated IVDs and not older than 3 years. This range of age results into a cohort of animals with degenerated IVDs with a Pfirrmann score of 2-3. Animals are ordered at Harlan or Marshal laboratories dependent on the availability and the age.

Regarding the sex of animals: In chondrodystrophic dogs, like the experimental Beagle, there is no male/female predisposition for the development of disc disease in canine patients (Smolders et al 2013), hormonal effects are considered irrelevant to the model. Even more so, there is no gender predeliction also for the human population of patients with IVD disease (Siemionow et al. 2011).

In short term studies (range of 3 months) we aim at having a sex ratio of 1:1 in the study cohort in order to have both sexes represented in the study. In long term studies of > 6 months, we preferably work with male dogs in order not to have issues with the cyclus of female dogs. Female dogs have twice a year an oestrus cycle and when not used for breeding castration is adviced in order to diminish the risk for development of breast cancer and other complications of irregularities of the cycle, including pseudopregnancy and pyometras, and accidental fertilisation can not be excluded during long term housing of the animals. For this reason, female dogs when not used for breeding should be sterilised, which would add another experimental procedure with moderate severity.

Over the course of the project we estimate to employ max 28 dogs, as we aim to test at least two-anti-inflammatory drugs and two known regenerative factors with max n=7 per study (4 studies)

4 studies: The anti-inflammatory drugs have already been selected on the basis of preceeding experimental work: [redacted] and [redacted] The regenerative drugs cannot be defined at this stage, given that preclinical work is being performed and will also be conducted within the course of this project before definite selection of new candidates. Over the course of this project we do not foresee combination of treatment. In follow up projects, based on the findings of this proposal, treatment strategies may contain combinatorial treatment of sequential treatment of anti-inflammatory and regenerative drugs.

References: Siemionow et al. The Effects of Age, Gender, Ethnicity, and Spinal Level on the Rate of Intervertebral Disc Degeneration. A review of 1712 Intervertebral Discs. Spine (Phila Pa 1976). 2011 Aug 1; 36(17): 1333–1339.
Smolders LA et al. Intervertebral disc degeneration in the dog. Part 2: chondrodystrophic and non-chondrodystrophic breeds. Vet J. 2013 Mar;195(3):292-9.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

After extensive development of treatment strategies based on in vitro and in vivo experimental studies in small animal models, it is essential that translation occurs in an animal model that resembles the human situation. In the chain of translation the Beagle dog is indispensable. The UU has developed a preclinical platform employing the Beagle dog as an experimental animal and veterinary patients with lower back pain (Figure in Appendix). In experimental animals the use of several IVDs per animal in combination with a randomized block design strengthen the power of the studies (Reduction) and longitudinal follow up of the animals is done by modern imaging techniques (1.5T Magnetic Resonance Imaging and 64-slice Computer Tomography), while in canine patients an additional read out parameters is objective gait analysis (force plate). For the laboratory dogs, post mortem analysis is performed by histology, biomolecular, and biochemical analysis from each IVD (Refinement).
In order to further minimize the number of animals we will also evaluate whether use of the IVDs in the tail of the Beagles is also feasible. In this respect, we would be able to have more experimental conditions or duplicates per animal. The tail is being employed in the IVD field in rats and mice, where IVD degeneration is induced either by puncture of the tail or looping of the tail. In this respect, inducing disc degeneration by puncture in the tail of the dog would minimize discomfort, given that the IVD in the tail is easily approached by a minimal invasive technique assisted by fluoroscopy. Nevertheless, one of the limitations of the "canine tail model" would be that the size of the IVDs is much smaller than the lumbar IVDs, and thereby determining all biochemical, biomolecular and histological parameters in each IVD (as described under the 3R section for the lumbar IVDs) would not be feasible.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals are allowed to accommodate to their new enviroment for at least 1 week, they are housed in groups, and only housed separately for one week post-induction of disc degeneration and post-treatment in order to prevent possible wound healing complications. Furthermore, animals are given toys as enrichment and are allowed to be for at least one hour outside their wards in order to play. Furthermore, animals are often examined by the researcher, which does not cause any pain but is seen as enrichment.
Supervision of surgeries and postoperative care, anesthesia, postoperative analgesia, imaging and force plate analysis are performed by European board-certified veterinary specialists in surgery (ECVS), neurology (ECVN), anesthesia (ECVA), and diagnostic imaging (ECVDI).

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Occasionally wound healing may be delayed; in that case the dog may have to be housed solitary for a short period of time.

Explain why these effects may emerge.

Animals are given an Elisabethian collar after IVD degeneration is induced. This is a plastic collar attached around their neck and is extending further than their mouth. In this way one can prevent them from licking their wound, but certain dogs have proven to be very inventive and still manage to get to their wound.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

proper suture techniques will be applied (subcutaneous sutures will prevent wound dehiscence)

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

We frequently observe general clinical signs, with higher frequency in the period pre- and post-induction of IVD degeneration and initiation of the treatment.

Clinical signs related to the animal model may include: paraparesis, paralysis, unilateral lameness of the hind limb, proprioceptive disorders, urinary incontinence. These neurologic signs are related to herniation of the IVD and can vary dependent on the location of the herniation. Humane endpoints include: acute paralysis. In case of the other neurologic signs, they are considered a human endpoint when the neurologic status of the dog is not improving after initiation of proper treatment that primarily consists of anti-inflammatory medication and if needed decompressive surgery of the allocated IVDs. In the latter, the decompressed IVDs will be excluded from the study, while the other IVDs and the allocated treatment group can be followed up until termination of the experiment. If general clinical signs indicate severe discomfort and animals cannot be treated adequately, animals are euthanized.

Indicate the likely incidence.

We do not expect this to occur. We already have performed similar experiments in the past with at least n=30 dogs and have not experienced any incidents that required the implementation of humane endpoints. Incidence less than 2%.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

moderate. The severity of the procedure would be mild if only one IVD would be employed per animal and becomes moderate in the described model where up to 8 IVDs can be implemented in the study. This project aims to perform 4 studies, per study 8 treatment. At an n=7 per treatment a total of 28 animals will be undergoing a procedure with moderate severity. If a single IVD would be studied per animal, that would result in the use of 224 dogs with a mild procedure. We believe that this reduction in number outweighs the increase in severity.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

in order to collect post-mortem information that is very important in order to define the effect of the regenerative treatment and also understand the underlying mechanism of action

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10800
1.2	Provide the name of the licenced establishment.	Universiteit Utrecht
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 5 Type of animal procedure: Degradation of biomaterial and release kinetics

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The degradation of the biomaterial used and release kinetics of the bioactive substances loaded (key objective 3) will be studied in experiments in which the effect of [redacted] and the presence of [redacted] process at that location are studied. To this end osteoarthritis and IVD degeneration are induced as described in appendices nr 2 and no 4, but also non diseased joints and IVDs will be included. Optimal doses of actives as identified in the procedures

described under objective 1 of this project will both be providing bolus controls for the latter experiments in terms of cartilage regeneration (histological scoring) and release profiles as also described in appendix no 2 (in order to reduce the number of animals employed). For IVD injection, the same animals can be used for biomaterial degradation studies by [redacted] or [redacted] labelling of biomaterials (readout parameter [redacted]), as the released actives will attain negligible concentrations inside the body after their release from the IVD. The[redacted] imaging methods will be validated in separate animals by tissue extraction, where biomaterial constituents measured by biochemical assays will be the major readout.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Pilot study: in order to validation of the imaging methods, biomaterials loaded with a range of known dosages of label will be injected into [redacted] and [redacted] of dead rats, n=3 per dose and per location. Surplus euthanized for other unrelated experiments will be employed for this purpose. After injection, the cadavers will be subjected to [redacted] and the signal will be correlated with the dose. Validation of each technique will not only determine the quantitative correlation between signal and dose, but will also give information on the threshold of the label that is still detectable by imaging. Thereafter, in vivo experiment with rats will be performed as described in study I.

Experimental groups Study I: to determine the effects o [redacted] and the presence of a [redacted] process on degradation rates of biomaterial-based carriers. Four biomaterial platforms can be studied in 12 rats. The platforms will be loaded with [redacted] or [redacted] label and injected separately in each location.

For the example presented for biomaterial 1 and biomaterial 2 below the major readout will be the NIR signal over time.
Experimental groups: based on locations, all locations are employed in each rat
- 1. [redacted]-loaded biomaterial 1
- 2. [redacted]-loaded biomaterial 1
- 3. [redacted]-loaded biomaterial 1 + [redacted]-loaded biomaterial 2 (each in one degenerated IVD per rat, n=1 per treatment).
- 4. [redacted]-loaded biomaterial 1 + [redacted]-loaded biomaterial 2 (each in one degenerated IVD per rat, n=1 per treatment).
- 5. [redacted]-loaded biomaterial 1 and 2 (separate locations)
- 6. [redacted]- loaded biomaterial 1 and 2 (separate locations)

NB: In order to reduce the number of animals for key objective 3, in these 6 rats additional degenerated IVDs per rat (n=3 rat tails) will be injected with one biomaterial loaded with active 1 in degenerated IVDs (n=3 rats). The release profile of the bioactive substances is studied with blood collection at predetermined time points

Procedure severity = moderate on the basis that more locations are injected per rat.

Relevant comparisons for [redacted] degradation: 1 vs 3-biomateral 1, 1 vs 5-biomaterial 1, 1 vs-6 biomaterial 1, 3-biomaterial 2 vs 5-biomaterial 2, and 3 vs 5-biomaterial 2.
Relevant comparison for [redacted] degradation: 1 vs 2, 3 vs 4 for biomaterial 1, and 3 vs 4 for biomaterial 2

Experimental groups study II:
Aim: to determine the [redacted]specificity of release of bioactive substances from loaded biomaterials. This is only done for a biomaterial of which it is known that it is sensitive to [redacted] processes.

Loading dose of the biomaterial: a single dose, highest dose without appreciable side effects from rat dose-study as described under objective 1 of this

project
Approach: OA is induced with anterior cruciate ligament transection and partial meniscectomy / IVD degeneration is induced by puncture as described in appendix description animal procedures no. 4 and 2, respectively. Procedure severity = moderate

Locations treated: knee, IVD; number of animals employed 22 (total of 28, given that n=6 is studied in study I to reduce number of animals employed), for two bioactives , that amounts to 58.
The experimental groups are:
- 1. Rats with [redacted] induced –biomaterial plus bioactive substance [redacted] injected with unloaded biomaterial 1 (n=7)
- 2. Rats [redacted] induced biomaterial + bioactive substance in [redacted] injected with unloaded biomaterial (n=7)
- 3. Rats [redacted] biomaterial + bioactive substance (n=7 of which 3 from study I)
- 4. Rats with [redacted] biomaterial + bioactive substance (n=7 of which 3 from study I) (see appendix no 2)

Relevant comparisons: 1 vs 2, 3 vs 4, 1 vs 3, 2 vs 4 ([redacted]- and [redacted]-dependent comparisons)
One of the first studies to be performed with this experimental design will include two anti-inflammatory compounds, i.e. [redacted] and [redacted].

Read out parameters:

In vivo:
- The rats of the OA groups will be assessed clinically: joint effusion will be measured, lameness will be scored and knee stability will be tested. Severity procedure = minor
- Pressure plate measurements will be performed for the OA groups on a regular basis to assess the amount of functional disability caused by the osteoarthritis that has been induced. Severity procedure = minor
- To measure release kinetics of the loaded compounds, blood samples will be collected on predetermined timepoints. To limit the burden for the animals only in bolus conditions and rats with induced IVD degeneration the release profile in blood circulation will be determined for the first 3 weeks of the study. The remaining of the group will be subjected to less time points than 12 of blood collection. The time points will be determined based on the release profile documented during the dose response study done under objective 1 of the project. Severity procedure = minor
- At the end of the experiment animals are euthanized to collect tissues for further analysis. Severity procedure = minor

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

These types of studies are not subjected to power analysis given that the primary read out parameter is not efficacy of the treatment. The aim of these studies is to better understand release kinetics in order to further fine tune the development of the controlled release systems adressed in this project. For this purpose a minimum of n=5 per treatment was chosen in order to have sufficient animals for the possible biological variation.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

We prefer to use female rats since male rats can increase a lot in size and weight during the experiment. During this long term follow up, the female rat knees will fit in the microCT scanner, while this will not be the case for the male rats. We use rats from the age of 12-14 weeks, because then they are large

enough for intra-articular injections and blood collection. Furthermore, skeletal growth does not influence IVD development any more at this age and both the OA and IVD model can be combined in one animal in this age range. Furthermore, female rats are preferred for this kind of experiment, since they are easy to keep and easy to handle. No effects on cartilage regeneration have been shown of fluctuating hormone levels, although in humans the frequency of OA in females is higher than males. However, as joint anatomy is also different in human females, it is not clear whether this is due to direct effects on cartilage metabolism or to the indirect effects of e.g. suboptimal alignment. Moreover, although ovariectomy was shown to have a clear effect on the development in various models of OA in female rodents, the administration of estrogens (=artificial fluctuation) to healthy reproductive female mice showed no clear effects, thereby suggesting that a total lack of estradiol rather than fluctuation affects disease progress (Sniekers YH, Weinans H, Bierma-Zeinstra SM, van Leeuwen JPTM, van Osch GJVM. Animal models for osteoarthritis: the effect of ovariectomy and estrogen treatment—a systematic approach. Osteoarthritis and Cartilage. 2008;16(5):533–541) Thus far there have been no reports on gender differences in IVD degeneration.

