Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-128

Ontvangen: 16-01-2012

DEC datum goedkeuring#	Type aanvraag [2]
16-01-2012	Nieuw

VROM/GGONR[3]

LNV/CBDNR[4]
n.v.t.

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

Financieel beheerder	Budgetnummer	31962192N

Titel van het onderzoek:

The role of inflammation in cutaneous wound healing: modulation of inflammation by induction of an anti-inflammatory M2 wound macrophage phenotype.

startdatum 01-11-2011 einddatum [9] 01-07-2012 *Duur van de proef [10]: 20 days*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegdheid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)				Art.9	
2. Vervanger VO (VVO)				Art.9	
3. Verantwoordelijk medewerker (VM) GGO[7]					
4. overige uitvoerenden				Anders[6]:	
5.				Art.9	

Diergroep	1	2	.	.	.	.	.
ctrl/exp/sham	experimental	experimental					
Diersoort	01	01					
Stam	C57BL/6	C57BL/6 db/db					
Construct / mutatie ?	wild-type	db/db					
Herkomst (leverancier) *	02	02					
Aantal	32	32					
Geslacht	female	female					
Dieren immuuncompetent ?	ja	ja	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]
Leeftijd/gewicht	10-12 wks	7-8 wks					
Doel van de proef *	33	33					
Belang van de proef *	01	01					
Toxicologisch onderzoek *	01	01					
Bijzondere technieken *	01	01					
Anesthesie *	03	03					
Pijnbestrijding *	02	02					
Mate ongerief *	03	03					
Toestand dier einde exp*	01	01					

* VHI-coderingen zie bijlage

1 Verantwoording

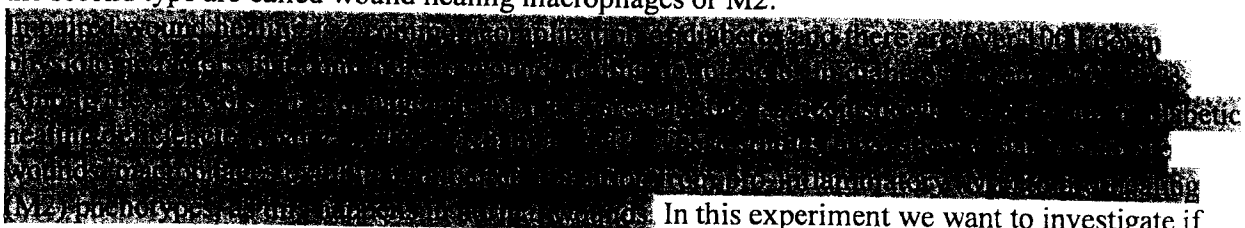
Aanvraag dierproef DEC-UM **(kaders zijn licht flexibel, maar het geheel is max. 5 pag. versie 2006)**

Titel: The role of inflammation in cutaneous wound healing: modulation of inflammation by induction of an anti-inflammatory M2 wound macrophage phenotype.

1. Doel van de proef.

Skin wounds on mammalian embryos heal perfectly without scars, whereas wounds to adult mammals scar. An important difference between embryonic and adult wound healing consists in the different inflammatory response that is elicited in embryonic and adult wounds, with a much milder inflammatory response in the first compared to the later.

An important cell that mediates inflammation is the macrophage. It has been shown that macrophages are present in the wounds already a few hours after injury and they persist for whole period of healing. Macrophages affect the healing process by secreting both factors that may hamper and delay healing, like pro-inflammatory cytokines and tissue destructive metalloproteases, but also factors that promote healing like growth factors, anti-inflammatory cytokines, angiogenic factors [2] [3]. In general, the first type of macrophages are called pro-inflammatory or M1 while the second type are called wound healing macrophages or M2.



In this experiment we want to investigate if induction of M2 macrophages in wounds can have a positive effect on wound healing.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

The primary goal of treatment of wounds is rapid wound closure and a functional and aesthetically satisfactory scar. Only in the US delayed wound healing is estimated to cost more than $9 billion each year. Understanding the process of wound healing can help improve and accelerate the repair and lead to reduced scarring. Apart from the consequences from the aesthetic point of view in patients undergoing surgery, improved wound healing will greatly decrease morbidity and mortality in diabetic patients and the elderly population, where this process is largely dysregulated.

