

	<b>Dossier: AVD11500202518775</b>	
		<b>Aanwezig</b>
<b>1</b>	<b>NTS</b>	<b>X</b>
<b>2</b>	<b>Aanvraagformulier</b>	<b>X</b>
<b>3</b>	<b>Projectvoorstel</b>	<b>X</b>
<b>4</b>	<b>Bijlage beschrijving dierproeven</b>	<b>2X</b>
<b>5</b>	<b>DEC-advies</b>	<b>X</b>
<b>6</b>	<b>Ontvangstbevestiging</b>	<b>X</b>
	<b>Evt. Vragen CCD aan aanvrager</b>	
	<b>Evt. antwoorden aanvrager</b>	
<b>7</b>	<b>Beschikking en vergunning</b>	<b>X</b>

## NIET-TECHNISCHE PROJECTSAMENVATTING

Naam van het project	Ontwikkeling van nieuwe methoden om hardnekkige bacteriële infecties te bestrijden
NTS-identificatiecode	NTS-NL-797198 v.1, 19-06-2025
Land	Nederland
Taal	nl
Duur van het project, uitgedrukt in maanden.	36
Trefwoorden	Biofilm Antimicrobiële resistentie Nanodeeltjes Aptameren Therapie
Doel(en) van het project	Fundamenteel onderzoek: Spier- en skeletstelsel Omzettinggericht en toegepast onderzoek: Spier- en botaandoeningen bij de mens

## DOELSTELLINGEN EN VERWACHTE VOORDELEN VAN HET PROJECT

Beschrijf de doelstellingen van het project (bijvoorbeeld het aanpakken van bepaalde wetenschappelijke onduidelijkheden, of wetenschappelijke of klinische behoeften).	<p>Bacteriële ontstekingen, oftewel ziektes door bacteriën, vormen een ernstig medisch probleem. Ze kunnen onder andere ontstaan wanneer bacteriën groeien op dingen die dokters in het lichaam plaatsen, zoals kunstgewrichten of hartkleppen. De huidige medicijnen werken vaak niet goed omdat bacteriën een kleverige laag vormen die als een schild werkt en waar gewone medicijnen niet doorheen kunnen komen. In zulke gevallen hebben patiënten meestal een nieuwe operatie nodig om het besmette onderdeel te verwijderen en te vervangen. Dit is gevaarlijk en duur.</p> <p>Ons project richt zich op twee grote problemen. Ten eerste willen we betere medicijnen ontwikkelen voor deze ontstekingen. We werken aan speciale kleine deeltjes die medicijnen rechtstreeks naar de ontsteking kunnen brengen en door die kleverige laag heen kunnen dringen. Je kunt ze zien als slimme pakketjes die specifiek de ontsteking aanpakken zonder gezond weefsel te beschadigen. Ten tweede willen we betere manieren ontwikkelen om deze ontstekingen vroeg te ontdekken. Op dit moment merken artsen vaak pas dat er een probleem is als de ontsteking al ernstig is. We ontwikkelen een nieuwe soort scan die veel eerder kan laten zien of er bacteriën zitten op wat in het lichaam is geplaatst. Het is vergelijkbaar met een speciale foto die specifiek bacteriën kan zien.</p> <p>Om deze nieuwe medicijnen en opsporingsmethoden te testen, gebruiken we muizen waarin we kleine voorwerpen plaatsen. Hoewel we veel tests in het lab hebben gedaan, is het belangrijk te begrijpen hoe deze medicijnen werken in levende wezens, waar veel factoren invloed kunnen hebben. We onderzoeken welke behandeling het beste werkt, of onze nieuwe scan echt kan zien waar de bacteriën zitten, en hoeveel medicijn het veiligst en best werkt. Dit onderzoek is belangrijk, omdat labproeven alleen niet kunnen voorspellen hoe goed deze medicijnen in levende lichamen werken, waar factoren zoals bloed, afweer en genezing een rol spelen.</p> <p>Deze studie kan leiden tot betere manieren om ontstekingen bij mensen te behandelen, mogelijk zonder extra operaties. Hierdoor kunnen patiënten sneller beter worden. Door zowel de medicijnen als de vroege ontdekken van ontstekingen te verbeteren, hopen we dat patiënten minder pijn hebben, dat de kosten lager worden en dat mensen die deze hulpmiddelen nodig hebben betere kansen hebben.</p>
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Welke potentiële voordelen kan dit project opleveren? Leg uit hoe de wetenschap vooruit kan worden geholpen of mensen, dieren of het milieu uiteindelijk	Dit onderzoeksproject biedt aanzienlijke voordelen voor zowel de medische wetenschap als de zorg voor patiënten. Op de korte termijn zal ons werk bijdragen aan een beter begrip van hoe bacteriën groeien op dingen die in het lichaam zijn geplaatst en hoe nieuwe, gerichte medicijnen deze ontstekingen kunnen bestrijden. Door deze ontstekingen te bestuderen in proefdieren, verkrijgen we waardevolle inzichten in hoe goed onze nieuwe behandelingen en scans werken.
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voordeel kunnen hebben bij het project. Maak, waar van toepassing, een onderscheid tussen voordelen op korte termijn (binnen de looptijd van het project) en voordelen op lange termijn (die mogelijk pas worden bereikt nadat het project is afgerond).