Sprague-Dawley are purchased from the Charles River laboratories on the basis that previous experiments are on the same strain.
We aim to study 2 anti-inflammatory bioactive substances ([redacted] and [redacted]). In total 4 biomaterials will be studied that will all be studied in terms of location-dependent degradation, but only two biomaterial-bioactive combinations will also be used to evaluate disease-dependent release. In the experimental study design as described above we employ 12 rats in Study I and 44 rats in Study II for 2 bioactive substances for the treatment of OA and IVD degeneration. We have also included 20 rats for pilot studies (if we do not manage to find cadavers for the pilot study described in this appendix) and max 10 rats for drop outs due to insufficient IVD degeneration induced. Altogether, we estimate to use 86 rats for this specific experimental procedure in the period of 2015-2020.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement: The joint and IVD are complex organs, consisting of different tissue- and cell types. Moreover, the homeostasis of the tissues is being regulated by biomechanical forces, both internally and externally. In the case of OA and IVD degeneration, changes occur in multiple areas and also the biomechanical loading can change, all influencing the disease progress. Up to now, it is not possible to mimic a whole joint/IVD in vitro, therefore animal experiments remain necessary in this phase of the development of drugs. However, a lot of tests regarding possible toxicity and release profiles have been performed in vitro, prior to the start of animal experiments.

Reduction: In order to further minimize the number of animals used, where applicable we adress the questions of the project in joined in vivo experiments. A typical example is Objective 3, in which we study the [redacted] and [redacted] degradation of biomaterials. In a single in vivo study, we employ for the unloaded biomaterials the same rats to study in parallel the degradation rate after [redacted] injection. Only when the biomaterials are loaded with the bioactive substance rats are employed for the study of a single location, in this case the joint, and investigate the release profile in the blood circulation and the effects locally. The latter is done on the basis that controlled release of the bioactive substance into the circulation and hence may influence all parts of the body.

Refinement: In order to decrease the stress levels of the rats, they will be trained to get used to handling, blood collection and pressure plate measurements. Moreover, the rats receive proper analgesia after OA and IVD induction injections, to inhibit acute pain. If necessary, analgesia will be continued.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals are allowed to accommodate to their new enviroment for at least 1 week, they are housed in groups. They will be trained prior to the experiment to get used to handling, fixation for blood collection (in a so-called blood collection 'sleeve') and pressure plate measurements.

Furthermore, animals are given cage enrichment and the animals are often examined and weighed by the researcher, which does not cause any pain but is seen as enrichment.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question 1.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Induced IVD degeneration model: occasionally wound healing may be delayed or wounds may become superficially infected; in that case the rat may have to be housed solitary for a short period of time.
OA rat model: complicated wound healing causing minor discomfort

Explain why these effects may emerge.

Induced IVD degeneration model: Occasionally rats tend to chew on each other's wounds when housed in groups. Given that the induction of IVD degeneration is done in a minimally invasive way whereby the skin and IVD is punctured with a 21G needle, there may be a small puncture hole that will have to heal secondarily.
OA rat model: occasionally rats tend to chew on their sutures (<10% of the rats). If there is wound dehiscence, we suture the skin again and have experienced that the rats do not further chew on their sutures.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Induced IVD degeneration model: During the first pilot experiment, the effect of the IVD puncture will be assessed. Puncture with a needle is not expected to induce substantial skin wounds and hence individuyal housing of the treated animals for one day after the treatment will most probably not be necessary.
Rat OA model: we use thin biodegradable sutures (size 4-0) that maintain their strength during the healing period of the skin; proper placement of the sutures prevets swelling of the skin and thereby prevents irritation to the animal. Surgery is performed by trained veterinarians for this procedure.

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

- General clinical signs will be frequently observed. If clinical signs indicate severe discomfort (e.g. body weight loss 15% in 2 days) and animals cannot be treated adequately, animals are euthanized. Furthermore if an animal is unable to stand or walk in spite of treatment with proper analgesia for at least one day, the humane endpoint is reached.

Indicate the likely incidence.

We already have performed similar experiments in the past with n=21 rats and have not experienced any incidents that required the implementation of humane endpoints. Incidence is expected to be < 5%.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

moderate

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

It is necessary to euthanized the rats at the end of the experiment, to gain insight in the histopathological and biochemical processes in the joint and IVD. furthermore, in order to determine the biomaterial left non degraded in each location, the surrounding tissues need to be examined. The latter is of importance in order to determine at which interval human and veterinary patients can safely receive locally the loaded biomaterial. This can only be studied in post mortem collected tissues.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

Project: 2015.II.813.025
Title: Local controlled release of medication for the treatment of degenerative joint diseases
Appendix: Coherence of the project

This project pursues biological repair of the diseased joint and IVD by combing both translation and basic research. The coherence between the different components and the different steps of the projects is illustrated in the figure.

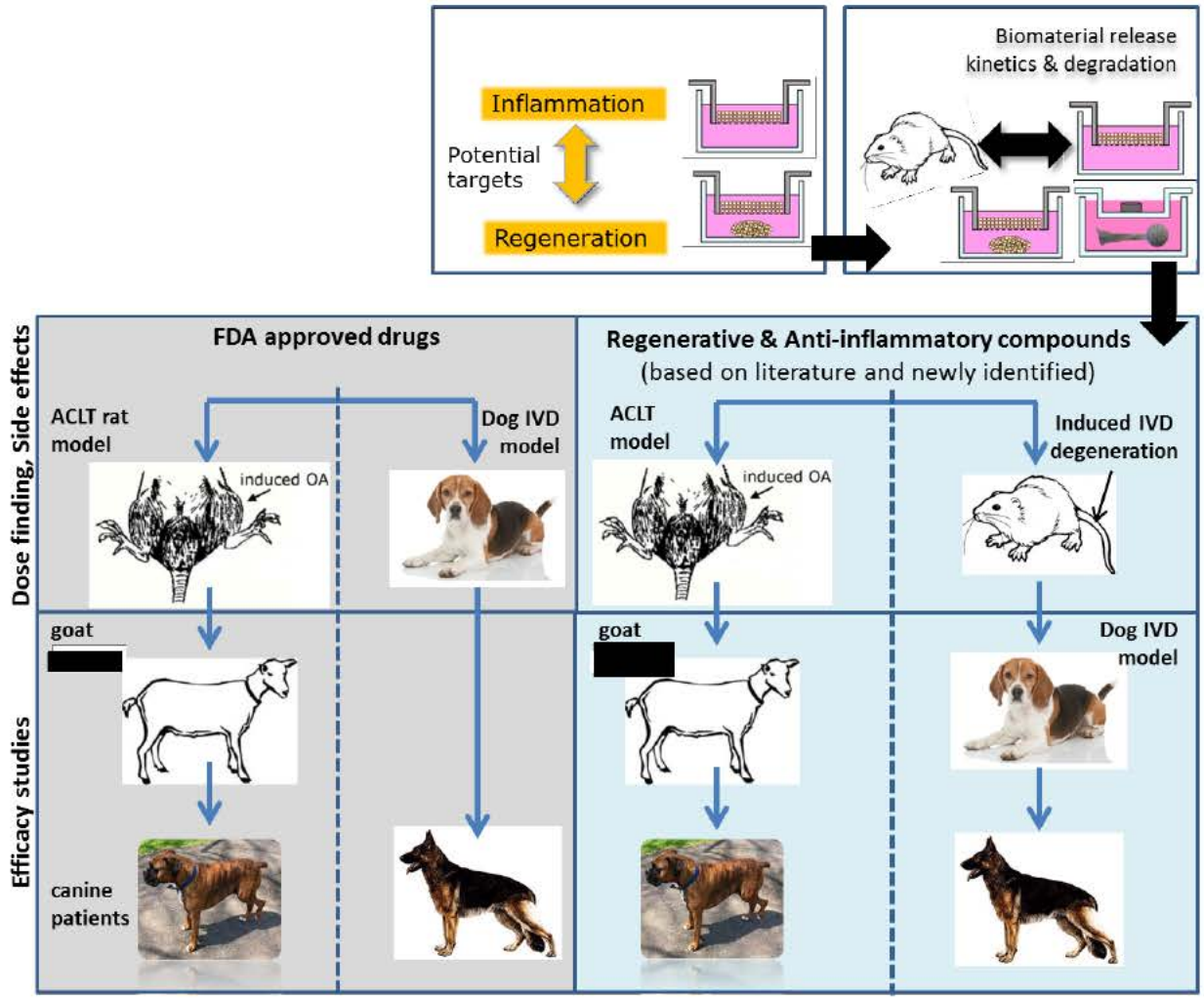


Figure legend: Animal studies largely consist of two distinct stages;
1) in small animals where proof of principle and therapeutic window (safety and optimal effectivity) will be determined and 2) large animals, where the effects in large and more clinically relevant models are being studied. As soon as safety and efficacy has been determined in the large animal models of OA and IVD, and release kinetics are comprehended, a first translational step will be undertaken in canine patients with OA or IVD disease within the 5-year period of the project. Likewise, a phase I clinical trial in humans will be feasible shortly after completion of the project, as soon as regulatory affairs and transfer of the newly developed treatment into a GMP-environment have been adressed

Therapeutic compounds that will be applied in animal study stages will have followed three different preceding trajectories;
1) FDA-approved drugs that have already been shown to be active in the clinic will directly enter the first stage, 2) compounds that have shown proof of principle in literature and
3) newly identified targets in the projects' fundamental research part, after validation in vitro of effectivity and optimal release profiles.

In addition, general information on the aspects of local controlled release will be answered by studying release by biomaterials loaded with labels at [redacted] ("Biomaterial release kinetics & degradation").

References used in the project proposal

Bellamy N, Campbell J, Welch V, Tl G, Bourne R, Ga W (2009) Intraarticular corticosteroid for treatment of osteoarthritis of the knee. The Cochrane Library. 2; 1-120

Blanquer SB, Sharifi S, Grijpma DW (2012) Development of poly(trimethylene carbonate) network implants for annulus fibrosus tissue engineering. J. Appl. Biomater. Funct. Mater. **10**: 177–184.

Gawri R, Antoniou J, Ouellet J, Awwad W, Steffen T, Roughley P, Haglund L, Mwale F, Hospital RV, Scoliosis M, Arabia S (2013) BEST PAPER NASS 2013 : LINK-N CAN STIMULATE PROTEOGLYCAN SYNTHESIS.

Hamilton DF, Howie CR, Burnett R, Simpson a HRW, Patton JT (2015) Dealing with the predicted increase in demand for revision total knee arthroplasty: challenges, risks and opportunities. Bone Joint J. **97-B**: 723–728.

Higashino K, Hamasaki T, Kim JH, Okada M, Yoon ST, Boden SD, Hutton WC (2010) Do the adjacent level intervertebral discs degenerate after a lumbar spinal fusion? An experimental study using a rabbit model. Spine (Phila. Pa. 1976). **35**: E1144–52.

Imai Y, Okuma M, An HS, Nakagawa K, Yamada M, Muehleman C, Thonar E, Masuda K (2007) Restoration of disc height loss by recombinant human osteogenic protein-1 injection into intervertebral discs undergoing degeneration induced by an intradiscal injection of chondroitinase ABC. Spine (Phila. Pa. 1976). **32**: 1197–205.

Jacobs WCH, Rubinstein SM, Koes B, van Tulder MW, Peul WC (2013) Evidence for surgery in degenerative lumbar spine disorders. Best Pract. Res. Clin. Rheumatol. **27**: 673–84.

Johnson K, Zhu S, Tremblay MS, Payette JN, Wang J, Bouchez LC, Meeusen S, Althage A, Cho CY, Wu X, Schultz PG (2012) A stem cell-based approach to cartilage repair. Science **336**: 717–21.

Lam J, Lu S, Kasper FK, Mikos AG (2015) Strategies for controlled delivery of biologics for cartilage repair. Adv. Drug Deliv. Rev. **84**: 123–34.

Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet **380**: 2197–223.

Pavlides S, Gutierrez-Pajares JL, Katiyar S, Jasmin J-F, Mercier I, Walters R, Pavlides C, Pestell RG, Lisanti MP, Frank PG (2014) Caveolin-1 regulates the anti-atherogenic properties of macrophages. Cell Tissue Res. **358**: 821–31.

Pereira CL, Gonçalves RM, Peroglio M, Pattappa G, D'Este M, Eglin D, Barbosa M a, Alini M, Grad S (2014) The effect of hyaluronan-based delivery of stromal cell-derived factor-1 on the recruitment of MSCs in degenerating intervertebral discs. Biomaterials **35**: 8144–53.

Seibel MJ, Cooper MS, Zhou H (2013) Glucocorticoid-induced osteoporosis: mechanisms, management, and future perspectives. lancet. Diabetes Endocrinol. **1**: 59–70.

Thavaneswaran P, Vandepeer M (2014) Lumbar artificial intervertebral disc replacement: a systematic review. ANZ J. Surg. **84**: 121–7.

