Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-101

Ontvangen: 09-08-2011

DEC datum goedkeuring#	Type aanvraag [2]
26-08-2011	Nieuw

VROM/GGONR[3]

LNV/CBDNR[4]

Hoofdproject	CARIM	NUTRIM	Hersenen en gedrag	GROW	biomaterialen	Ander UM *CAPHRI*	Geen UM
Deelproject	1. 2. 3.	1. 2. 3. 4.	1. 2. 3.	1. 2. 3.			

Financieel beheerder	

Budgetnummer	*Not allocated yet*

Titel van het onderzoek:

Assessment of) incorporation and remodeling in an unloaded critical sized distal femoral condyle model in NZW rabbits.

startdatum ***01/09/2011*** einddatum [9] ***01/09/2012*** *Duur van de proef [10]:* *56 dagen*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegd-heid[5]	Cap. groep /afdeling
1. Verantwoordelijk onderzoeker (VO)				*Art.9*	
2. Vervanger VO 1 (VVO)				*Art.9*	
Vervanger VO 2 (VVO)				*Art.9*	
3. Verantwoordelijk medewerker (VM) GGO[7]					
4. overige uitvoerenden					
5.					

Diergroep	1	2	3	4			
ctrl/exp/sham	*Exp*						
Diersoort	*Konijn*						
Stam	*NZW*						
Construct / mutatie ?	-						
Herkomst (leverancier) *	*Harlan*						
Aantal	*39*						
Geslacht	*Vrouwelijk*						
Dieren immuuncompetent ?	*ja*						
Leeftijd/gewicht	*> 6 mnd*						
Doel van de proef *	*05*						
Belang van de proef *	*01*						
Toxicologisch onderzoek *	*01*						
Bijzondere technieken *	*01*						
Anesthesie *	*04*						
Pijnbestrijding *	*04*						
Mate ongerief *	*04*						
Toestand dier einde exp*	*01*						

* VHI-coderingen ~~zie~~ bijlage

1 Verantwoording

Aanvraag dierproef DEC-UM (kaders zijn licht flexibel, maar het geheel is max. 5 pag. versie 2006)

Titel: Assessment of) incorporation and remodeling in an unloaded critical sized distal femoral condyle model in NZW rabbits.

1. Doel van de proef. Version 8: Reply after wijzigingsbrief DEC 04-08-2011 CA, PE, JO

Total joint replacement due to serious degeneration of hip and knee joints is a very successful medical intervention of which roughly 18000 are performed yearly in the Netherlands. Over time a small number of these implants will fail, which is most often accompanied by severe bone loss around the implanted prosthesis. These bone defects make fixation of the newly implanted prosthesis during revision total joint replacement surgery a real problem.

The use of autograft bone is most effective however the amount that can be harvested from the iliac crest is limited. Also iliac crest harvesting is known for its morbidity and complications due to the additional intervention and surgery time. Bone transplants with allograft are a good alternative but they also have some undesirable qualities such as the risk of virus and disease transmission, variability in quality (donor specific). Due to an aging population and an expected surge of primary and revision surgery in the Netherlands the demand will quickly outgrow the supply and bone allograft alternatives need to be developed. Due to the expected shortage of bone grafts for surgical procedures in the future and the risk of virus transfer when using allograft bone, there has been an increased interest in bone substitutes [LeGeros RZ CORR 2002].

The approach of using solely TCP-HA granules has the advantage of being purely synthetic, excluding the need of bone grafts and its associated drawbacks such as virus transfer and donor site morbidity. From a biological point of view ceramic calcium phosphates, such as tri-calcium phosphate (TCP) and hydroxyapatite (HA) are widely considered as promising bone graft substitute and there is already clinical experience in trauma surgery and orthopaedic surgery. It appeared that the handling characteristics of pure TCP-HA granules were inadequate and needed to be improved before purely synthetic reconstructions could be applied clinically.

It was hypothesized that the handling characteristics can be improved by combining the materials with a) paste (as such a material will adhere the TCP-HA granules together into a cohesive plug. Additional advantages of such a material are the easy handling, the interdigitation and close contact with surrounding tissue and the quick resorption characteristics. Also such a paste may be used as a carrier for pharmaceutical release.