3. Alternatieven

Wound healing is a complex process that involves the participation of different immune and non immune cell types like keratinocytes, fibroblasts, macrophages, neutrophils, T cells. Although there have been developed *in-vitro* skin models (*http://www.epistem.co.uk/invitroskinmodels.asp*), these are only composed of human keratinocytes and human dermal fibroblasts within a collagen gel. This means that studies involving influx of leukocytes from the circulation into the wound site and the determination of the phenotype of wound macrophages during the different phases of the healing process, like the studies we want to perform, are not possible with these models. For this reason, in our case it is not possible to study in an *in-vitro* system the wound healing process, and animal models are required. Therefore, no biologically relevant *replacement* to study this process can be used.

However, as a *refinement approach,* the discomfort of the animals will be minimized as possible. Experience from other researchers working in this field has shown that *"mice cope well with the injuries; mice start to climb, clean and feed soon after the end of anesthesia"* [1]. However upon any noticeable signs of excessive discomfort, "humane endpoints" will be respected.

We believe that the potential benefits of this research in reducing morbidity and increasing patient's quality of life in the very big number of patients that undergo surgical operations every day but also in diabetic patients and the elderly population that show wound healing problems, justifies the use of these animals in our experiment.

4. Ethische afweging

Impaired wound healing is a major source of morbidity in patients undergoing surgery and in diabetic patients that due to wound healing complications are more prone to infections. Eventually, if the wound becomes serious, tissues can be damaged and infection cannot be controlled and it becomes necessary to remove the affected part in order to save the life of the patient. For this reason, diabetic patients are more prone to limb amputations. Therefore, the implications of a research that would improve wound healing rates are important.

Wound healing experiments in mice are performed though induction of small (3-5 mm) incisions on the dorsal surface of the mice under anesthesia (see picture in section 13). Upon recovery, mice behave normally, they eat and move as before and in general do not show any big signs of discomfort. As other researchers working on this field have published before, the discomfort provoked is not extreme and in general mice cope well with the injuries and do not die due to complications of the wound. In addition, infections of the wounds are never observed.

Unfortunately, no alternatives exist to study this complex process in another non-animal or *in-vitro* model. We think that the potential benefits to patients' quality of life and reduction of morbidity or even mortality in diabetic patients, largely outweigh the discomfort.

5. Wetenschappelijke onderbouwing

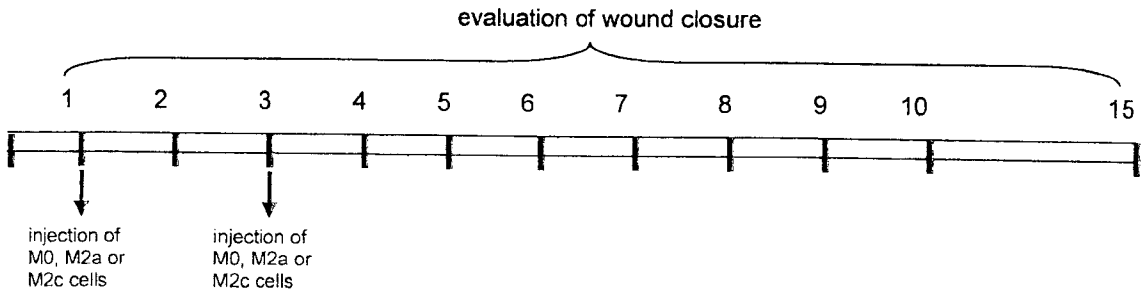
Wound healing is a dynamic, interactive process involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells. Wound healing has three phases — inflammatory, proliferative, and tissue remodeling — that overlap in time. In the inflammatory phase, bacteria and debris are phagocytized and removed, and factors are released that cause the migration and division of cells involved in the proliferative phase. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. In angiogenesis, new blood vessels are formed by vascular endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix (ECM) by excreting collagen and fibronectin. Concurrently, re-epithelialization of the epidermis occurs, in which epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue. In contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. When the cells' roles are close to complete, unneeded cells undergo apoptosis [2] [3].

Factors secreted by macrophages can affect all the above mentioned stages of the healing process. Indeed macrophages were shown to be both detrimental in the initial stages of healing due to secretion of pro-inflammatory factors and beneficial in the later stages due to secretion of pro-healing factors (growth factors, angiogenic factors, anti-inflammatory cytokines).