Trampuz A. & Widmer AF (2006) Infections associated with orthopedic implants. Curr. Opin. Infect. Dis. **19**, 349–56

A. Algemene gegevens over de procedure

1. Aanvraagnummer : 2015.II.813.025
2. Titel van het project : Gecontroleerde locale afgifte van medicatie voor de behandeling van rugpijn en artrose
3. Titel van de NTS : Medicijnen voor plaatselijke behandeling van rugpijn en artrose

4. Type aanvraag:
   - ☒ nieuwe aanvraag projectvergunning
   - ☐ wijziging van vergunning met nummer :

5. Contactgegevens DEC

Naam DEC	: DEC Utrecht
Telefoonnummer contactpersoon	: 088 – 75 59 247
Emailadres contactpersoon	: dec-utrecht@umcutrecht.nl

6. Adviestraject (data dd-mm-jjjj):
   - ☒ ontvangen door DEC: 26-06-2015
   - ☐ aanvraag compleet:
   - ☒ in vergadering besproken: 13-07-2016 en 26-08-2015
   - ☒ anderszins behandeld: per email 10-09-2015
   - ☒ termijnonderbreking(en) van / tot : 16-07-2015 tot 13-08-2015 en
     03-09-2015 tot 10-09-2015 en
     29-09-2015 tot 01-10-2015
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met max. 15 werkdagen:
   - ☐ aanpassing aanvraag:
   - ☒ advies aan CCD: 12-10-2015

7. Eventueel horen van aanvrager
   - Datum: 26-08-2015
   - Plaats: Utrecht
   - Aantal aanwezige DEC-leden: 6 DEC-leden
   - Aanwezige (namens) aanvrager: Aanvrager
   - Strekking van de vraag / vragen: zie punt A8.
   - Strekking van het (de) antwoord(en):
     Weergave presentatie onderzoeker:
     De onderzoekster presenteerde haar project en ging in op de vragen die haar voorafgaand aan het project waren toegestuurd (zie daarvoor vraag A8). Additioneel kwamen de volgende punten aan de orde.

De meest voorkomende rugklachten zijn lage rugklachten en artrose. De huidige behandelingen kosten veel geld en omdat men ouder wordt zal de mens er steeds meer hinder van gaan ondervinden. De behandeling die nu beschikbaar zijn, zijn pijnstilling en allerlei operaties, maar zijn helaas niet afdoende. Bij operaties gaat het om het aanbrengen van protheses voor gewrichten en protheses voor tussenwervelschijven. De gewrichtsprotheses zijn slechts voor een bepaalde duur bruikbaar, tussen de 10 en 15 jaar, waarna ze vervangen moeten worden. Voor tussenwervel protheses treden er nog andere problemen op en ook de duur van het gebruik van deze protheses is beperkt.
Artrosemodellen en tussenwervel modellen hebben veel overeenkomsten al zijn er ook enkele verschillen, daarom onderzoekt men beide modellen. Biochemisch is het allemaal goed vergelijkbaar en genetisch gezien zijn er ook veel overeenkomsten; de pijnmechanismen zijn bijvoorbeeld vergelijkbaar. Bij beide aandoeningen is gebleken dat plaatselijke medicatie een goede behandeling is.
Het is de bedoeling dat via een injectie een depot met medicatie geplaatst gaat worden welke gecontroleerd afdoende pijnstilling zal afgeven voor een langere periode zodat de patiënt niet om de 3 maanden terug hoeft voor een nieuwe (pijnlijke) injectie. Onderzocht zal worden wat de exacte dosering moet zijn en welke dosering veilig is en voor welke periode dit zal moeten zijn.

In het project wordt gewerkt met 3 niveaus:

1) Dit is een Clinical Treatment studie. Dit onderdeel bevat een dose finding studie in de rat en de hond. Er wordt gewerkt met medicatie die FDA goedgekeurd is zodat er een snellere vertaalslag gemaakt kan worden naar de mens. De hond wordt gebruikt als tussenwervelschijf model. Tevens bevat dit deel een efficacy studie met de geit. Reden voor het gebruiken van de geit, in plaats van de hond, berust op haalbaarheid van de uitvoering, zowel financieel als organisatorisch, terwijl translatie van de resultaten naar de 'target species' (hond) geen belemmering vormt. Diergeneeskundig heeft de hond de voorkeur, maar humaan de geit. Omdat de hond en de geit qua grootte ongeveer gelijk zijn zal dezelfde dosering gebruikt kunnen worden. Na de studie met de geit kan de hondenpatiënt gebruikt worden in een proof of principle studie en de een humane studie in de kliniek als fase 1 onderzoek.
2) Dit deel is een proof of concept studie met nieuwe targets.
3) Dit deel bestaat uit fundamenteel onderzoek om nieuwe targets te identificeren. Met name de relatie tussen inflammatie en regeneratie wordt bestudeerd. Er wordt ook gekeken naar de werking van de afbraak van de biomaterialen en wanneer het veilig is om een nieuwe injectie te geven.

- Het horen van de aanvrager heeft geleid tot aanpassing van de aanvraag: Ja

8. Correspondentie met de aanvrager
   - Datum: 16-07-2015, 03-09-2015 en 29-09-2015

- Strekking van de vraag / vragen:
  Projectvoorstel
  - 3.1, achtergrond: De DEC mist informatie over onderzoek dat op dit gebied al door andere groepen uitgevoerd is. Graag toevoegen.
  - 3.4, onderzoeksstrategie: De onderzoeksstrategie is niet helder. De DEC ziet graag een duidelijkere opbouw van de verschillende stappen die u wilt nemen in uw onderzoek, waarbij u de keuze voor dier en model onderbouwt. Daarbij begrijpt de DEC de samenhang tussen de verschillende stappen niet goed. Graag herschrijven en toelichten.
  - 3.4, onderzoeksstrategie: De motivatie voor het gebruik van het [redacted] model voor de geit mist. Waarom beperkt u zich niet tot de rat? Wat verwacht u nog aan additionele gegevens?

  Bijlage 3:
  - De DEC begrijpt dat het gebruik van de hond niet goed ligt bij het publiek, maar de DEC vindt dat onvoldoende motivering voor het gebruik van de geit. Wat zou het in aantallen dieren schelen? Bij gebruik van de geit moet een extra translatieslag gemaakt worden.

- Datum: 03-09-2015
- Strekking van de vraag / vragen:
  Projectvoorstel:
  - Naar aanleiding van uw presentatie is het de DEC nu helder waarom u kiest voor het gebruik van de geit. De DEC verzoekt u ook in het projectvoorstel nog meer aan te scherpen waarom u kiest voor de geit in plaats van de hond (of het paard).

- Datum: 29-09-2015
- Strekking van de vraag / vragen:
  - In bijlage 2 vraagt u vrouwelijke ratten aan, maar de motivatie daarvoor is onvoldoende. Graag nader motiveren.

- Datum antwoord: 13-08-2015
- Strekking van het (de) antwoord(en):
  Projectvoorstel:
  - 3.1, achtergrond: The background has been extended with additional information regarding the field of controlled delivery of biologics for the treatment of osteoarthritis and back pain. Recent work by collaborators highlights the progress achieved in the field of joint cartilage repair with the aid of controlled delivery of biologics (Lam J, Lu S, Kasper FK, Mikos AG. Strategies for controlled delivery of biologics for cartilage repair. Adv Drug Deliv Rev. 2015 Apr;84:123-34). Thus far only a limited number of publications on solid or hydrogel based-systems specifically developed for intra-discal controlled release is present [redacted], we have conducted a systemic review on Biomaterials for intervertebral disc regeneration. This work has been resubmitted after minor revision

[redacted] Biomaterials for intervertebral disc regeneration; past performance and possible future strategies, *e Cells and Materials Journal,* Manuscript ID: eCM-Dec-2014-REV-0166.R2). Based on this work it appears that the concept of employing biomaterials for the sustained delivery of agents in IVD regeneration is just emerging. However, the concept of local prolonged exposure to factors modulating regeneration and degeneration holds great promise by reduction of systemic side effects and increasing effectivity.

- 3.4, onderzoeksstrategie: The project encompasses strategies for the treatment of OA and IVD degeneration that are at different levels in the chain of translation:
  a) those that are based on readily available FDA-approved compounds and are further investigated and validated in preclinical studies for dose finding in small and safety and efficacy in large animals, in order to accelerate their translation in to "first-in-man/dog" studies, and
  b) those that explore targets shown in vitro and in vivo to have a positive biologic effect. Safety and dose finding is then done in rat models. The next step of translation is undertaken in large animal models (goat for OA and dog for IVD).
  c) those that are based on new compounds identified in fundamental research. Herein, we identify potent targets based on animal-free in vitro experiment. The promising bioactive substances are then further developed, incorporated in suitable controlled release biomaterial platforms. Proof of principle studies will be done in small animals, followed by dose and safety studies, after which also large animal studies will be prepared for human and canine translation.

  In the revised proposal we discuss the outline of the different components with a clear focus on the rationale behind the choice of animal model. Furthermore, in the Appendix of the proposal a figure has been included illustrating the steps undertaken and their coherence.
- 3.4, onderzoeksstrategie: In the rat OA model a wide dose range will be studied for dose finding and determination of possible side effects. The optimal dose as obtained from the studies in the rat OA model will be translated towards the goat [redacted] model of OA, to determine safety and efficacy before canine and human patients will be treated. The goat [redacted] model consists of [redacted] due to which a mild osteoarthritis develops over a period of 20 weeks without inducing joint instability. The latter does occur in the rat OA model, while joint instability is not common in canine and human patients with chronic OA. As such the [redacted] model is closer to the clinical situation. Moreover, biochemistry of cytokines in synovial fluid for the detection of the effect of the injected materials is not possible in small animal models.

Bijlage 3:

- The [redacted] model in experimental Beagle dogs entails several disadvantages. By employing the canine model we would have to perform the studies with only half of the

number of animals and thus would be unable to complete the project as planned and approved by the financing body.

- Datum antwoord: 10-09-2015
- Strekking van het (de) antwoord(en):

Justification of the choice of the animal model is given.
(a) in the basic outline of the different components and type of procedures:
Goat [redacted] model: Optimal dosages in the rat model will be translated towards the goat [redacted] model of OA, to determine safety and efficacy before canine and human patients can be treated. The goat [redacted] model consists of [redacted], due to which a mild osteoarthritis develops over a period of 20 weeks. This model for OA closely resembles human and canine OA, as it does not involve ligament transection or injection of enzymes as done in small animal models, but is based on mechanical damage to the cartilage. The gross anatomy of the goat knee is fairly similar to both canines and humans, and in both canine and human patients the knee is the most frequently affected joint. Additional readout parameters to those used in small animal models will be cartilage tissue degeneration and synovial inflammation, in addition to biochemistry of ECM proteins and cytokines in synovial fluid.
As soon as safety and efficacy has been determined in the large animal models of OA and IVD a first translational step will be undertaken in canine patients treated at the Academic Veterinary Hospital of The Netherlands. Such studies will be performed within the 5-year period of the project. As a spin-off this project, a phase I clinical trial in humans will be feasible as soon as regulatory affairs and transfer of the newly developed treatment into a GMP-enviroment have been addressed.
(b) This justification is further extended with additional arguments in the revised Appendix-experimental animal procedure no 4 as follows:
In the section of proposed animal procedures: OA tends to develop insidiously, sometimes without evidence of prior injury or a clear inflammatory component and is primarily driven by focal cartilage damage which increases in severity by biomechanical loading. This is best simulated in the [redacted] model. This model for OA closely resembles human and canine OA, as it does not involve ligament transection or injection of enzymes as done in small animal models, but is based on mechanical damage to the cartilage.
In section B. The animals: The [redacted] model for goats has been set up and validated (manuscript submitted) by the department of Rheumatology and department of Orthopaedics at the UMCU. The gross anatomy of the goat knee is fairly similar to both canines and humans, and in both canine and human patients the knee is the most frequently affected joint. Also in terms of function and biomechanics the goat knee joint resembles the human and canine knee joint more than rats. In horses, an accepted animal for research on regeneration of osteochondral defects, the metacarpophalangeal joint is studied, which is analogous to the human wrist with two layers of carpal bones. However, developing a [redacted]

model of the metacarpophalangeal joint in horses would be hampered by the limited access to the dorsal aspect of the metacarpophalangeal joint and the narrowed space between phalanx 1 (P1) and the metacarpus (MC3) and in addition, this is not the joint most affected in either canine or human patients. Although the goat is ruminant and hence not ideal for studying enteral therapies, this project concentrates on local delivery of medication and hence this is not an issue. Goat models have been used as cartilage regeneration models before by the research group. Due to the size, the joint of goats is easily accessible for surgery and multiple read out parameters can be obtained from one animal. Female animals will be used as they are easier to handle and house together. Goats older than 1 year, and hence skeletally mature, will be purchased from a registered Dutch breeder in order not to have issues with skeletal remodelling seen during growth. There is only limited evidence regarding the role of gender hormones in OA in large animal models.
Furthermore, in order to communicate clearly that after the studies in goats [redacted] model), studies in both canine and human patients with OA can be initiated, the "coherence of the project" within the main project proposal and the respective appendix has also been adjusted accordingly. This is done as follows within these sections:
…..As soon as safety and efficacy has been determined in the large animal models of OA and IVD, and release kinetics are comprehended, a first translational step will be undertaken in canine patients with OA or IVD disease within the 5-year period of the project. Likewise, a phase I clinical trial in humans will be feasible shortly after completion of the project, as soon as regulatory affairs and transfer of the newly developed treatment into a GMP-environment have been addressed…….