Aim of study:

The aim of this study is to assess :

- Biological incorporation, remodeling and resorption of Puregel HA paste

Further, we will assess whether an based paste ( ) added to TCP-HA granules can improve:

- Handling and cohesion of TCP-HA granules
- Incorporation and remodeling speed of the TCP-HA granules into a new bony structure
- Resorption speed of TCP-HA granules

2. Maatschappelijke relevantie en/of wetenschappelijk belang

The need for better understanding of bone substitute biomaterial clinical implementation and behavior in the body is obvious. We aim to obtain more insight in biomaterial incorporation, remodeling and resorption characteristics in an well established animal model. When successful this project will offer surgeons better tools (combination of biomaterials allow tuning of incorporation and resorption speed) and better handling properties to treat bone defects in future implant revision surgery or trauma surgery. Data from these experiments can eventually be extrapolated to all other calcium phosphate based implants (skeletal reconstructions, dental implants and medical devices).

3. Alternatieven

Bone healing is a complex biological process in which several biological parameters play a crucial role. Bone cells such as osteocytes and osteblasts moderate bone tissue incorporation and remodelling. Cell likes osteoclasts, macrophages / giant cells moderate bone resorption. Bone healing treatment success with biomaterials can only be evaluated in a biological environment (*in vivo*), because of a lack of reliable *in vitro* models. An in vivo animal study is the gold standard to study bone healing and incorporation, remodeling and resorption of Ca-P biomaterials.

4. Ethische afweging

When successful this project will generate valuable information about in vivo behavior of a new generation of calcium phosphate biomaterials. It will establish a pathway towards clinical use of a nano-particulate | based biomaterials. In respect to current materials this material is better suited to fill irregular defects and has a quicker resorption time. Also it is possible to combine this material with antibiotics (yet outside the scope of this study).

For the animals this project implies one operation in which a biomaterial is placed bilateral in a pre-drilled distal femoral condyle defect. Together with direct post-op antibiotics, additional feeding and painkilling, the discomfort for the animals is classified as **"matig/ernstig" during surgery and first day post-operative**, In general 6 hours after surgery the animal will have no apparent discomfort of the surgery.

Since bone healing (biomaterial incorporation, remodeling and resorption) is a complex process involving both local biological and mechanical and systematic physiological stimuli this research cannot be performed in the lab. An in vitro or ex vivo model which covers all these properties does not exist. The proposed animal model in New Zealand white rabbits is the method of choice (and well established in literature) to study the process bone healing.

3 Wetenschap

5. Wetenschappelijke onderbouwing

Bone repair is a multi-dimensional process that requires osteogenic cells, an osteoconductive matrix, osteoinductive signaling, mechanical stability and vascularization. In clinical practice, bone substitute materials are being used for reconstructive purposes, bone stock augmentation, and bone repair. Over the last decade, the use of calcium phosphate (CaP) based bone substitute materials has increased exponentially. These bone substitute materials vary in composition, mechanical strength and biological mechanism of function, each having their own advantages and disadvantages. It is known that intrinsic material properties of CaP bone substitutes have a profound effect on their mechanical and biological behavior and associated biodegradation. These material properties of bone substitutes, such as porosity, composition and geometry change the trade-off between mechanical and biological performance. The choice of the optimal bone substitutes is therefore not always an easy one, and largely depends on the clinical application and its associated biological and mechanical needs. Not all bone graft substitutes will perform the same way, and their performance in one clinical site may not necessarily predict their performance in another site. CaP bone substitutes unfortunately have yet to achieve optimal mechanical and biological performance and to date each material has its own trade-off between mechanical and biological performance.

The approach of using solely TCP-HA granules has the advantage of being purely synthetic, excluding the need of bone grafts and its associated drawbacks such as virus transfer and donor site morbidity. From a biological point of view ceramic calcium phosphates, such as tri-calcium phosphate (TCP) and) are widely considered as promising bone graft substitute and there is already clinical experience in trauma surgery and orthopaedic surgery. It appeared that the handling characteristics of pure TCP-HA granules were inadequate and needed to be improved before purely synthetic reconstructions could be applied clinically.