We believe that by modulating the macrophage phenotype in a way to have a less strong pro-inflammatory response in the initial phases and a more powerful and sustained anti-inflammatory response in the later stages, we can improve the parameters of healing.

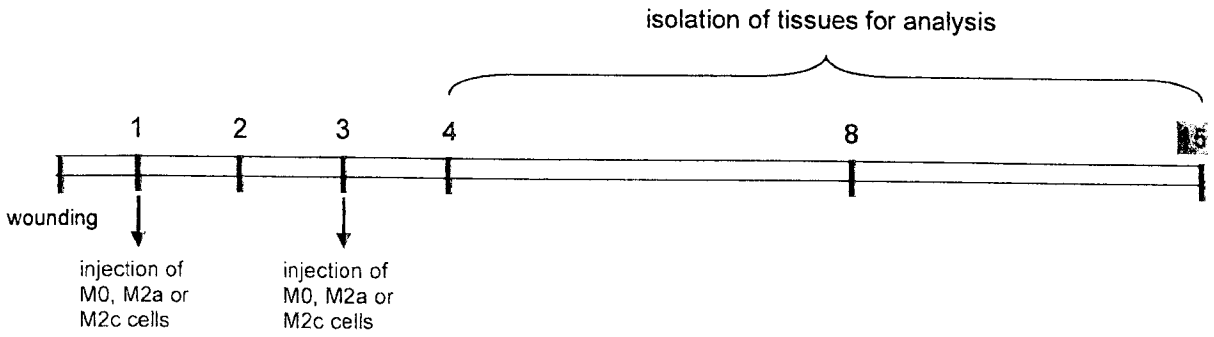
In order to modify the macrophage phenotype, we want to isolate macrophages from mice, polarize them *in-vitro* to make them acquire an anti-inflammatory (wound healing) phenotype (M2 macrophages) and inject them to wounds made in normal or diabetic mice. We hypothesize that the injected M2 macrophages will secrete pro-healing factors in the wound environment that will accelerate healing. We would like address this question in both normal healing conditions but also in diabetic conditions since wound healing is a major problem in diabetes and our research is highly relevant for the millions of diabetic patients.

A scheme of our experiment is outlined below. We will first generate 2 types of M2 macrophages *in-vitro* (M2a or M2c), and inject these cells in wounds made in mice after 1 or 3 days of wounding. As control group we will use mice injected with non-polarized macrophages (M0). We will evaluate closure and make pictures every 2 days until day 15 after wounding.



If our hypothesis is correct and injection of either of the 2 or both M2 macrophage subtypes improves indeed healing, we will repeat this experiment in order to isolate tissues after different time points to study the mechanisms that mediate this effect.

We would like to study the process in at least 3 different time points (day 4, 8 and 15). Tissues samples will be isolated for histology, immunohistochemistry, ELISA, RNA isolation, for the determination of the cellular and molecular differences between treatments.



6. Wetenschappelijke beoordeling

Principal investigator ( ) is responsible for this research in collaboration with from the . Both scientific validity and social relevance of the research questions were assessed. The experiments planned have been considered suitable to answer our questions. The whole project has therefore been assessed as positive.

5 Proefdier

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

Mus musculus domesticus, strain: C57BL/6, 10-12 wks of age or db/db, 7-8 wks of age. Since diabetic db/db mice become obese very early in life (already at the 3rd week of age) and at the age of 10-12 weeks can reach 50-60 grams of weight, we will make use of younger mice compared to the C57BL/6 to avoid working with excessively big and heavy mice. Source: Charles River. Mice will be sacrificed at different time points during the experiment.

7b. Sexe

In contrast to the beneficial effects of estrogen, most of the recent evidence suggests that androgens have a negative effect on wound repair. For this reason we will make use of female mice.

7.c. Aantallen

Power analysis (according to Sachs):
Our read out parameter in the wound healing experiments is the degree of wound closure upon M2 (M2a or M2c) macrophages administration compared to controls (M0 and salines), as measured by the reduction in wound area calculated by the digital images of wounds recorded every 2 days. According to previous published experiments, a variation (σ) of approximately 20% is expected. If we want to demonstrate a difference in the wound healing response (δ) of at least 25% between treatment and control groups according to Sachs: n=15.7*(20/25)^2=10.04=11 mice per group. For our initial experiment we have 2 experimental groups (M2a and M2c) and a control group (M0, as internal control for the administration of non polarized macrophages). Thus we will need: 11*3=33 mice + 11 (saline control for all 3 groups) = 44 mice.