- Datum antwoord: 1-10-2015
- Strekking van het (de) antwoord(en):

In bijlage 2 is in overleg met de IvD de onderbouwing voor gebruik van vrouwelijke dieren verder toegelicht als volgt:
We prefer to use female rats for three reasons:
(1) In order to reduce the number of animals employed we will, where possible, combine the current animal procedure with animal procedure no. 1 (rat OA model) where female mice are employed so that they fit into the micro-CT scanner for analysis knee-bone pathology.
(2) Male rats can increase in size and gain a lot of weight during the longer term experiments with 6 months follow up.
(3) In order to reduce variability and make comparisons between experiments possible we will need to concentrate on female rats within this project proposal. Gender is expected not to influence the study. No effects have been described of progesterone on regeneration of tissue by NP cells and only two studies mention that estradiol can protect against induced cell death. As this is very limited and restricted to artificially induced cell death, and in human nor canine patients a connection between gender and disc degeneration was found, also at menopausal age, it is very unlikely that any effect of cycle will be evident.

Verder is bijlage 4 is de onderbouwing van gebruik van vrouwelijke of mannelijke honden ook aangescherpt als volgt:
Regarding the sex of animals: In chondrodystrophic dogs, like the experimental Beagle, there is no male/female predisposition for the development of disc disease in canine patients (Smolders et al 2013), hormonal effects are considered irrelevant to the model. Even more so, there is no gender predilection also for the human population of patients with IVD disease (Siemionow et al. 2011). In short term studies (range of 3 months) we aim at having a sex ratio of 1:1 in the study cohort in order to have both sexes represented in the study. In long term studies of > 6 months, we preferably work with male dogs in order not to have issues with the cyclus of female dogs. Female dogs have twice a year an oestrus cycle and when not used for breeding castration is advised in order to diminish the risk for development of breast cancer and other complications of irregularities of the cycle, including pseudopregnancy and pyometras, and accidental fertilization can not be excluded during long term housing of the animals. For this reason, female dogs when not used for breeding should be sterilized, which would add another experimental procedure with moderate severity.

References:

- Siemionow et al. The Effects of Age, Gender, Ethnicity, and Spinal Level on the Rate of Intervertebral Disc Degeneration. A review of 1712 Intervertebral Discs. Spine (Phila Pa 1976). 2011 Aug 1; 36(17): 1333–1339.
- Smolders LA et al. Intervertebral disc degeneration in the dog. Part 2: chondrodystrophic and nonchondrodystrophic breeds. Vet J. 2013 Mar;195(3):292-9.

- De antwoorden hebben geleid tot aanpassing van de aanvraag: Ja

9. Eventuele adviezen door experts (niet lid van de DEC)
   - Aard expertise:
   - Deskundigheid expert:
   - Datum verzoek:
   - Strekking van het verzoek:
   - Datum expert advies:
   - Expert advies:

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er zijn geen DEC-leden betrokken bij het betreffende project.

C. Beoordeling (inhoud):

1. Het project is:
   - ☒ uit wetenschappelijk oogpunt verantwoord.
   - ☐ uit onderwijskundig oogpunt verantwoord.
   - ☐ uit het oogpunt van productiedoeleinden verantwoord.
   - ☐ wettelijk vereist.

2. De in de aanvraag aangekruiste doelcategorie(ën) zijn / is in overeenstemming met de hoofddoelstelling(en).

3. De DEC onderschrijft het belang van de doelstelling. Het wordt ingeschat als een substantieel belang, omdat het onderzoek kan bijdragen aan het ontwikkelen en valideren van een nieuwe behandelmethode, waarbij via een injectie anti-inflammatoire medicijnen en/of biomaterialen gecontroleerd worden afgegeven in het gewricht, ter behandeling van artrose en chronische rugpijn bij mens en hond. De behandeling van patiënten met artrose of chronische rugpijn bestaat in eerste instantie uit het slikken van ontstekingsremmers. Deze medicijnen hebben echter, bij langdurig gebruik, meer risico op maagklachten, botontkalking of zelfs hartklachten. De enige andere optie is dan nog een operatie. Bij artrose patiënten wordt het versleten gewricht dan vervangen door een prothese en bij patiënten met chronische rugpijn wordt de versleten discus ofwel vervangen door een prothese of in zijn geheel weggehaald, waardoor de wervels aan elkaar groeien. Deze operaties gaan gepaard met een lange herstelperiode en in veel gevallen herstelt de patiënt niet volledig. Een alternatieve behandeling zou kunnen zijn om een medicijn te verpakken in een biomateriaal en in het versleten gewricht/discus te injecteren, waardoor het medicijn geleidelijk over langere tijd vrij komt, waardoor de ontsteking voor langere tijd geremd wordt en de afbraak van het weefsel van het gewricht of de discus wordt geremd. Door middel van deze deels translationele en deels fundamentele studie kan onderzocht worden of deze techniek een werkzame en veilig toepasbare techniek is en op termijn toepast kan worden in de kliniek bij de mens en de hond.

4. De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De DEC is ervan overtuigd dat de aanvrager over voldoende expertise en voorzieningen beschikt om de projectdoelstelling met de gekozen strategie/aanpak binnen de gevraagde termijn te realiseren.

5. Er is geen sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren:
   - ☐ Bedreigde diersoort(en) (10e lid 4)
   - ☐ Niet-menselijke primaten (10e)
   - ☐ Dieren in/uit het wild (10f)
   - ☐ Gefokt voor dierproeven (11)
   - ☐ Zwerfdieren (10h)

☐ Hergebruik (1e lid 2)
☐ Huisvesting en verzorging
☐ Locatie: instelling vergunninghouder (10g)
De keuze hiervoor is voldoende wetenschappelijk onderbouwd.

6. Het ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Het ongerief in de bijlagen als gevolg van de (be)handelingen in de bijlage varieert van licht tot matig ongerief. Cumulatief komt het ongerief in alle bijlagen op matig. In bijlage 1 is het cumulatieve ongerief matig o.a. als gevolg van het induceren van osteoartritis, het vooraf onderzoeken van het kniegewricht, de force plate metingen, het toedienen van biomaterialen via een injectie in de knie en het euthanaseren van de dieren. In bijlage 2 is het cumulatieve ongerief ingeschat als matig als gevolg van het induceren van degeneratie van de tussenwervelschrijf in de staart, het toedienen van injecties onder anesthesie en het euthanaseren van de dieren. Het cumulatieve ongerief in bijlage 3 is ingeschat als matig als gevolg van het induceren van osteoartritis, het toedienen van biomaterialen en het euthanaseren van de dieren. In bijlage 4 is het cumulatieve ongerief als gevolg van het induceren van degeneratie in meerdere tussenwervelschijven, het toedienen van injecties onder anesthesie en het euthanaseren van de dieren ingeschat als matig. En in de vijfde bijlage is het cumulatieve ongerief matig als gevolg van het meten van de kreupelheid, de force plate metingen, bloedafname, diverse injecties met biomaterialen en het euthanaseren van de dieren. De DEC is van mening dat deze ongeriefinschattingen realistisch zijn.

7. Er zijn geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen vervangen. In dit project wordt onderzocht hoe weefsels reageren op de biomaterialen en de langdurige afgifte van medicijnen. Omdat hiervoor de bloedtoevoer met afweercellen nodig is en activiteit van de lever (vanwege het afbreken en afvoeren van de biomaterialen), kan dit alleen in levende dieren onderzocht worden. De meeste proeven m.b.t. mogelijke toxiciteit en afgifte van de biomaterialen zijn vooraf in in vitro experimenten getest. Ten behoeve van de translatie is het bovendien vereist dat deze nieuwe behandeling getest wordt op proefdieren, eerst in kleine proefdieren, daarna op grote proefdieren.

8. In het project wordt optimaal tegemoet gekomen aan de vereiste van de vermindering van dierproeven. Het maximale aantal te gebruiken dieren is realistisch ingeschat. Voor het berekenen van het aantal benodigde dieren zijn statistische methoden toegepast.
Waar mogelijk worden voor verschillende vraagstellingen dezelfde dieren gebruikt en worden verfijnde laboratoriumtechnieken toegepast, waarmee per dier meer kennis verkregen kan worden. Doordat gebruik wordt gemaakt van MRI en röntgen doorlichting kunnen de processen in het levende dier in de tijd gevolgd worden. Daarnaast worden, indien mogelijk, meerdere discussen per dier (rug en staart) gebruikt i.p.v. slechts één discus, waardoor er minder dieren nodig zijn. Door gebruik te maken van een verfijnde post mortem analyse kunnen meerdere uitleesparameters van elk gewricht bestudeerd worden.

In bijlage 1 en 5 zullen alleen vrouwelijke ratten gebruikt worden omdat mannelijke ratten gedurende de studie en de follow up periode een stuk groter en zwaarder worden, waardoor de knie van mannelijke ratten niet in microCT scanner past. In bijlage 2 zullen eveneens alleen vrouwelijke ratten gebruikt worden. Indien mogelijk zal bijlage 2 gecombineerd worden met bijlage 1, zodat dezelfde dieren gebruikt kunnen worden, wat tot vermindering van het aantal benodigde dieren leidt. Daarnaast nemen mannelijke ratten gedurende de lange termijn experimenten en de follow up periode te veel in gewicht en omvang toe. Een derde reden voor het gebruik van vrouwelijke ratten is gelegen in het feit dat door het gebruik van vrouwelijke ratten de variatie kleiner wordt en het mogelijk is de verschillende experimenten met elkaar te vergelijken. In bijlage 3 zullen alleen vrouwelijke geiten gebruikt worden omdat vrouwelijke geiten beter hanteerbaar zijn en mannelijke geiten moeilijker gezamenlijk te huisvesten zijn, wat van negatieve invloed is op het ongerief voor de geit. En in bijlage 4 zullen voor de korte termijn studies (<3 maanden) vrouwelijke en mannelijke honden gebruikt worden. Voor de lange termijn studies (>6 maanden) worden mannelijke honden gebruikt vanwege de cyclus van vrouwelijke honden. Daarnaast wordt geadviseerd om vrouwelijke honden te steriliseren om de kans op borstkanker en andere onregelmatigheden van de cyclus te verkleinen, wat een extra experimentele handeling met matig ongerief betekend. Bovenstaande maakt dat de DEC van mening is dat het maximale aantal te gebruiken dieren realistisch is ingeschat en proportioneel is ten opzicht van de gekozen strategie en looptijd.

9. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven en het project is zo opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. Vanwege de aard van de experimenten zullen adequate pijnbestrijding en humane eindpunten worden toegepast. Om de stress van de ratten te beperken zullen de dieren in bijlage 1 en 5 eerst getraind worden, zodat zij gewend raken aan het hanteren ten behoeve van de bloedafname en de force plate metingen. In bijlagen 1, 2 en 5 worden ratten gebruikt omdat er reeds veel ervaring is met ratten in onderzoek naar artrose en discusslijtage. Hierdoor kunnen onderzoekers de beste doses bepalen en zijn er verfijnde technieken beschikbaar om de afgifte van medicijnen en slijtage te volgen in het levende dier. Geiten zullen in bijlage 3 worden gebruikt om de nieuwe behandelingen en medicijnen voor artrose verder te testen. De ratio voor het gebruik van geiten is gelegen in het feit dat het te gebruiken [redacted] model een bekend en gevalideerd model is en de knie van de geit in grote mate vergelijkbaar is met die van de mens en de hond. In bijlage 4 zullen honden gebruikt worden voor het verdere onderzoek naar discusslijtage. Met honden is veel ervaring in onderzoek naar discusslijtage. Daarnaast dient de hond als ‘doeldier’ omdat discusslijtage ook bij honden optreedt en deze vorm van slijtage vergelijkbaar is met de slijtage zoals die bij mensen voorkomt.

10. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd.

D. Ethische afweging

Op grond van de onder C, punt 3, genoemde overwegingen is de DEC van mening dat het belang van de doelstelling, namelijk het ontwikkelen en valideren van een nieuwe behandelmethode, waarbij via een injectie anti-inflammatoire medicijnen en/of biomaterialen gecontroleerd worden afgegeven in het gewricht, ter behandeling van artrose en chronische rugpijn bij mens en hond, substantieel is. Ten gevolge van de inductie van osteoartritis en degeneratie van de tussenwervelschrijf in de staart dan wel de rug treedt bij een aanzienlijk deel van de dieren matig ongerief op, maar de DEC is van mening dat voor de juiste onderzoeksstrategie gekozen is, en dat de verschillende (be)handelingen noodzakelijk zijn voor het bereiken van het gewenste doel. De DEC heeft gediscussieerd over het gebruik van het [redacted] model in de geit en is na overleg met de onderzoeker van mening dat het [redacted] model noodzakelijk is om de translatie van de rat naar de mens dan wel de hond te maken en de geit daarvoor het meest geschikte diermodel is.
Er is voldaan aan de vereisten van verfijning en vermindering. Het is nog niet mogelijk om dit onderzoek bij mensen uit te voeren, en er zijn evenmin in vitro of ex vivo alternatieven beschikbaar. De onderzoekers hebben goed beargumenteerd waarom zij in dit onderzoek alleen vrouwelijke ratten en geiten en vrouwelijke dan wel mannelijke honden willen gebruiken. Dit alles brengt de DEC tot het oordeel dat het belang van de doelstelling opweegt tegen het ten hoogste matige ongerief dat de dieren in dit project zullen ondervinden. Zij acht gebruik van de dieren ethisch aanvaardbaar.