It was hypothesized that the handling characteristics can be improved by combining the materials with a paste ( ) as such a material will adhere the TCP-HA granules together into a cohesive plug. Additional advantages of such a material are the easy handling, the interdigitation and close contact with surrounding tissue and the quick resorption characteristics. Also such a paste may be used as a carrier for pharmaceutical release.

The combined application of paste (') with HA/TCP 60/40 400-700 µm granules is unknown relative to its mechanical and biological performance. In an earlier mechanical pilot experiment [Arts JJ Eng Med 2005], a related material Ostim HA-paste was already used with a mixture of MCB and TCP-HA granules. During this study an improvement of the handling characteristics was observed.

TCP-HA bone substitute materials have been shown to be osteoconductive and they have performed well in animal and clinical trials as a standalone material or in combination with allograft.

However, also some critical downsides have been reported.

- Handling is not optimal and cohesion of individual granules is hard to achieve
- Incorporation and remodeling into a new bone structure is slow
- Resorption is slow

Aim of study:

The aim of this study is to assess :

- Biological incorporation, remodeling and resorption of

Further, we will assess whether an based paste (') added to TCP-HA granules can improve:

- Handling and cohesion of TCP-HA granules
- Incorporation and remodeling speed of the TCP-HA granules into a new bony structure
- Resorption speed of TCP-HA granules

To assess the aims of this study several different materials (allograft bone, HA paste, HA/TCP 60/40 400-700 µm) or mixtures of materials will be used. From a biological perspective, it is hypothesized that the resorption characteristics and the osteoconductive properties of will not be changed when combining with HA/TCP 60/40 400-700 µm granules in an well established unloaded defect model in the femoral condyle of rabbits [ref: Voor MJ 2004). Furthermore, we hypothesize that has an positive effect on speed of remodeling , incorporation and resorption of the HA/TCP 60/40 400-700 µm granules due to an improved angiogenesis.

6. Wetenschappelijke beoordeling

This study protocol **Version 8: Reply after wijzigingsbrief DEC 04-08-2011 CA, PE** has been reviewed and approved by researcher of ( ) and by the ( ).

The principal investigator of this project is:

5 Proefdier

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

New Zealand White SPF Rabbits (Harlan, Specified Pathogen Free) (skeletal mature) will be used for these experiments, all animals will be euthanized at the end of the experiment.
Skeletal mature animals with closed growth plate as assessed by X-ray
This animal model has been chosen because it is well established and frequently reported in literature. Also the principal investigator (have much experience with this animal model and biomaterials research in general.

7b. Sexe

Female (due to fighting for dominance of male rabbits in Group housing)

7.c. Aantallen

Number of animals.

Group	(n)animals (legs)	Code	Description
0	3(6)	DR	Animals will be used to harvest donor allograft from femur condyles and at the same time animals will be used to practice surgical technique
1	3(6)	ED	Control group. *Negative control for model validation* Defect will not be filled during surgery. Sacrifice after 8 weeks.
2	3(6)	MCB	Control group. *Positive control as gold standard treatment.* Defect will be filled with 100% allograft during surgery. Sacrifice after 8 weeks.
3	6 (12)	CC	Intervention group. Defect will be filled with 100% HA/TCP 60/40 400-700 µm granules. Sacrifice after 8 weeks.
4	6 (12)	PST	Intervention group. Defects will be filled with 100% Puregel HA paste. Sacrifice at 4 weeks.
5	6 (12)	PLT	Intervention group. Defects will be filled with 100% Puregel HA paste. Sacrifice at 8 weeks.
6	6 (12)	MIXST	Intervention group. Defects will be filled with 70% Puregel HA paste and 30% HA/TCP 60/40 400-700 µm granules Sacrifice at 4 weeks
7	6 (12)	MIXLT	Intervention group. Defects will be filled with 70% Puregel HA paste and 30% HA/TCP 60/40 400-700 µm granules Sacrifice at 8 weeks
TOTAL	**39 (78)**		

Power calculation based on two-tailed testing:

Power calculation according to Sachs L.:
1e met de formule van L. Sachs: $n = 2(z_{0.05/2} - z_{\pi})^2 * (\sigma/\delta)^2$

The formula of Sachs was used (n=21.02*(sigma/delta)^2).
We expect a deviation of 25%, a significant difference of 80% between subgroups (power 90%, α=0.05). This gives n=10.1.