De directe voordelen voor de wetenschap omvatten het ontwikkelen en testen van nieuwe methoden om ontstekingen vroeg te ontdekken, voordat ze ernstig worden. Deze kennis helpt ons te begrijpen hoe bacteriën zich gedragen in het echte leven en hoe onze medicijnen werken in levende wezens. We leren ook belangrijke informatie over hoeveel medicijn we moeten geven en wanneer.

De voordelen van dit onderzoek zijn dat op lange termijn de behandeling voor ontstekingen verbeterd kan worden. Als de nieuwe methode succesvol is, kan dit leiden tot nieuwe medicijnen die ontstekingen effectief opruimen zonder dat een extra operatie nodig is. Dit zou minder pijn voor patiënten betekenen, het risico op problemen en de kosten verminderen. Onze beeldvormingstechniek kan een waardevol hulpmiddel worden voor artsen om ontstekingen vroeg te vinden, zodat snelle behandeling mogelijk is, voordat de ontstekingen ernstig worden.

Dit alles kan miljoenen patiënten wereldwijd helpen die jaarlijks kunstonderdelen in hun lichaam krijgen. Betere medicijnen en methoden voor vroege ontdekking kunnen vooral oudere patiënten en mensen met een zwakke afweer helpen, omdat zij een hoger risico lopen op ontstekingen. Het geldelijke effect kan ook groot zijn, omdat minder operaties en kortere ziekenhuisopnames geld besparen.

De kennis uit dit onderzoek kan ook bijdragen aan een breder begrip van ontstekingen door bacteriën en hoe deze bestreden kunnen worden. Wat we leren over hoe we medicijnen precies kunnen brengen waar ze nodig zijn en hoe we kunnen zien of er schadelijke bacteriën zijn, kunnen we misschien ook gebruiken bij andere ziektes. Dit kan nieuwe wegen openen voor onderzoek en de ontwikkeling van medicijnen in andere medische gebieden, wat uiteindelijk zowel mens als dier helpt.

## VOORSPELDE SCHADE

In welke procedures worden de dieren gewoonlijk gebruikt (bijvoorbeeld injecties, chirurgische procedures)? Vermeld het aantal en de duur van deze procedures.

De dieren zullen procedures ondergaan in twee modellen, waarbij we op gecontroleerde manier ontstekingen veroorzaken en bestuderen. In het eerste onderzoek maken we een wond bij de dieren. Dit gebeurt terwijl ze in slaap zijn gebracht met verdoving via de luchtwegen. Dit duurt vijftien minuten. Daarna krijgen ze een pijnstillertjes ingespoten. Op dag 2 brengen we bacteriën aan op de wond met een injectie. Tussen dag 4 en 9 geven we één keer een behandeling direct op de wond (dit duurt 5 tot 10 minuten).