E. Advies

1. Advies aan de CCD

   ☐ De DEC adviseert de vergunning niet te verlenen vanwege:

   ☐ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden

   ☒ De DEC adviseert de vergunning te verlenen.

2. Het uitgebrachte advies is gebaseerd op consensus.

11

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Utrecht
[redacted]
Postbus 12007
3501 AA UTRECHT

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD108002015282

Bijlagen
2

Datum 19 oktober 2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Geachte [redacted],
Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 13 oktober 2015.
Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD108002015282. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	10800
Naam instelling of organisatie:	Universiteit Utrecht
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	30275924
Postbus:	12007
Postcode en plaats:	3501AA UTRECHT
IBAN:	NL27INGB0000425267
Tenaamstelling van het rekeningnummer:	Universiteit Utrecht

Gegevens verantwoordelijke onderzoeker	
Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 september 2015
Geplande einddatum:	30 augustus 2020
Titel project:	Local controlled release of medication for the treatment of degenerative joint diseases
Titel niet-technische samenvatting:	Medicijnen voor plaatselijke behandeling van rugpijn en artrose
Naam DEC:	DEC Utrecht
Postadres DEC:	Postbus 85500 3508 GA Utrecht
E-mailadres DEC:	dec-utrecht@umcutrecht.nl

Betaalgegevens

De leges bedragen:	€ 741,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting
Overige bijlagen:	[x] DEC-advies

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Utrecht
Datum:	15 oktober 2015

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Utrecht
[redacted]
Postbus 80011
3508 TA UTRECHT

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD108002015282
Bijlagen
2

Datum 19 oktober 2015
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 19 oktober 2015
Vervaldatum: 18 november 2015
Factuurnummer: 15700282
Ordernummer: CB.841910.3.01.011

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD108002015282	€ 741,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL28RBOS 056.99.96.066 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 20401, 2500 EK te 's Gravenhage.

12

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Utrecht
t.a.v. Instantie voor Dierenwelzijn Utrecht
[redacted]

Centrale Commissie Dierproeven

Postbus 20401
2500 EK Den Haag
www.centralecommissiedierproeven.nl

T 0900-28 000 28 (10 ct /min)

info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD108002015282

Uw referentie

Bijlagen

Datum 2 november 2015
Betreft Aanvulling Aanvraag projectvergunning dierproeven

Geachte [redacted], leden van IvD Utrecht,

Op 13 oktober 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project 'local controlled release of medication for the treatment of degenerative joint disease' met aanvraagnummer AVD108002015282. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben.

Deze brief sturen wij u om enerzijds nadere toelichting te vragen over enkele inhoudelijke onduidelijkheden en anderzijds om met u van gedachte te wisselen over argumentatie die u in uw projectaanvraag aanvoert. Wij zouden graag zien dat u zich beperkt tot uitsluitend wetenschappelijk onderbouwde argumentatie van uw keuzes.

Wij willen u vragen om de volgende vragen inhoudelijk toe te lichten:

U beargumenteert in bijlage dierproeven 3.4.4.1 en 3.4.4.2 dat u vrouwelijke ratten in wilt zetten omdat mannelijke dieren gedurende het experiment teveel in gewicht toenemen om in de micro CT scan geplaatst te worden. Uit de tekst maken wij op dat dit probleem zich alleen voordoet bij de laatste post mortem meting (niet bij t=-4 en t=0). Kunt u meer uitwerken waarom het niet mogelijk is om, gezien dit een post mortem meting betreft alleen de achterpoot of het kniegewricht te scannen?

U beschrijft het individueel huisvesten van de geiten in bijlage 3.4.4.3, u beschrijft dat de dieren kreupel of licht verlamd zijn na de operatie en dat het individueel huisvesten nodig is om complicaties met wondgenezing te voorkomen. De door u beschreven kooiverrijking als autobanden en ballen lijken voor dieren met dergelijk letsel niet van nut in relatie met de beschreven klinische verschijnselen en dus niet voor verlichting van het ongerief van de individuele huisvesting te zorgen. Kunt u deze keuze nader toe lichten of eventueel andere keuzes beschrijven?

Kunt u nader toelichten hoe de dierproeven uit bijlage 3.4.4.5 in de strategie van het project zijn opgenomen? Uit het door u bijgevoegde schema lijkt het alsof u deze dierproeven uitvoert voor u aanvangt met de dierproeven uit de bijlagen 3.4.4.1 t/m 3.4.4.4. Uit de beschrijving van bijlage 3.4.4.5 menen wij op te maken dat u deze dierproeven uitvoert met componenten die succesvol zijn gebleken in de dierproeven uit de bijlagen 3.4.4.1 t/m 3.4.4.4. Graag zien wij dit beter uitgelegd.

In meer algemene termen zouden wij met u van gedachten willen wisselen over het onderstaande:
U voert in de bijlagen dierproeven op dat de controle door de onderzoeker en het wegen van de dieren wordt gezien als verrijking. Wij vragen ons af of dit voor ratten het geval is aangezien dit in de lichtperiode plaatsvindt en dus eerder als een verstoring kan worden gezien. Voor de geiten en honden kan dit gelden mits dit niet plaatsvind in de periode net na de operatie en het klinisch onderzoek mogelijk pijn veroorzaakt en u werkelijk tijd besteedt aan socialisatie van de dieren.
Wij willen u vragen deze generieke formulering in de bijlagen te heroverwegen en in elk geval per bijlage meer te specificeren of te verwijderen.

In bijlage dierproeven 3.4.4.4 beargumenteert u dat de voorkeur uitgaat naar mannelijke dieren voor de experimenten die langer dan 6 maanden duren omdat de cyclus van teven interfereert met de uitvoer van het experiment. U beschrijft daarna de noodzaak om teven te steriliseren om de ontwikkeling van borsttumoren en schijndracht te voorkomen. Gezien de looptijd van het experiment, de honden zijn bij aanvang van het experiment max. 3 jaar oud, lijkt het ontwikkelen van borsttumoren niet veelvoorkomend. Wij willen u vragen uw argumentatie van de noodzaak van steriliseren met bijkomend extra ongerief voor de teven te heroverwegen. Daarnaast is deze argumentatie is ons inziens niet van toepassing in uw overweging om alleen mannelijke dieren in te zetten. De noodzaak om enkel mannelijke of enkel vrouwelijke dieren in te zetten lijkt volgens uw beschrijving alleen een praktische achtergrond te hebben. Zou u willen toelichten of u kunt overwegen om voor de kortdurende experimenten (3 maanden) alleen vrouwelijke dieren in te zetten in plaats van 1:1 zoals nu beschreven en voor de experimenten die < 6 maanden duren mannelijke dieren in te zetten?

U beargumenteert in bijlage 3.4.4.1 en 3.4.4.2 dat de voorkeur uitgaat naar vrouwelijke ratten omdat deze makkelijker te hanteren zijn dan mannelijke ratten. Dit is een persoonlijke voorkeur en wij willen u vragen deze stelling te heroverwegen.

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Stuurt u het per post op, gebruik dan het formulier dat u bij deze brief krijgt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlage:
formulier Melding Bijlagen via de post

Centrale Commissie Dierproeven

Melding

Bijlagen via de post

- U wilt één of meerdere bijlagen naar ons versturen? Voeg *altijd* deze Melding Bijlagen toe. Wij weten dan welke documenten van u zijn en hoeveel documenten u opstuurt.
- Meer informatie vindt u op www.centralecommissiedierproeven.nl
- Of bel met ons: 0900 28 000 28 (10 ct/min).

1 Uw gegevens

1.1 Vul de gegevens in.

Naam aanvrager			
Postcode		Huisnummer	

1.2 Bij welke aanvraag hoort de bijlage?
Het aanvraagnummer staat in de brief of de ontvangstbevestiging.

Aanvraagnummer	

2 Bijlagen

2.1 Welke bijlagen stuurt u mee?
Vul de naam of omschrijving van de bijlage in.

☐	
☐	
☐	

3 Ondertekening

3.1 Onderteken het formulier en stuur het met alle bijlagen op naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Naam	
Datum	- - 20
Handtekening	

Betreft: response op vragen CCD over AVD108002015282

Utrecht, 11 november 2015

Geachte leden van de Centrale Commissie voor Dierproeven,

Op 2 november 2015 hebben wij uw response op een aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om het project 'Local controlled release of medication for the treatment of degenerative joint disease' met aanvraagnummer AVD108002015282. In uw brief bleek nadere toelichting nodig te zijn op enkele inhoudelijke onduidelijkheden. Er werd verzocht om van gedachten te wisselen over argumentatie die gebruikt werd in de aanvraag. Tevens heeft de DEC-Utrecht omtrent deze aanvraag ook een brief ontvangen waarin om nadere toelichting gevraagd werd.

Naar aanleiding van uw verzoek is een vergadering belegd waarbij de voorzitter van de DEC-Utrecht, de verantwoordelijke onderzoeker en de IvD-Utrecht aanwezig waren. Uw punten zijn uitvoerig besproken. Deze brief sturen wij u om enerzijds nadere toelichting te geven over de inhoudelijke onduidelijkheden en anderzijds om onze argumentatie uit te leggen. In de bijlage treft uw puntsgewijs de toelichting op de vragen die de CCD gesteld heeft. Hierin worden ook de bijbehorende wijzigingen in de formulieren aangegeven met rode tekst. Tevens zijn de gewijzigde documenten van de aanvraag bijgesloten.

Betreffende uw punt over het gebruik van mannelijke en vrouwelijke dieren in dierproeven zoals beschreven onder 3.4.4.4 in deze aanvraag (AVD108002015282): hierover is tijdens de vergadering gesproken. In projectvoorstel AVD108002015285 (ook onlangs bij de CCD ingediend) worden alleen vrouwelijke dieren ingezet waarbij stamcellen gebruikt worden in de regeneratieve strategie. Aangezien het geslacht geen invloed lijkt te hebben op het hebben van tussenwervelschijfslijtage, kunnen in de overige experimenten binnen het project AVD108002015285, dat parallel wordt uitgevoerd, meer mannelijke dieren ingezet worden. Hiermee kan de onderzoeker zorgen dat beide geslachten gebruikt worden en bij de leverancier geen overschot van mannelijke dieren ontstaat.

We hopen uw hiermee voldoende te hebben geïnformeerd,

Hoogachtend,

[redacted]

[redacted]

Utrecht University

[redacted]

Betreft: puntsgewijs response op vragen van aanvraag AVD108002015282

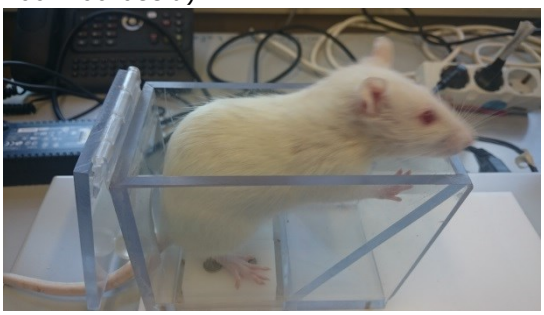
U beargumenteert in bijlage dierproeven 3.4.4.1 en 3.4.4.2 dat u vrouwelijke ratten in wilt zetten omdat mannelijke dieren gedurende het experiment teveel in gewicht toenemen om in de micro CT scan geplaatst te worden. Uit de tekst maken wij op dat dit probleem zich alleen voordoet bij de laatste post mortem meting (niet bij t=-4 en t=0). Kunt u meer uitwerken waarom het niet mogelijk is om, gezien dit een post mortem meting betreft alleen de achterpoot of het kniegewricht te scannen?