We also calculated a drop out percentage based on a groupsize of 10 and dropout of 15%.
This gives: 0,85a = 10 *of* 10/0,85 = a a = 11,764 rabbits which becomes 12.

8. Experiment

In the present study we will use an established animal model to assess bone healing by various materials and combination of materials. Several outcome measurements will be used in the course of this trial.

1. Temperature and bodyweight:

It is well known that temperature increase and weight loss can indicate active infection in the NZW rabbit model.
Both parameters will be assessed pre-and post-operatively on routine time points by CPV personnel.

Timepoints: Day 0-3-7-14-21-28-35-42-49-56.

2.

X-rays:

To assess correct implantation of materials/ correct closure of defect and to disregard occurrence of fracture post-op.
In order to guarantee proper implant placement and fixation X-rays will be made pre- and postoperatively and after sacrifice. Both AP and lateral X-rays will be taken.

Timepoints: Day 0 - Postmortem.

3. Histology and histomorphometry:

To assess macroscopic material distribution in the defect
To assess incorporation and remodeling of biomaterials
To assess resorption of biomaterials
To assess new bone formation in the defect
Bone healing (incorporation, remodeling and resorption) will be scored according to Vogely et al. [11] on the post-mortem retrieved tissue samples.
Angiogenesis: Paraffine coupes and VEGF STAINING

Post-operatively the animals will receive fluorchrome staining, typically Calcein green (CG, 20 mg/kg LG, s.c.), Calcein blue (CB, 20 mg/kg LG, s.c.), Xylenol orange (XO, 30 mg/kg LG, s.c.). Such markers bind to calcium on the day of injection and therefore adhere to freshly formed bone. The markers will be used at different time periods of the experiment so bone formation can be assigned to these periods.
In the four week follow-up group fluorchrome markers will be implemented at 7, 14, 21, 27 days post-op
In the eight week follow-up group fluorchrome markers will be implemented at 14,21, 27, 55 days post-op

The bone growth in these periods can be measured quantitatively by measuring the distance between the fluorescent lines and by using computer assisted histomorphometry on slides.

Timepoint: Postmortem

4.Micro CT

To assess macroscopic material distribution in the defect
To assess cohesion of materials
To assess bone structure and bone density parameters
Structure

TV	total volume [mm^3]
BV	bone volume [mm^3]

BV/TV	relative bone volume to tissue volume (%)
Tb.N	number of trabeculae
Tb.Th	trabecular thickness
Tb.Sp	trabecular separation
C.Th	cortical thickness

Density

D100	average bone density
Dtrab	trabecular bone density
Dcort	cortical bone density
BMD	bone mineral density

Timepoint: Postmortem

5. SEM

To assess cell and tissue reactions. 4 and 8 weeks parallel to histology
To assess biomaterial morphology

Timepoint: Postmortem

6. Liver biopt and kidney biopt

To assess biocompatibility and toxiticity tests.

Timepoint: Postmortem

This study is a RCT design with various biomaterials and two follow-up time periods (4,8 weeks):
A randomization scheme will be predetermined using an online tool. The resulting scheme will be written down on paper sheets separate for left and right leg of each rabbit to be operated and thereafter presealed.
1 hour before every surgery the surgeon will open an envelope to learn which biomaterials will be inserted in the rabbit. The scientist that examines the histology and histomorphometry will be blinded to the groups.
Two control groups will be used:

1. A negative control group in which a defect will be created but not be filled with biomaterials. Negative control for critical sized model validation.
2. A positive control group in which the defect will be created and filled with 100% allograft. Positive control as gold standard treatment.