If our experiment is successful, we would like to perform kinetics studies in which we will isolate tissues after 4, 8 and 12 days to study mechanisms of improved healing. Therefore we will need 44*3 time points=132 mice.

Given the societal relevance of improving diabetic wound healing, we would like to perform the same experiment in db/db mice that are established models for human type 2 diabetes. Therefore the same number of db/db mice will be required.

Dierproef

8. Experiment

Mice are anesthetized by isofluorane inhalation and the dorsal surface is shaved, washed with betadine solution and 70% ethanol. Using a disposable 3 mm diameter skin punch biopsy tool 4 full-thickness wounds are made in the dorsum of each mouse (see picture in section 13). Mice will be placed in cages individually, under a warming lamp, and observed until they wake up. Following wake up mice will be housed in groups of 3-4. It is important to avoid standard animal bedding as long as the wounds are not covered by a stable and dry scab. For this reason, a special bedding material will be used consisting of paper bedding (*7089 Teklad Diamond Soft Bedding).* This avoids the possibility that bedding particles will become enclosed within the drying wound. Mice will be monitored for the following 4-6 hours and humane endpoints will be applied in case of signs of distress (i.e. behavioral problems like the ability to move in the cage, intake of food and water). Wounds are left to heal by secondary intention and 1 and 3 days after the generation of the wounds, *in-vitro* generated M2a and M2c macrophages are going to be injected around each wound. Healing will be monitored every 2 days by acquisition of digital photographs of the wounds, while mice are under isofluorane anesthesia, that will be later measured in a computerized way. If we are going to see a difference in the healing pattern and an improvement between the groups, then we would like to investigate the mechanisms of this effect. For the second experiment then, we would like to repeat the experiment as in the first setting in 3 groups of mice, but this time we would like to isolate wound tissue for the different analysis (histology, immunohistochemistry, RNA, protein). Each group of mice will be sacrificed at the indicated time points (d4, d8, d14) by CO2/O2 inhalation and analyzed for wound closure and other parameters listed above.

9. Experimentele condities

9a. Anesthesie

For the induction of dorsal wounds, mice will be anesthetized by isofluorane inhalation. Anesthesia will be performed every 2 days in order to inject the macrophages or control solution around the wound and also to take digital images of the wounds. After wounding, the mice will be placed in cages individually, under a warming lamp, and observed until they wake up.

9b. Pijnbestrijding

In similar published experiments studying wound healing, no analgesics are given to the mice during the course of the experiment. This is done for 2 reasons: first, application of analgesics in the area of the wounds will very probably affect the course of our experiments since it can interfere in ways that cannot be predicted with the molecular and cellular events taking place in the wounds. This would make the final evaluation of our experiment not valid and we could come to wrong conclusions **[1] [4]**. A second reason for not application of analgesics in mice is that from other published reports it is considered that the discomfort provoked is not extreme and generally mice seem to cope well with the wounds. Although the mouse behavior does not exactly reflect the pain conditions in the mice, we will anyway monitor their behavior and in case we notice signs of pain or discomfort we will evaluate the administration of analgesics.

9c. Euthanasie en Humane eindpunten

Unconsciousness is induced in the animals by CO_2/O_2 (6:4) and subsequently sacrificed by raising the CO_2 to 100%. Any animal that shows signs of distress, behavioural problems, body weight decrease, reduced intake of food or water or diminished urine/faeces production will be prematurely sacrificed by euthanasia (CO_2/O_2) inhalation combined with cervical dislocation.

10a. Ongerief

In the procedure of wound healing, mice experience a medium level of distress due to the generation of wounds in the dorsal surface. This can be quantified in a scale of 03. However mice seem to cope well with the injuries as can be deduced from their normal behavior after they wake up from the anesthesia. In addition, subsequent handling of the mice such as taking digital pictures (distress 03) or injection of macrophages (distress 02) is done under isofluorane anesthesia. In this way the discomfort is greatly reduced.