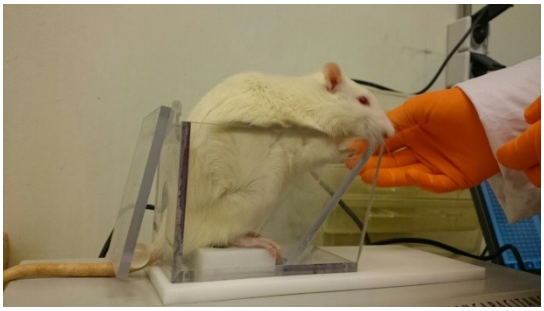
Dit project onderzoekt de locatie- en ziekte-afhankelijke afgifte van medicatie van het geladen biomateriaal. De lading van het biomateriaal met de specifieke factoren wordt bepaald op basis van geschatte volume van de locatie (in dit geval de kniegewricht/tussenwervelschijf) en het distributie volume van het dier zelf. Deze twee parameters zijn afhankelijk van de grootte/gewicht van het dier. Vrouwelijke en mannelijke ratten op dezelfde leeftijd hebben een duidelijk verschil in grootte/gewicht, en inherent eraan ook verschillen in volume van kniegewricht/tussenwervelschijf. Door beide geslachten in het onderzoek te gebruiken introduceren we extra variabelen (volume van knie/tussenwervelschijf en distributie volume van het dier) en daardoor een grotere standard deviatie in de primaire read out. Dit project heeft niet als doel het finetunen van een dosering op basis van volume locatie (knie/tussenwervelschijf) en gewicht van de patiënt. Voordat deze twee parameters onderzocht kunnen worden, moeten we eerst beter begrijpen hoe [redacted] op zichzelf invloed hebben op de afgifte van medicatie. **Door alleen een geslacht te gebruiken, beperken we de variabelen tot de dosering die geladen is op het afgifte systeem en locatie/ziekte. Hiermee kunnen we de vraagstelling van het project beantwoorden met een beperkt aantal dieren.**

In dierproef 3.4.4.1 gaat onze voorkeur uit naar vrouwelijke dieren omdat

(a) we reeds een experiment afgerond hebben met vrouwelijke ratten in een vergelijkbare proef zoals beschreven in 3.4.4.1. De studie is uitgevoerd om te kijken welke doseringsrange geschikt is voor twee anti-inflammatoire factoren [redacted] en [redacted]. De eerste proef binnen dit project aanvraag, is een vervolg studie waarbij we binnen de gekozen range van medicatie de dosering verder verfijnen en de bijeffecten onderzoeken. Om de nieuwe verkregen data goed te kunnen interpreteren is het van belang dat we gebruik kunnen maken van de reeds verkregen historische data.

(b) gedurende het onderzoek (ante mortem) worden dieren wekelijks onderzocht door middel van de "pressure plate" (Incapacitance meter). Hiermee kunnen we de gewichtsverdeling in de achterpoten onderzoeken. Dit is een objectieve maat van belasting van de behandelde achterpoot. Onze studie loopt door voor een periode van 12-30 weken na injectie om de lange termijn effecten te onderzoeken. Vrouwelijke dieren passen in dit onderzoekssysteem gedurende de gehele periode van het onderzoek, terwijl mannelijke dieren in de tweede helft van de periode (vanaf 12 weken en later) zodanig gegroeid zijn dat ze niet meer goed in de pressure plate opstelling passen en de meting onbetrouwbaar verloopt (zie figuur voor voorbeeld).





Pressure plate (Incapacitance meter): Links: een vrouwelijke rat die goed in de opstelling staat. Het is de bedoeling dat de rat met elke poot op een sensor staat en dat er verder geen gewicht op de sensor geplaatst wordt. Er is daarom ook een gat voor de staart in de achterkant, zodat het dier die niet op de sensoren kan positioneren. Rechts: Als een rat te groot is, dan zit hij klem in het bakje en zal hij of met zijn hele lijf op de sensoren rusten, of hij hangt als het ware tussen de zijkanten. Beide scenario's zorgen voor onbetrouwbare meetresultaten. Hier ziet u een mannelijke rat in het begin van de studie. Na 1,5 maand zijn ze ruim 100 gram zwaarder (van 430 naar 560 gram) en kan de meeting niet betrouwbaar uitgevoerd worden.

(c) micro-CT scan wordt in procedure 3.4.4.1 inderdaad post-mortem uitgevoerd. Direct na euthanasie en voordat rigor mortis optreed, worden de dieren in een liggende houding bevestigd met de knieën parallel aan elkaar, gestrekt en met de patella in de juist positie. Hiermee kunnen we op een gestandaardiseerde manier de achterpoten fixeren en scannen in de micro-CT. Door de gestandaardiseerde positionering bereiken we minder standard deviatie in de metingen van de microstructuren die onderzocht wordt door middel van de microCT. In een eerder experiment met mannetjesdieren hebben wij ondervonden dat 11 en 16 weken na injectie met microspheren niet meer mogelijk was deze dieren onder de micro-CT te leggen. Hoewel we in 3.4.4.1 van deze aanvraag uitgaan van metingen op t-4, t0 en t12-30 weken na injectie en dus alleen de poten van de dieren post mortem gescand zouden kunnen worden, lopen wij het risico bij het gebruik van mannetjes dat wanneer uit het dan lopende onderzoek blijkt dat het beter is een tussentijdstip te incorporeren, dit niet mogelijk zal zijn om longitudinaal uit te voeren in het zelfde dier. Zo'n overweging zouden we maken wanneer blijkt dat er verschillen de osteophyt formatie te zien zijn en dat osteophyt formatie zo uitgebreid is dat het ook intra-articulair te vinden is. In het laatste is het van belang dat we in een vroegere stadium kijken waar de osteophyt-formatie begint en of dit gerelateerd is aan de artrose of aan het intra-articulair injecteren van het biomateriaal+medicatie.
Indien we een eerder tijdpunt nemen en ook mannetjes in de studie hebben lopen, zijn we genoodzaakt om dubbel zo veel mannetjes gebruiken om post-mortem microCT scan te kunnen doen van de achterpoten. Een alternatief hierop is alsnog op alleen vrouwtjesdieren overgaan om minder dieren te gebruiken en longitudinaal de metingen te doen. Tevens, ivm verschillen in grote van dier (mannelijk vs vrouwelijk) en, inherent eraan, verschillen in volume van knie en distributie volume in het lichaam die invloed hebben op de gecontroleerde afgifte van medicatie, is het niet wenselijk om mannetjes te gebruiken voor vroege tijdstippen en vrouwtjes voor de latere tijdstippen van de studie. Dit aspect wordt nu beter uitgelegd in de aangepaste versie van 3.4.4.1 en 3.4.4.2 als volgt:

"Post mortem:
- In the initial studies microCT will be performed to assess radiologic changes in the knee joints at the end of the experiment (between 12-30 weeks after injection). Knee joints that have received a specific treatment will be compared to knee joints that have received a sham treatment. It may appear necessary to incorporate an earlier time point in the study to detect radiologic changes in the knee joint and define the initiating event. Ideally, these changes are followed up in a longitudinal manner. In that case, micro-CT scan will be conducted with the rats under anaesthesia to minimize the number of animals used. "

In dierproef 3.4.4.2 worden ook vrouwelijke dieren geprefereerd. In het kader van proefdiergebruik verminderen zijn er studies gepland waar dierproeven 3.4.4.1 en 3.4.4.2 gecombineerd worden in een en hetzelfde dier om hiermee dieren te verminderen zonder het ongerief toe te laten nemen. Een voorbeeld hiervan zijn de studies beschreven onder 3.4.4.5. Ook voor dierproef 3.4.4.2 is het van belang dat de staart uniform en gestandaardiseerd gepositioneerd wordt om de standard deviatie van de gemeten microstructuren (zoals discushoogte en trabekeldikte en -richting) te verminderen.

Dit wordt nu beter toegelicht in sectie A van 3.4.4.2 als volgt: "Post-mortem: Radiography and micro-computed tomography to evaluate the presence of extradiscal mineralization, one of the possible complications of intradiscal treatment with bioactive factors. Thereafter, rat tails will be harvested and IVDs will be accordingly processed for biochemical analyses of the tissues and/or after macroscopic evaluation will be fixed, decalcified, and thereafter subjected to histopathological evaluation (Boos scoring and immunohistochemical stainings). It may appear necessary to incorporate an earlier time point in the study to detect radiologic changes in the IVD and surrounding tissues and define the initiating event. Ideally, these changes are followed up in a longitudinal manner. In that case, micro-CT scan will be conducted with the rats under anaesthesia to minimize the number of animals used."

Sectie B van 3.4.4.2. is als volgt aangepast:
"We prefer to use female rats for three reasons:
(1) in order to reduce the number of animals employed we will, where possible, combine the current animal procedure with animal procedure no. 1 (rat OA model) where female mice are employed so that they fit into the micro-CT scanner and the incapacitance meter for analysis knee-bone pathology and for load distribution of the hind legs in the OA model, respectively.

(2) male rats can increase in size and gain a lot of weight during the longer term experiments with 6 months follow up and will not allow longitudinal measurements of the microstructures of the tail in a longitudinal manner.
(3) in order to reduce variability and make comparisons between experiments possible we will need to concentrate on female rats within this project proposal. Note that male and female rats differ in dimensions of their skeleton. The loading dose of the medication in the biomaterial platform that will effectuate controlled release of the medication is calculated based on an estimation of the volume of the IVD and the distribution volume of the total body. Given that the main aim of this project is dose finding and efficacy and is further exploring the effect of location (knie/IVD) and disease (healthy/degenerated), we concentrate on one gender to exclude the variable of volume distribution and achieve hereby reduction in the standard deviation of the primary read out parameters. The effect of volume distribution (local and total body) will be the topic of follow up projects that will concentrate on fine tuning the translation towards humans and dogs in the clinic."

U beschrijft het individueel huisvesten van de geiten in bijlage 3.4.4.3, u beschrijft dat de dieren kreupel of licht verlamd zijn na de operatie en dat het individueel huisvesten nodig is om complicaties met wondgenezing te voorkomen. De door u beschreven kooiverrijking als autobanden en ballen lijken voor dieren met dergelijk letsel niet van nut in relatie met de beschreven klinische verschijnselen en dus niet voor verlichting van het ongerief van de individuele huisvesting te zorgen. Kunt u deze keuze nader toe lichten of eventueel andere keuzes beschrijven?
In bijlage 3.4.4.3 beschrijven we dat dieren kreupel kunnen zijn (=lameness) en niet dat ze licht verlamd zijn na de operatie. Inderdaad, in de periode direct na operatie (inductie van artrose) zal de kooiverrijking niet aangereikt worden. Nadat de hechtingen zijn verwijderd, dat wil zeggen 7 dagen na de operatie, zal de kooiverrijking zoals beschreven aangereikt worden en functioneel kunnen zijn.

Het text in "sectie D' is aangepast als volgt: "….They are only housed separately immediately after induction of the OA and enrichment, including car tires and balls, will be withheld from them, in order to prevent possible wound healing complications. Furthermore, except for the immediate post-operative period of 7 days, animals are given toys as enrichment, including licking stones, car tires, and balls. ~~Animals are often examined by the researcher, which does not cause any pain but is seen as enrichment.~~ All intraarticular injections will be done by a veterinarian ~~diplomate of the European College of Veterinary Surgeons~~ with expertise in orthopaedics."

The sentence "Animals are often examined by the researcher, which does not cause any pain but is seen as enrichment" is verwijderd aangezien we de geiten na inductie van artrose alleen onderzoeken in het kader van gezondheids- and pijnmanagement.

Het tekst in "sectie I" is aangepast als volgt:
Describe which other adverse effects on the animal's welfare may be expected: "In the goat, unilateral OA is induced and hence moderate unilateral lameness is expected to occur. Immediately after induction of OA, animals will receive pijn medication over the course of the 1st week for pain relief and enrichment is temporarily removed (car tires and balls are removed for a maximum of 7 days; licking stones remain in place). Other adverse effects that may occur is delayed or complicated wound healing."

Kunt u nader toelichten hoe de dierproeven uit bijlage 3.4.4.5 in de strategie van het project zijn opgenomen? Uit het door u bijgevoegde schema lijkt het alsof u deze dierproeven uitvoert voor u aanvangt met de dierproeven uit de bijlagen 3.4.4.1 t/m 3.4.4.4. Uit de beschrijving van bijlage 3.4.4.5 menen wij op te maken dat u deze dierproeven uitvoert met componenten die succesvol zijn gebleken in de dierproeven uit de bijlagen 3.4.4.1 t/m 3.4.4.4. Graag zien wij dit beter uitgelegd.
De dierproeven in bijlage 3.4.4.5 zijn onderdeel van Objective 3 waar we onderzoek doen naar het effect van locatie en ziekte op de kinetiek van afgifte van verscheidene biomaterial-platformen. Hiermee kunnen we beter begrepen hoe lokatie (bv knie of tussenwervelschijf) en hoe ziekte (gezond vs verslijten) invloed hebben op de afgifte. Dit zal ons helpen bij het verder verfijnen van de systemen voor verder gebruik bij regeneratieve geneeskunde. Dit objective bevat een *in vitro* gedeelte dat niet verbonden is in de tijd met de *in vivo* studies beschreven onder Objective 1 & 2. Om diergebruik te verminderen, hebben we gekozen om eerst binnen Objective 1 te kijken naar de optimale dosering die een positief effect heeft op artrose en tussenwervelschijfslijtage en daarna

deze dosering te gebruiken om het effect van locatie en ziekte verder te onderzoeken. In de Appendix van het project voorstel, de pijl tussen Objective 3 "Biomaterial release kinetics & degradation" en Objective 1&2 illustreert niet een "tijds-afhankelijkheid". De pijl illustreert dat dit objective inhoudelijk zal bijdragen aan het verfijnen van de efficacy studies bij de grote diermodellen en klinische patiënten.