All results will be compared to established results in literature. [Orr 2001, Voor 2004 and Arts 2006, 2007]

Day	Intervention	Time	4 week FU group	[redacted]
0	Weight measurement	3 min	X	
0	Shaving and prepare surgery	10 min	X	
0	Surgery	25 min	X	
0	X-ray	5 min	X	
0	Pain medication and antibiotics	2 min	X	
0	Additional feeding ( )	3 min	X	
3	Weight measurement	3 min	X	
7	Weight measurement	3 min	X	
7	X-ray + Fluorchrome label 1	10 min	X	

14	Weight measurement	3 min	X
14	X-ray + Fluorochrome label 2	10 min	X
14	[redacted]	10 min	-
21	Weight measurement	3 min	X
21	X-ray + Fluorochrome label 3	10 min	X
21	[redacted]	10 min	-
27	Fluorochrome label 4	10 min	X
27	[redacted]	10 min	-
28	Weight measurement	3 min	X
28	Sacrifice and X-ray post-mortem	20 min	X
28	Harvest liver and kidney of rabbits	15 min	X
35	Weight measurement	3 min	-
42	Weight measurement	3 min	-
49	Weight measurement	3 min	-
55	[redacted]	10 min	-
56	Weight measurement	3 min	-
56	Sacrifice and X-ray post-mortem	20 min	-
56	Harvest liver and kidney of rabbits	15 min	-
>56	Postmortem analysis of - Histology - Histomorphometry - Micro-CT - SEM - Toxicity test liver and kidney		X X X X X

9. Experimentele condities

9a. Anesthesie

Standard anaesthetic protocol for surgeries of NZW rabbits.
Do not use finadyne!! NSAID may be used according to veterinarian but will influence ectopic bone formation
Preference for Buprenorphine! (Temgesic)
Premedication/induction: ketamine 15 mg/kg LG i.m. and medetomidine 0,25 mg/kg LG i.m, in combination with isoflurane.
Maintenance: midazolam 1 mg/kg LG/4h i.v. and fentanyl 0.5-2 µg/kg LG/h i.v.
Fluid administration: ringers lactate 20 ml/kg LG/h i.v

Sedation for X-rays: 5 mg/kg LG teletamine/zolazepam i.m. (Zoletil) will be administered.

9b. Pijnbestrijding

Post-operatively buprenorphine 0.05 mg/kg LG s.c will be administered every 8-12 hours for the first 3 days. The **animal will receive its first dose at the end of the surgery.**
If the animals have clear signs of pain, the buprenorphine treatment can be continued. Always after consulting venetarian.

9c. Euthanasie en Humane eindpunten

At the end of the experiment, the rabbits are euthanized using an overdose of pentobarbital (Euthanasate) 200 mg/kg LG i.v.

Human endpoints are:

(1)	fracture of the femur at the implant site	as proven on X-ray
(2)	weight loss of more than 30%	as proven with weight measurements

(3) In case of infection of the implant area, the veterinarian will be consulted, for further treatment.

In all these cases and in case of other diseases, the veterinarian will be consulted.

Zorg

10a. Ongerief

The discomfort will be severe (code 04) for the first week after surgery, due to the implant procedure and wound healing. After the first week the discomfort is expected to decrease to mild (code 02). The X-ray and fluorochrome injection (day 7, 14, 21 and 27 or day 14, 21, 27 and 55) are classified as mild (code 02).

Procedure	Discomfort	Frequence
Surgery	4	1
X-rays	2	2
Fluorochrome	2	4

Total discomfort is estimated as severe (code 04)

10b. Welzijnsevaluatie

The well being of the animals will be monitored and reported throughout the study.
Every material, which will be implanted in the animals, has been tested and approved *in vitro*, in this way the risk for additional complications and inconvenience will be minimal.

11. Verzorging en huisvesting

The rabbits will be housed in groups before and after operation under standard conditions of the CPV. (Straw on the floor, ability to walk around freely, separate group housing from the animals of other experiments. They should have water and food available ad libitum. Surgery will be performed at the CPV.