Aard	Duur	Frequentie	Ongerief (code)
generation of wounds [redacted]	15 min.	one time	03
Anesthesia [redacted]	5-10 min.	every 2 days	03
injection of macrophages [redacted]	10 min.	on day 1 and 3	02
Totale ongerief [redacted]			03

Aard	Duur	Frequentie	Ongerief (code)
generation of wounds [redacted]	15 min.	one time	03
Anesthesia [redacted]	5-10 min.	every 2 days	03
injection of macrophages [redacted]	10 min.	on day 1 and 3	02
Totale ongerief [redacted]			03

10b. Welzijnsevaluatie

[redacted]

11. Verzorging en huisvesting

The care and housing of the animals will be in accordance to the standards of the Centrale Proefdier Voorzieningen Maastricht. The mice will be housed conventionally in groups of 3-4/cage and will eat and drink at libitum. In consultation with the laboratory animal facility, a special paper bedding will be applied (*7089 Teklad Diamond Soft Bedding*) to improve

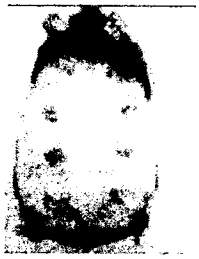
experimental conditions and animal well being. The test animals will be daily visited by the VO during the whole course of the experiment. At calamities please contact

12. Deskundigheid
Both principal investigator (VO) and VVO are art.9 competent and have extensive experience with interventions in mice.

13. Standard Operation Procedures (SOP)
Materials:
anesthesia by isofluorane inhalation
shaving device
betadine solution and 70% ethanol
sterile operation blades and forceps
sterile gauzes
3 mm diameter skin punch biopsy tool by Miltex

Procedure:
The mice are anesthetized by isofluorane inhalation, the skin is cleaned with betadine solution and 70% ethanol, and the animal is covered with autoclaved Boeklon plastic foil, with the hole over the surgery area. Using a disposable 3mm diameter skin punch biopsy tool 4 full-thickness wounds are made in the dorsum of each mouse. After wounding, the mice will be placed in cages individually, under a warming lamp, and observed until they wake up. Cell or control saline solutions will be injected around the wounds 1 and 3 days after generation of wounds while mice are under isofluorane anesthesia. Digital pictures will be taken every 2 days while mice are under isofluorane anesthesia.

Example of induction of 6 excisional wound in the back of the mice as published but other groups [1].



End of the experiment:
Unconsciousness is induced in the animals by CO_2/O_2 (6:4) and subsequently sacrificed by raising the CO_2 to 100%. Wounds are resected for analysis. For histology wounds are placed in 1% paraformaldehyde solution and embedded for cryosections. For RNA or protein analysis wounds are stored in liquid nitrogen.

Relevante literatuur

1. *Frank, S. and H. Kampfer, Excisional wound healing. An experimental approach. Methods Mol Med, 2003. **78**: p. 3-15.*
2. *Singer, A.J. and R.A. Clark, Cutaneous wound healing. N Engl J Med, 1999. **341**(10): p. 738-46.*
3. *Martin, P. and S.J. Leibovich, Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol, 2005. **15**(11): p. 599-607.*
4. *Eming, S.A., T. Krieg, and J.M. Davidson, Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol, 2007. **127**(3): p. 514-25.*

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

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

Uw referentie:

Onze referentie :

Maastricht, 26-10-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*The role of inflammation in cutaneous wound healing: modulation of inflammation by induction of an anti-inflammatory M2 wound macrophage phenotype*", is op de DEC vergadering van 21 oktober 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt de doelstelling beter te formuleren, de invloed van diabetes op wondgenezing en het mogelijke gunstige effect van het macrofaag phenotype dient hierin meegenomen te worden.
- De DEC verzoekt u te bevestigen dat "**dit**" DEC protocol wetenschappelijk beoordeeld en goedgekeurd is door...
- Punt 5- De DEC wenst een toelichting van de toegevoegde waarde van de metingen op dag 12, ten opzichte van dag 15, aangezien een zelfde eindpunt ertoe zou leiden dat 1 diergroep minder nodig is.
- Punt 7- De DEC is van mening dat de gekozen proefopzet niet geheel overeen lijkt te komen met de doelstelling van het protocol. De DEC wenst een toelichting waarom in eerste instantie vastgesteld zal worden wat het effect van macrofaag phenotype op wondgenezing in gezonde BL6 muizen is. De beschrijvingen bij 1 en 5 doet namelijk vermoeden dat een belangrijk effect wordt verwacht in de diabete dieren, waar de wondgenezing slecht verloopt. Indien begonnen wordt met het experiment met de diabete dieren en hieruit blijkt dat dit de wondgenezing gunstig beïnvloedt, wat is dan de meerwaarde van het experiment met de gezonde BL6 dieren?
- Punt 7c- groepsgrootte wordt uitgerekend met "10.04", en afgerond naar 10, de DEC adviseert 11 dieren per groep aan te houden (afronden naar boven). De DEC verzoekt dit aan te passen.
- Punt 8- De DEC vraagt zich af of de muizen 4 of 6 wonden krijgen? (in de tekst wordt gesproken over 4 wonden, de foto toont een dier met 6 wonden). De DEC vraagt zich af of het mogelijk is om in één muis verschillende behandelingen kunnen worden toegepast, met het oog op reductie van het aantal dieren.