Om dit helderder te communiceren is de legenda van het schema aangepast als volgt: "In addition, general information on the aspects of local controlled release will be answered by studying release by biomaterials loaded with labels at different body locations and disease stages ("Biomaterial release kinetics & degradation"). These studies will help fine tune the delivery systems for the efficacy studies in large animal models and clinical patients".

In meer algemene termen zouden wij met u van gedachten willen wisselen over het onderstaande: U voert in de bijlagen dierproeven op dat de controle door de onderzoeker en het wegen van de dieren wordt gezien als verrijking. Wij vragen ons af of dit voor ratten het geval is aangezien dit in de lichtperiode plaatsvindt en dus eerder als een verstoring kan worden gezien. Voor de geiten en honden kan dit gelden mits dit niet plaatsvind in de periode net na de operatie en het klinisch onderzoek mogelijk pijn veroorzaakt en u werkelijk tijd besteedt aan socialisatie van de dieren.
Wij willen u vragen deze generieke formulering in de bijlagen te heroverwegen en in elk geval per bijlage meer te specificeren of te verwijderen.

Ratten: De onderzoekers zullen in de periode voorafgaand aan het experiment de dieren habitueren aan de handelingen die ze ondergaan tijdens de studie (wegen en pressure plate) zodat de dieren minder stress ervaren tijdens de studie en de metingen betrouwbaar verlopen. Na inductie van artrose of tussenwervelschijfslijtage zullen de handelingen bij de ratten zich beperken tot handelingen die gerelateerd zijn aan de read out parameters van de onderzoeksvraagstelling.

Geiten & Honden: Inderdaad de onderzoekers werken aan socialisatie van de dieren met de mens gedurende het gehele onderzoek. Dit begint voorzichtig al tijdens de eerste twee weken van acclimatisatie, zodat de dieren minder stress ervaren gedurende het onderzoek. Rondom de direct postoperatieve periode (7 dagen na inductie van slijtage knie of tussenwervelschijf) worden deze handelingen beperkt tot wat strikt noodzakelijk is voor de gezondheid van het proefdier om onnodig ongerief te voorkomen.

Dit aspect is verder gespecificeerd en aangepast als volgt:
3.4.4.1: "Animals are allowed to accommodate to their new environment for at least 1 week, they are housed in groups. They will be trained prior to the experiment to get used to handling, weighing, fixation for blood collection (in a so-called blood collection 'sleeve') and pressure plate measurements. The first week after OA induction, the rats will be checked daily and if necessary, extra analgesia will be given animal handling is limited to the necessary handling in relation to health and pain management. ~~Furthermore, animals are given cage enrichment and the animals are often examined and weighed by the researcher, which does not cause any pain but is seen as enrichment.~~"

3.4.4.2: "Animals are allowed to accommodate to their new environment for at least 1 week, they are housed in groups, and only housed separately for one day post-induction of disc degeneration and post-treatment in order to prevent possible wound healing complications. Furthermore, animals are given cage enrichment ~~and the animals are often examined and weighed by the researcher, which does not cause any pain but is seen as enrichment~~. In order to decrease the stress levels of the rats, they will be trained to get used to handling and blood collection. Moreover, the rats receive proper analgesia after induction of IVD degeneration, to inhibit acute pain. If necessary, analgesia will be continued. During the experiment animal handling is limited to the necessary handling in relation to health and pain management. Supervision of surgeries and postoperative care, anesthesia, postoperative analgesia, and imaging are performed by European board-certified veterinary specialists in surgery (ECVS), anesthesia (ECVA), and diagnostic imaging (ECVDI). The body weight of the rats will be recorded weekly to obtain an impression of the overall health and wellbeing of the animal."

3.4.4.3: "Animals are allowed to accommodate to their new environment for at least 2 weeks, they are housed in groups, freely walking in pens of approximately 20 square meters. There will be no dietary restrictions and the goats will have access to water ad libitum. They are only housed separately immediately after induction of the OA and enrichment, including car tires and balls withheld from them, in order to prevent possible wound healing complications. Furthermore, except

for the immediate post-operative period of 7 days, animals are given toys as enrichment, including licking stones, car tires, and balls. ~~Animals are often examined by the researcher, which does not cause any pain but is seen as enrichment.~~ All injections will be done by a veterinarian diplomate of the European College of Veterinary Surgeons with expertise in orthopedics. Except for the post-operative period of 7 days after induction of OA, animal care-takers and the researcher spend time with the animals during general, orthopaedic examination, and weighing; this helps the animals to socialize and experience less stress during the regular follow-ups throughout the study. During the direct-post-operative period animal handling is limited to the necessary handling in relation to health and pain management"

3.4.4.4: "Animals are allowed to accommodate to their new environment for at least 1 week, they are housed in groups, and only housed separately for one week post-induction of disc degeneration and post-treatment in order to prevent possible wound healing complications. Furthermore, except for the direct post-operative period of 7 days, animals are given toys as enrichment and are allowed to be for at least one hour outside their wards in order to play. Furthermore, animals are often examined by the researcher, which does not cause any pain but is seen as enrichment. Specifically for the direct post-operative period, animal handling is limited to the necessary handling in relation to health and pain management. Supervision of surgeries and postoperative care, anesthesia, postoperative analgesia, imaging and force plate analysis are performed by European board-certified veterinary specialists in surgery (ECVS), neurology (ECVN), anesthesia (ECVA), and diagnostic imaging (ECVDI)."

3.4.4.5: "Animals are allowed to accommodate to their new environment for at least 1 week, they are housed in groups. They will be trained prior to the experiment to get used to handling, fixation for blood collection (in a so-called blood collection 'sleeve') and pressure plate measurements.

Furthermore, animals are given cage enrichment. During the experiment animal handling is limited to the necessary handling in relation to health and pain management. ~~and the animals are often examined and weighed by the researcher, which does not cause any pain but is seen as enrichment.~~"

In bijlage dierproeven 3.4.4.4 beargumenteert u dat de voorkeur uitgaat naar mannelijke dieren voor de experimenten die langer dan 6 maanden duren omdat de cyclus van teven interfereert met de uitvoer van het experiment. U beschrijft daarna de noodzaak om teven te steriliseren om de ontwikkeling van borsttumoren en schijndracht te voorkomen. Gezien de looptijd van het experiment, de honden zijn bij aanvang van het experiment max. 3 jaar oud, lijkt het ontwikkelen van borsttumoren niet veelvoorkomend. Wij willen u vragen uw argumentatie van de noodzaak van steriliseren met bijkomend extra ongerief voor de teven te heroverwegen. Daarnaast is deze argumentatie is ons inziens niet van toepassing in uw overweging om alleen mannelijke dieren in te zetten. De noodzaak om enkel mannelijke of enkel vrouwelijke dieren in te zetten lijkt volgens uw beschrijving alleen een praktische achtergrond te hebben.
Inderdaad worden schijndracht, pyometra en borsttumoren voornamelijk gezien bij dieren ouder dan 6 jaar en de kans dat vrouwelijke intacte dieren rond de leeftijd van 3 jaar dit soort klachten krijgen is klein. Daarbij zijn er ook praktische overwegingen bij het inzetten van enkel vrouwelijke of mannelijke dieren en is er geen wetenschappelijke achtergrond; tussenwervelschijfslijtage wordt niet beïnvloedt door geslacht. De onderzoeker wil hier graag nog het volgende onder de aandacht brengen: een gemiddelde studie met 6 honden waar alle read out parameters verzameld worden kost ~90.000€. Hierbij is het van belang dat het onderzoek niet het risico van complicaties loopt. Daarom worden ook praktische aspecten overwogen in de keuze van het geslacht bij lange termijn studies.

Onder B, is formulier 3.4.4.4 als volgt aangepast:
"In short term studies (range of 3 months) we aim at having a sex ratio of 1:1 ~~in the study cohort~~ in order to have both sexes represented in the project. In long term studies of > 6 months, we preferably work with male dogs in order not to have issues with the cyclus of female dogs. Female dogs have twice a year an oestrus cycle and when not used for breeding castration is adviced in order to diminish the risk for development of breast cancer and other complications of irregularities of the cycle, including pseudopregnancy and pyometras, and accidental fertilisation can not be excluded during long term housing of the animals. ~~For this reason, female dogs when not used for breeding should be sterilised, which would add another experimental procedure with moderate severity.~~ "

Zou u willen toelichten of u kunt overwegen om voor de kortdurende experimenten (3 maanden) alleen vrouwelijke dieren in te zetten in plaats van 1:1 zoals nu beschreven en voor de experimenten die < 6 maanden duren mannelijke dieren in te zetten?

Mannelijke dieren zijn groter, hebben grotere tussenwervelschijven dan vrouwelijke dieren. Indien beide geslachten in een proef genomen worden, zorgt dit voor toename van het aantal variabelen en daarmee toename van de standard deviatie van de primaire read out parameters: het volume van de tussenwervelschijf en het distributie volume van de medicatie is afhankelijk van de grootte/gewicht van het dier. Inherent hieraan, zullen deze variabelen invloed hebben op het profiel van afgifte van de medicatie vanuit de biomateriaal platform en het lokale effect op weefsel niveau. Het doel van dit project is om de juiste dosering te identificeren en te begrijpen hoe het ziekte proces invloed heeft op de afgifte en indirect dus ook effect heeft op weefsel niveau. Om deze reden, is het in een longitudinale studie onwenselijk om voor het eerste meetpunt vrouwelijke dieren te gebruiken en voor tweede meetpunt mannelijke dieren. Deze zullen niet met elkaar te vergelijken zijn en daarnaast zal de wetenschappelijke output niet te publiceren zijn. Dit laatste is ook een aandachtspunt binnen de ethische afweging. Daarom voor korte termijn studies zullen we met 1:1 verhouding werken waar bv vrouwelijke dieren voor medicatie A gebruikt worden en mannelijke dieren voor medicatie B. Hiermee kunnen we wel binnen de vrouwelijke dieren de dose response onderzoeken voor medicatie A; hetzelfde geldt voor medicatie B. Medicatie A en B worden niet met elkaar vergelijken.

U beargumenteert in bijlage 3.4.4.1 en 3.4.4.2 dat de voorkeur uitgaat naar vrouwelijke ratten omdat deze makkelijker te hanteren zijn dan mannelijke ratten. Dit is een persoonlijke voorkeur en wij willen u vragen deze stelling te heroverwegen.

Deze stelling is verwijderd uit het project voorstel.

Project: 2015.II.813.025

Title: Local controlled release of medication for the treatment of degenerative joint diseases

Appendix: Coherence of the project

This project pursues biological repair of the diseased joint and IVD by combing both translation and basic research. The coherence between the different components and the different steps of the projects is illustrated in the figure.

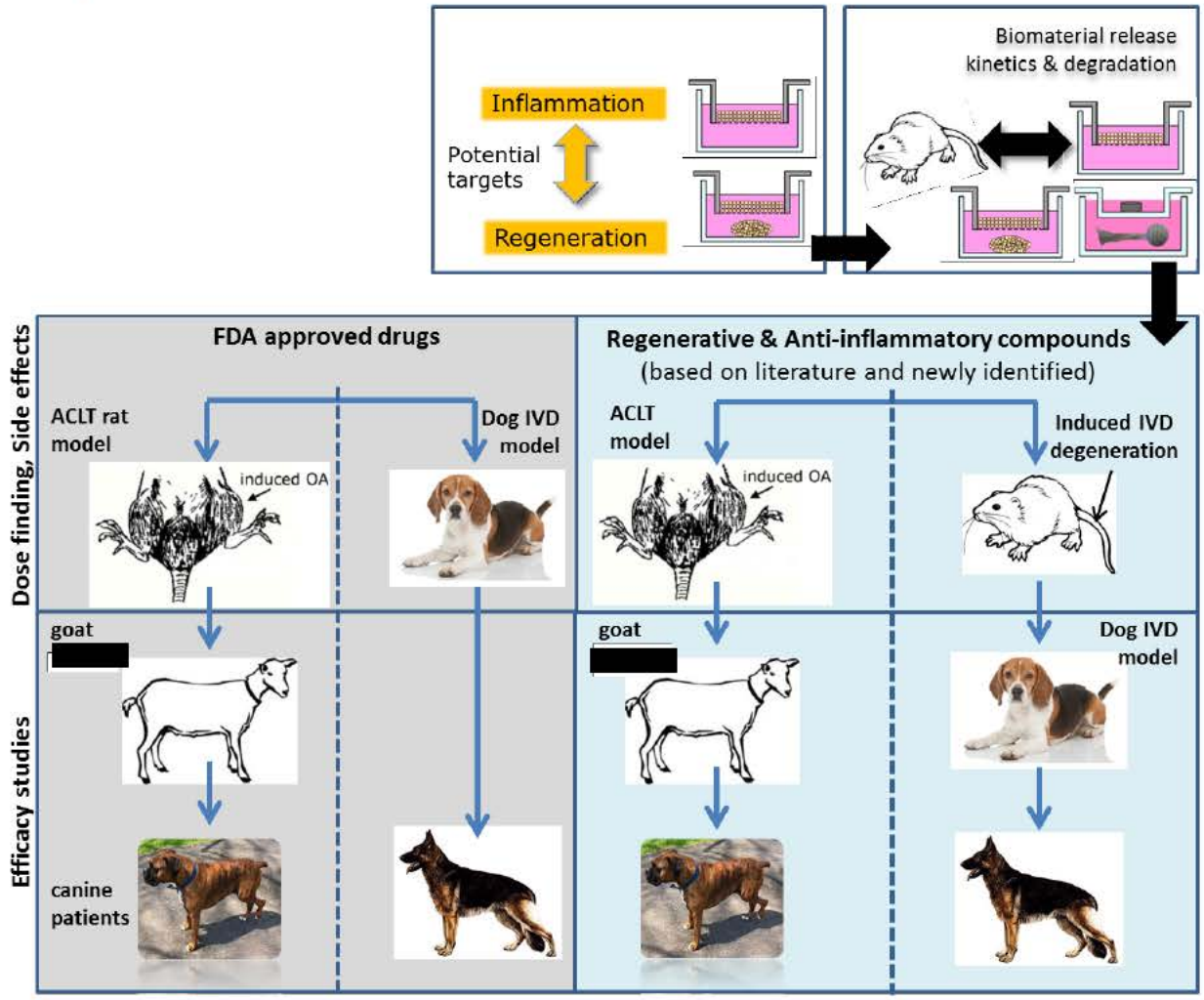


Figure legend: Animal studies largely consist of two distinct stages;
1) in small animals where proof of principle and therapeutic window (safety and optimal effectivity) will be determined and 2) large animals, where the effects in large and more clinically relevant models are being studied. As soon as safety and efficacy has been determined in the large animal models of OA and IVD, and release kinetics are comprehended, a first translational step will be undertaken in canine patients with OA or IVD disease within the 5-year period of the project. Likewise, a phase I clinical trial in humans will be feasible shortly after completion of the project, as soon as regulatory affairs and transfer of the newly developed treatment into a GMP-environment have been adressed