12. Deskundigheid

The principal investigator ..... are art.9 licensed.
Both ; have clinical experience with animal model research.
is a well known expert in Europe about research and treatment procedures with bone substitute Ca-P materials.

13. Standard Operation Procedures (SOP)

SOP 1- Anaesthesia

Premedication/induction: ketamine 15 mg/kg LG i.m. and medetomidine 0,25 mg/kg LG i.m, in combination with isoflurane. According to SOP CPV
Maintenance: midazolam 1 mg/kg LG/4h i.v. and fentanyl 0.5-2 µg/kg LG/h i.v.
Fluid administration: ringers lactate 20 ml/kg LG/h i.v

SOP 2 – Surgery

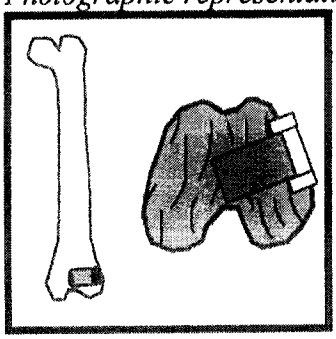
Pre-operatively, the animals will receive an antibiotic injection (Ampicilline 15mg/kg; Alfason, Woerden, the Netherlands). One day prior to surgery the MCB will be taken from the -80°C storage and placed into a refrigerator (6°C). Four hours prior to surgery the MCB is placed in room temperature. Surgery will be performed with the animals under anaesthesia according to SOP 1

The incision site is shaved and thoroughly cleaned with betadine. After locating the knee joint space, a 2-3 cm lateral skin incision will be made to expose the distal femur. Subsequently, a 2 mm diameter hole will be drilled 4 mm deep in the lateral condyle and a guidepin is inserted afterwards. Using a custom made drill, a final drill hole with a diameter of 5.5 mm and a depth of 8 mm will be created without damaging the lateral collateral ligament. The defect must be cleaned using a sharp spoon and thorough irrigation with a saline solution.

All groups are prepared by placing particles of allograft, paste or HA/TCP 60/40 400-700 µm granules or a mixture of the last two in the required volume ratio (determined by placing in a standardized size syringe) into a 5.0 mm inner diameter stainless steel impactor tube. This impactor tube is designed to allow controlled compression of the reconstructive mixes while forming an impacted construct to fit into the drilled defect. A standardized pressure of 6.73 MPa shall be applied to the construct for two minutes. This pressure was also used in previous impaction experiments [Arts biomaterials 2004]. After the compression period of two minutes, the tube is removed from the compression device and the bottom cap will be unscrewed. The tube is placed on the defect and the sample will be inserted press-fit into the defect using a plunger. A standardized depth of the defect must be filled and afterwards a polyethylene (PE) plug is placed press fit on top of the

implanted material to seal the defect. Thereafter, the area surrounding the defect will be irrigated with a saline solution to remove any remaining bone or biomaterial debris and the soft tissues will be closed in layers. Subsequently, the procedure will be performed on the contra-lateral limb. Postoperatively, the animals are allowed to walk freely. Each rabbit will receive a subcutaneous injection of fluorchrome markers at 8, 15 and 55 days postoperative (calcein green/red/orange solution (25 mg/kg)). The rabbits are killed eight weeks postoperatively, with an overdose of 1ml/kg barbiturate (Nembutal 1 ml/kg;
). Standard roentgen photographs will be taken from the implant sites to verify position and to exclude fractures. The distal
femurs are harvested, cleaned from all soft tissue and fixed in a 4% buffered formaldehyde solution at 4°C for at least ten days prior to microCT scanning and histological analysis.

Photographic representation of surgery steps: Exposure, drilling, open- and sealed defect.



Schematic overview of defect in distal femoral condyle.