- Bij punt 10a verzoekt de DEC in een tabel, de aard, ernst, duur en frequentie beter te definiëren per handeling en groep, het totale ongerief aan te geven en het voorblad in overeenstemming te brengen. Tevens verzoekt de DEC de copy/paste fouten (van het vorige DEC protocol) te verwijderen.
- Punt 10b- De DEC merkt op dat er eerder experimenten gedaan zijn. De DEC verzoekt deze experimenten in de welzijnsevaluatie te beschrijven.

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 **2011-128**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Reply to DEC for project number 2011-128

- *De DEC verzoekt de doelstelling beter te formuleren, de invloed van diabetes op wondgenezing en het mogelijke gunstige effect van het macrofaag phenotype dient hierin meegenomen te worden.*
  The influence of diabetes in wound healing has been more clearly described and also the effect of macrophage phenotype in determining diabetic wound healing has been included in paragraph 1.

- *De DEC verzoekt u te bevestigen dat "**dit**" DEC protocol wetenschappelijk beoordeeld en goedgekeurd is door...*
  A sentence has been added in paragraph 6 stating that the scientific validity of THIS DEC has been approved by

- *Punt 5- De DEC wenst een toelichting van de toegevoegde waarde van de metingen op dag 12, ten opzichte van dag 15, aangezien een zelfde eindpunt ertoe zou leiden dat 1 diergroep minder nodig is.*
  The final time point has been changed to day 15 since indeed this requires fewer mice.

- *Punt 7- De DEC is van mening dat de gekozen proefopzet niet geheel overeen lijkt te komen met de doelstelling van het protocol. De DEC wenst een toelichting waarom in eerste instantie vastgesteld zal worden wat het effect van macrofaag phenotype op wondgenezing in gezonde BL6 muizen is. De beschrijvingen bij 1 en 5 doet namelijk vermoeden dat een belangrijk effect wordt verwacht in de diabete dieren, waar de wondgenezing slecht verloopt. Indien begonnen wordt met het experiment met de diabete dieren en hieruit blijkt dat dit de wondgenezing gunstig beïnvloedt, wat is dan de meerwaarde van het experiment met de gezonde BL6 dieren?*
  Since wound healing in diabetic mice is a very slow process, we have decided to perform the first experiment in diabetic mice in order to be able to see if there is even a small improvement in the healing process after injection of M2 macrophages.
  If we do see that the healing is greatly improved in the diabetic mice, the next step will be to determine if injection of M2 macrophages is also beneficial in wild-type mice in which healing is faster. For this reason we are going to repeat the experiment in wild-type mice only if the improvement that we see in diabetic mice is very big.

- *Punt 7c- groepsgrootte wordt uitgerekend met "10.04", en afgerond naar 10, de DEC adviseert 11 dieren per groep aan te houden (afronden naar boven). De DEC verzoekt dit aan te passen.*
  The number has been changed in the DEC.

- *Punt 8- De DEC vraagt zich af of de muizen 4 of 6 wonden krijgen? (in de tekst vordt gesproken over 4 wonden, de foto toont een dier met 6 wonden). De DEC vraagt zich af of het mogelijk is om in één muis verschillende behandelingen kunnen worden toegepast, met het oog op reductie van het aantal dieren.*
  The mice will receive 4 wounds. The picture is from a publication and is used as an example to show how these type of experiments are performed.
  It is not possible to use one mouse for different treatments since it has been shown before that there is interaction of the treatment in one wound to the healing of the wound nearby.