Therapeutic compounds that will be applied in animal study stages will have followed three different preceding trajectories;
1) FDA-approved drugs that have already been shown to be active in the clinic will directly enter the first stage, 2) compounds that have shown proof of principle in literature and
3) newly identified targets in the projects' fundamental research part, after validation in vitro of effectivity and optimal release profiles.

In addition, general information on the aspects of local controlled release will be answered by studying release by biomaterials loaded with labels at [redacted] and [redacted] ("Biomaterial release kinetics & degradation"). These studies will help fine tune the delivery systems for the efficacy studies in large animal models and clinical patients

References used in the project proposal

Bellamy N, Campbell J, Welch V, Tl G, Bourne R, Ga W (2009) Intraarticular corticosteroid for treatment of osteoarthritis of the knee. The Cochrane Library. 2; 1-120

Blanquer SB, Sharifi S, Grijpma DW (2012) Development of poly(trimethylene carbonate) network implants for annulus fibrosus tissue engineering. J. Appl. Biomater. Funct. Mater. **10**: 177–184.

Gawri R, Antoniou J, Ouellet J, Awwad W, Steffen T, Roughley P, Haglund L, Mwale F, Hospital RV, Scoliosis M, Arabia S (2013) BEST PAPER NASS 2013 : LINK-N CAN STIMULATE PROTEOGLYCAN SYNTHESIS.

Hamilton DF, Howie CR, Burnett R, Simpson a HRW, Patton JT (2015) Dealing with the predicted increase in demand for revision total knee arthroplasty: challenges, risks and opportunities. Bone Joint J. **97-B**: 723–728.

Higashino K, Hamasaki T, Kim JH, Okada M, Yoon ST, Boden SD, Hutton WC (2010) Do the adjacent level intervertebral discs degenerate after a lumbar spinal fusion? An experimental study using a rabbit model. Spine (Phila. Pa. 1976). **35**: E1144–52.

Imai Y, Okuma M, An HS, Nakagawa K, Yamada M, Muehleman C, Thonar E, Masuda K (2007) Restoration of disc height loss by recombinant human osteogenic protein-1 injection into intervertebral discs undergoing degeneration induced by an intradiscal injection of chondroitinase ABC. Spine (Phila. Pa. 1976). **32**: 1197–205.

Jacobs WCH, Rubinstein SM, Koes B, van Tulder MW, Peul WC (2013) Evidence for surgery in degenerative lumbar spine disorders. Best Pract. Res. Clin. Rheumatol. **27**: 673–84.

Johnson K, Zhu S, Tremblay MS, Payette JN, Wang J, Bouchez LC, Meeusen S, Althage A, Cho CY, Wu X, Schultz PG (2012) A stem cell-based approach to cartilage repair. Science **336**: 717–21.

Lam J, Lu S, Kasper FK, Mikos AG (2015) Strategies for controlled delivery of biologics for cartilage repair. Adv. Drug Deliv. Rev. **84**: 123–34.

Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet **380**: 2197–223.

Pavlides S, Gutierrez-Pajares JL, Katiyar S, Jasmin J-F, Mercier I, Walters R, Pavlides C, Pestell RG, Lisanti MP, Frank PG (2014) Caveolin-1 regulates the anti-atherogenic properties of macrophages. Cell Tissue Res. **358**: 821–31.

Pereira CL, Gonçalves RM, Peroglio M, Pattappa G, D'Este M, Eglin D, Barbosa M a, Alini M, Grad S (2014) The effect of hyaluronan-based delivery of stromal cell-derived factor-1 on the recruitment of MSCs in degenerating intervertebral discs. Biomaterials **35**: 8144–53.

Seibel MJ, Cooper MS, Zhou H (2013) Glucocorticoid-induced osteoporosis: mechanisms, management, and future perspectives. lancet. Diabetes Endocrinol. **1**: 59–70.

Thavaneswaran P, Vandepeer M (2014) Lumbar artificial intervertebral disc replacement: a systematic review. ANZ J. Surg. **84**: 121–7.

Trampuz A. & Widmer AF (2006) Infections associated with orthopedic implants. Curr. Opin. Infect. Dis. **19**, 349–56

[redacted]

Van: [redacted]
Verzonden: dinsdag 10 november 2015 22:04
Aan: info@zbo-ccd.nl
Onderwerp: Fwd: aanhouden beoordelen AVD108002015280
Bijlagen: AVD108002015282 aanhouden beoordelen.doc; ATT00001.htm

Categorieën: Dossier [redacted]

Geachte [redacted]

Dezer dagen hebt U het antwoord ontvangen van de onderzoeker op de vragen door U gesteld over boven genoemd project. U vraagt in uw brief ook naar de visie van de DEC Utrecht en ik wil daar gaarne kort op ingaan .
De door U genoemde punten zijn grotendeels in de bespreking van dit project in de DEC vergadering aan de orde geweest en hebben niet geleid tot vragen omdat deze punten niet van direct belang bleken voor de ethische afweging . Daar moet wel een kanttekening bij gemaakt worden want het niet stellen van vragen over een aantal van de door U genoemde punten heeft ook te maken met het feit dat het hier gaat om lopend onderzoek waarvan de DEC reeds in eerdere projecten kennis had genomen. De onderzoeker heeft dat in haar antwoord aan U , waar ze ook ingaat op een aantal aan de DEC bekende praktische redenen voor de keuzes die gemaakt zijn , ook aangegeven.

Met vriendelijke groet ,

[redacted]

Van: Info-zbo [mailto:info@zbo-ccd.nl]
Verzonden: maandag 2 november 2015 14:42
Aan: dec-utrecht
Onderwerp: aanhouden beoordelen AVD108002015280

Geachte leden van DEC Utrecht,

Op 13 oktober hebben wij een aanvraag tot projectvergunning ontvangen waarover uw DEC advies heeft uitgebracht. Het betreft het project AVD108002015282 getiteld: Local controlled release of medication for the treatment of degenerative joint disease.

Wij willen de onderzoeker om toelichting vragen over een aantal onduidelijkheden. Daarnaast onderbouwt de onderzoeker een aantal keuzes met argumentatie waarvoor geen wetenschappelijke onderbouwing is. Wij willen de onderzoeker vragen om nadere wetenschappelijke onderbouwing voor deze argumentatie.

Graag horen we ook de visie van de DEC hoe in het adviseringstraject hier tegenaan is gekeken. En of u dit in uw discussie heeft betrokken.

Met vriendelijke groet, [redacted]

Centrale Commissie Dierproeven

www.centralecommissiedierproeven.nl

.....

Postbus 20401 | 2500 EK | Den Haag

.....

T: 0900 2800028

E: info@zbo-ccd.nl (let op: nieuw emailadres!)

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Utrecht
[redacted]
Tav. Instantie voor Dierenwelzijn
Postbus 12007
3501 AA UTRECHT

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
www.centralecommissiedierproeven.nl
T 0900-28 000 28 (10 ct /min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD108002015282

Uw referentie

Bijlagen
1

Datum: 25 NOV. 2015
Betreft Beslissing Aanvraag projectvergunning dierproeven

Geachte [redacted]

Op 13 oktober 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Local controlled release of medication for the treatment of degenerative joint diseases" met aanvraagnummer AVD108002015282. Wij hebben uw aanvraag beoordeeld.

U heeft uw aanvraag aangevuld na vragen van de CCD op 13 november 2015. In een brief heeft u de vragen beantwoord en de bijlagen dierproeven heeft u op 13 november 2015 aangepast en opnieuw ingediend.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning. Deze voorwaarden zijn algemene voorwaarden die de CCD stelt bij meerjarige projecten om te voldoen aan datgene wat voortvloeit uit artikel 10 van de wet.
U kunt met uw project "Local controlled release of medication for the treatment of degenerative joint diseases" starten. De vergunning wordt afgegeven van 1 december 2015 tot en met 30 augustus 2020. De startdatum wijkt af van uw aanvraag omdat deze in het verleden ligt.
Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC Utrecht gevoegd. Dit advies is opgesteld op 12 oktober 2015. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Wij hebben de DEC om nadere toelichting gevraagd en hebben op 10 november 2015 antwoord van de DEC ontvangen. Wij nemen dit advies van de commissie over, inclusief de daaraan ten grondslag liggende motivering. Aangevuld met de bovengenoemde algemene voorwaarden.
Dit advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze gegevens in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

De Centrale Commissie Dierproeven
namens deze:

[redacted]

Ir. G. de Peuter
Algemeen Secretaris

Dit besluit is genomen met inachtneming van het Besluit mandaat, volmacht en machtiging van de Centrale Commissie Dierproeven CCD 2014 zoals de Centrale Commissie Dierproeven heeft vastgesteld op 19 december 2014, ref 2014-04 en is gepubliceerd in de Staatscourant van 2 januari 2015, Nr. 163

Bijlagen
- Vergunning
  Hiervan deel uitmakend: - DEC-advies
  - Weergave wet- en regelgeving

Projectvergunning

gelet op artikel 10a van de Wet op de dierproeven

Verleent de Centrale Commissie Dierproeven aan
Naam: Universiteit Utrecht
Adres: postbus 12007
Postcode en woonplaats: 3501AA Utrecht
Deelnemersnummer: 10800

deze projectvergunning voor het tijdvak 01 december 2015 tot en met 30 augustus 2020, voor het project "Local controlled release of medication for the treatment of degenerative joint diseases" met aanvraagnummer AVD108002015282, volgens advies van Dierexperimentencommissie DEC Utrecht.
De functie van de verantwoordelijk onderzoeker is [redacted]

De aanvraag omvat de volgende bescheiden:

1. een aanvraagformulier projectvergunning dierproeven, ontvangen op 13 oktober 2015
2. de bij het aanvraagformulier behorende bijlagen:
    a. Projectvoorstel, zoals ontvangen bij digitale indiening op 13 oktober 2015;
    b. Niet-technische Samenvatting van het project, zoals ontvangen bij digitale indiening op 13 oktober 2015;
    c. Advies van Dierexperimentencommissie dd 12 oktober 2015, ontvangen op 13 oktober 2015.
    d. De aanvullingen: aangepaste bijlagen dierproeven, ontvangen op 13 november 2015

Dierproeven

Naam dierproef	Diersoort	Aantal dieren	Ernst
Rat model for mild osteoarthritis	Ratten (Rattus norvegicus) /	132	Matig/ moderate
Rat model of induced IVD degeneration	Ratten (Rattus norvegicus)	110	Matig/ moderate
[redacted] model for the induction of mild osteoarthritis (goat)	Geiten (Capra aegagrus hircus)	48	Matig/ moderate
Canine model of IVD degeneration	Honden (Canis familiaris) / beagle	28	Matig/ moderate
Degradation of biomaterial and release kinetics	Ratten (Rattus norvegicus)	86	Matig/ moderate

Op grond van artikel 10a1 lid 2 Wet zijn aan een projectvergunning voorwaarden te stellen
De vergunning wordt verleend onder de voorwaarde dat eventuele go/no go beslissingen worden genomen met instemming van de IvD.
In artikel 10, lid 1a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is.
Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning.

Indien bovenstaande zich voordoet dient aanvrager dit in overleg met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarden wijzigen of intrekken.

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister van Economische Zaken een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade

zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13c volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13d is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.

Levensloopdossier
Voor iedere hond, kat en niet-menselijke primaat moet volgens artikel 15a van de wet een levensloopdossier bijgehouden worden.