SOP 3 - X-ray

Sedation for X-rays: 5 mg/kg LG tiletamine/zolazepam (Zoletil) will be administered i.m. Standard x-ray setup will be used to make x-rays of the tibia and implants

Materials:
Zoletil
Fluorescope

SOP 4 – Administration of fluorescent markers

Calcein green (20 mg/kg LG) / Xylenolol orange (90 mg/kg LG) / Calcein blue (20 mg/kg LG) are administered subcutaneously under anesthesia according to SOP 3.
In the four week follow-up group fluorchrome markers will be implemented at 7, 14, 21, 27 days post-op
In the eight week follow-up group fluorchrome markers will be implemented at 14,21, 27, 55 days post-op

Materials:
Calcein green (20 mg/kg LG s.c.) (provided by pi)
Xylenolol orange (30 mg/kg LG s.c.) (provided by pi)
Calcein blue (20 mg/kg LG s.c.) (provided by pi)
Syringe + needles

SOP 5 – Euthanasia

The animals are euthanized by means of an intravenous overdose of barbiturate 200 mg/kg LG i.v. Both femurs are removed in a non-sterile manner and fixated in 4% formaldehyde/PBS.

Materials

Scalpel nr. 10
Blad nr. 10
Euthanasate
4% formaldehyde in PBS

Relevante literatuur

1. *Arts JJ et al: (2006) The use of a bioresorbable nano-crystalline hydroxyapatite paste in acetabular bone impaction grafting. Biomaterials 27(7):1110-8.*
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3. De Long WG, Einhorn TA, Koval K, McKee M, Smith W, Sanders R, et al. Bone grafts and bone graft substitutes in orthopaedic trauma surgery. A critical analysis. J Bone Joint Surg Am 2007;89:649-58.
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8. Voor MJ et al (2004): Is hydroxyapatite cement an alternative for allograft bone chips in bone grafting procedures? A mechanical and histological study in a rabbit cancellous bone defect model. (2004) J Biomat Mater Res B Appl Biomater. 71B: 398-407.
9. Orr TE, Villars PA, Mitchell SL, Hsu HP, Spector M. Compressive properties of cancellous bone defects in a rabbit model treated with particles of natural bone mineral and synthetic hydroxyapatite. Biomaterials. 2001 Jul;22(14):1953-9.
10.

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

, voorzitter
p/a Secretariaat DEC-UCVi
Postbus 616
NL-6200 MD Maastricht
Telefoon: 043-

Aan:

Uw referentie: Onze referentie : Maastricht, 19-07-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*BMM NANTICO: Assessment of Puregel (Hydroxyl-Apatite paste) incorporation and remodeling in an unloaded critical sized distal femoral condyle model in NZW rabbits*", is op de DEC vergadering van 15 juli 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt van punt 5 het deel "In the present study we will use, enzovoort" bij punt 8 te vermelden.
- De DEC verzoekt bij punt 6 . te verwijderen (de VO of de VVO mag zijn eigen aanvraag niet wetenschappelijk beoordelen en goedkeuren).
- Punt 7c- De DEC vraagt zich waarom er gerekend wordt met een power van 90%. Een hogere standaarddeviatie en uitval moeten meegenomen worden in de berekening en zijn geen argumentatie voor het verhogen van de power.
- De DEC vraagt zich af of groep 8 nodig is, omdat deze dieren al berekend zijn in het uitvalspercentage. De DEC denkt dus dat groep 8 niet nodig is.
- De DEC verzoekt bij punt 9a de anesthesie aan te passen in overleg met de proefdierdeskundige.
- Graag de humane eindpunten bij punt 9b beter definiëren (mogelijk ontstaan van ontstekingen vermelden).
- Bij punt 10a verzoekt de DEC in een tabel, de aard, ernst, duur en frequentie beter te definiëren per handeling en groep, en het totale ongerief aan te geven en in overeenstemming te brengen met het voorblad.

Conclusie:
Het project wordt aangehouden

Gelieve eventuele vragen te beantwoorden in een brief en indien noodzakelijk Uw project aan te passen en duidelijk de aanpassingen grijs te markeren.

Uw project staat bij de DEC geregistreerd onder nummer 2011-101, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Maastricht, 04-08-2011

Geachte Leden van de dierexperimenten commissie van Universiteit Maastricht

In deze brief vind uw onze reactie op uw wijzingingsbrief betreffende onze projectaanvraag: "*BMM NANTICO: Assessment of . (-.) incorporation and remodeling in an unloaded critical sized distal femoral condyle model in NZW rabbits*", welke op de DEC vergadering van 15 juli 2011 besproken is.