- *Bij punt 10a verzoekt de DEC in een tabel, de aard, ernst, duur en frequentie beter te definiëren per handeling en groep, het totale ongerief aan te geven en het voorblad in overeenstemming te brengen. Tevens verzoekt de DEC de copy/paste fouten (van het vorige DEC protocol) te verwijderen.*
  The table has been added to the DEC.

- *Punt 10b- De DEC merkt op dat er eerder experimenten gedaan zijn. De DEC verzoekt deze experimenten in de welzijnsevaluatie te beschrijven.*
  The paragraph has been changed in the protocol.

University Maastricht

Faculty of Health, Medicine
and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

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

Uw referentie: Onze referentie : Maastricht, 19-12-2011

Geachte Onderzoeker,

De **herziene versie** van uw projectaanvraag: "*The role of inflammation in cutaneous wound healing: modulation of inflammation by induction of an anti-inflammatory M2 wound macrophage phenotype*", is op de DEC vergadering van 16 december 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) Punt 5- Het aangeven van de toegevoegde waarde van de metingen op dag 12, ten opzichte van dag 15, werd gevraagd bij de 1ste aanhouding, dit is nog niet volledig aangepast.
2) De DEC heeft eerder verzocht om het ongerief bij punt 10a per groep aan te geven, dit is echter niet gebeurd. De DEC verzoekt dit alsnog te doen en het ongerief van de anesthesie schat de DEC in op code 02.

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 **2011-128**, gelieve dit nummer in verdere correspondentie te vermelden.

De DEC-UM wenst u en uw familie fijne feestdagen en een voorspoedig en vooral gezond 2012!

Hoogachtend,

Voorzitter DEC-UM

Reply to DEC for project number 2011-128

1) *Punt 5- Het aangeven van de toegevoegde waarde van de metingen op dag 12, ten opzichte van dag 15, werd gevraagd bij de 1ste aanhouding, dit is nog niet volledig aangepast.*

We have changed the day of isolation of biopsies from day 12 to day 15. So we are going to isolate biopsies on days 4, 8 and 15 after wounding as indicated in the figure in page 4.

2) *De DEC heeft eerder verzocht om het ongerief bij punt 10a per groep aan te geven, dit is echter niet gebeurd. De DEC verzoekt dit*

A different table has been added for each group of wild-type and diabetic mice.

From:
Sent: maandag 16 januari 2012 13:57
To:
Subject: FW: Project 2011-128-herziene versie-w
Attachments: answers to DEC questions 2011-128.doc; 2011-128 DEC.doc

Geachte Onderzoeker, beste ,

De DEC en de proefdierdeskundige hebben je herziene versie nagekeken en hebben nog de volgende vragen/opmerkingen:

Opmerking bij vraag 2 van de DEC:
Er is een tabel toegevoegd (identiek voor wt en db/db mice).
In de tekst daarboven staat dat "taken digital pictures' (onder anesthesie) 01 is, in de tabel staat 02 in grijs, dit moet in overeenstemming zijn.
Qua totaal ongerief: elke 2 dagen anesthesie kan wellicht leiden tot totaal 03. De DEC verzoekt de tabel bij 10a en het voorblad in overeenstemming te brengen.

Opmerking bij punt 6 van de aanvraag:
De DEC verzoekt de namen te verwijderen. Het is niet de bedoeling dat verwezen wordt naar personen in de aanvraag, in verband met de Wet Openbaarheid van Bestuur. Het is voldoende als de PI op het voorblad staat.

Graag je reactie zodat ik de aavraag kan afhandelen.

Met vriendelijke groet namens DEC-UM:

Ambtelijk Secretaris Dierexperimentencommissie

Postbus 616-UNS 50-Box 48, 6200 MD Maastricht
T 043
E-mail:

Werktijden: Ma-Di-Wo-Don van 08.00 uur tot 16.00 uur

From:
Sent: donderdag 5 januari 2012 12:40
To:
Subject: FW: Project 2011-128-herziene versie-w

Dear
Here is the changed DEC and the answers to the questions of the DEC.
Hopefully this time is ok.
Greetings,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	19-01-2012

Project: *The role of inflammation in cutaneous wound healing: modulation of inflammation by induction of an anti-inflammatory M2 wound macrophage phenotype.*

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:	**2011-128**
Diersoort:	**muis**
Aantal dieren:	**264**
Einddatum:	**16-01-2016**

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

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

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6200 MD Maastricht