Alleereerst willen we de DEC verzoeken "BMM NANTICO" uit de titel van het project te verwijderen daar dit verwijst naar een ander lopend project. We hebben deze correctie zelf ook doorgevoerd in onze documenten

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt van punt 5 het deel "In the present study we will use, enzovoort" bij punt 8 te vermelden.

Punt is aangepast in protocol naar de wensen van de DEC

- De DEC verzoekt bij punt 6 te verwijderen (de VO of de VVO mag zijn eigen aanvraag niet wetenschappelijk beoordelen en goedkeuren).

Punt is aangepast in protocol naar de wensen van de DEC

- Punt 7c- De DEC vraagt zich waarom er gerekend wordt met een power van 90%. Een hogere standaarddeviatie en uitval moeten meegenomen worden in de berekening en zijn geen argumentatie voor het verhogen van de power.

Er zijn twee redenen om de power van deze studie te verhogen.

1. Histomorpometrie (meten van nieuwe bot formatie is een vooraf bepaalde region of interest (ROI) is deels een subjectieve maat aangezien deze ROI in hisologie onderling veel kunnen verschillen. Samenhangend hiermee is het feit dat in deze studie botvervangende materialen worden vergeleken met een compositie die zeer veel overeenkomt tussen producten. Het is volgens de onderzoekers een goed argument om hierop de power te verhogen naar 0.9 om na analyse verzekerd te zijn van voldoende discriminatie in gegevens tussen groepen en daarbij een statistische hoge power te kunnen hanteren wat duidt op hoge betrouwbaarheid van de studie gegevens.

2. Een power van 90% wordt door de afdeling orthopaedioe ook regelmatig in klinische studie gehanteerd als er sprake is van subjectieve uitkomstmaten. Een voorbeeld hiervan is het artikel van Caroline Wyers et al (BMC Public Health. 2010 Apr 27;10:212) betreffende een klinische studie van heupfractuur patienten.

- De DEC vraagt zich af of groep 8 nodig is, omdat deze dieren al berekend zijn in het uitvalspercentage. De DEC denkt dus dat groep 8 niet nodig is.

Punt van DEC is geaccepteerd en groep 8 is verwijderd uit huidig protocol.

- De DEC verzoekt bij punt 9a de anesthesie aan te passen in overleg met de proefdierdeskundige.

Anesthesie is aangepast in overleg met proefdierdeskundige volgens SOP van het CPV

- Graag de humane eindpunten bij punt 9b beter definiëren (mogelijk ontstaan van ontstekingen vermelden).

Toelichting uitgebreid met toevoeging van infectierisico.

- Bij punt 10a verzoekt de DEC in een tabel, de aard, ernst, duur en frequentie beter te definiëren per handeling en groep, en het totale ongerief aan te geven en in overeenstemming te brengen met het voorblad.

Tabel ingevoegd en toelichting toegevoegd, totaal ongerief in overeenstemming gebracht met het voorblad.

Met vriendelijke groet namens de onderzoekers van de afdeling

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	30-08-2011

Project: *Assessment of [illegible] incorporation and remodeling in an unloaded critical sized distal femoral condyle model in NZW rabbits.*

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht

Verantwoordelijk onderzoeker (VO):

Namens de Vergunninghouder van de DEC-UM, delen wij u mede dat voornoemd project aan de ethische toetsingscriteria voor proefdiergebruik voldoet.
De DEC maakt geen bezwaar tegen uitvoering van dit project zoals aangevraagd en geeft een positief advies.

Projectnummer:	2011-101
Diersoort:	konijn
Aantal dieren:	39
Einddatum:	26-08-2015

Uw project staat bij de DEC en CPV geregistreerd onder bovenstaand nummer. Gelieve dieren, die voor dit project bestemd zijn, ook onder dit nummer aan te vragen.

Voorzitter DEC-UM

Vicevoorzitter DEC-UM