In het tweede onderzoek plaatsen we een metalen implantaat bij de dieren. Ook dit gebeurt terwijl ze in slaap zijn gebracht met verdoving via de luchtwegen (15 minuten), gevolgd door een pijnstillertjes. Het implantaat is van tevoren besmet met bacteriën. De behandeling is hetzelfde als bij het eerste onderzoek, maar nu krijgen de dieren tussen dag 4 en 9 één injectie in een ader in hun staart. Op dag 9 nemen we wat bloed af uit hun staart. We maken ook vier keer foto's van de dieren (op dag 0, 1, 3 en 7 na de injectie). Hiervoor worden de dieren steeds kort in slaap gebracht (15 tot 20 minuten). Bij beide onderzoeken worden de dieren op dag 9 gedood om hun weefsels te kunnen onderzoeken.

Alle procedures worden uitgevoerd door goed opgeleide mensen onder schone omstandigheden waar nodig, en de dieren krijgen goede verdoving en pijnstilling tijdens het hele onderzoek.

Wat zijn de verwachte gevolgen/nadelige effecten voor de dieren, bijvoorbeeld pijn, gewichtsverlies, inactiviteit/verminderde mobiliteit, stress, abnormaal gedrag, en wat is de duur van die effecten?

De dieren kunnen bijwerkingen ervaren. Na het maken van een wond of plaatsen van een implantaat wordt gemiddelde pijn verwacht gedurende 2–48 uur, behandeld met pijnstillertjes. Ontsteking en licht ongeriefongemak op de plek van de operatie kunnen 3–4 dagen aanhouden, met minder activiteit, eetlust en mogelijk tijdelijk gewichtsverlies van 5–10%.

Na toediening van bacteriën kunnen lokale infectiesymptomen optreden (zwellen, roodheid, verhoogde temperatuur). Algemene klachten kunnen slaperigheid, vermoeidheid, verminderde eetlust en lichte koorts omvatten, die 2–3 dagen kunnen duren en verbeteren na start behandeling op dag 4.

Herhaalde staartaderprikken kunnen tijdelijke stress en lokaal ongeriefongemak veroorzaken, enkele minuten per procedure. Ook verwachten we korte stressmomenten tijdens beeldvorming door hantering en inhalatie-anesthesie (15–20 minuten), met herstel binnen 10–15 minuten en tijdelijke verwarring.

Doordat dieren, die van nature sociaal leven, soms alleen komen te zitten (2–9 dagen), kan dat extra ongerief veroorzaken.

Bij de dieren kan er zogeheten sepsis optreden als complicatie van de besmetting met bacteriën, hierbij zal in het hele dier een afweerreactie op de bacterie zijn. Dat is net als bij mensen, een ernstige complicatie, waarbij het dier slap wordt en spontaan kan overlijden. We monitoren hier strikt op en zullen de dieren waarbij dit (mogelijk) optreedt direct doden om ongerief verder in te perken. Mocht dit toch bij dieren optreden, dan zullen die dieren ernstig ongerief ondergaan. In eerdere studies kwam dit niet voor, we denken dat het bij maximaal 15 dieren in dit project zou kunnen gebeuren.

Bloedafnames kunnen kort ongeriefongemak en stress geven. Het kleine afgenomen volume (100 µL) zal naar verwachting geen merkbare veranderingen in het lichaam hebben. Dieren worden goed gemonitord op stress, pijn, gewichtsverlies (>20%) of ander ongeriefongemak. Bij ernstige bijwerkingen of lijden (in overleg met dierverzorgers) worden dieren onmiddellijk gedood.

Alle bijwerkingen zijn tijdelijk en beheersbaar met goede zorg en pijnstilling. Dieren hervatten binnen 24–48 uur normaal gedrag en eetpatroon. Volledige genezing wordt verwacht, met herstel van conditie aan het einde van de studie.

Welke soorten en aantallen dieren zullen naar verwachting worden gebruikt? Wat zijn de verwachte ernstgraden en de aantallen dieren in elke ernstcategorie (per soort)?

Soort:	Totaal aantal	Geraamde aantallen naar ernstgraad			
		Terminaal	Licht	Matig	Ernstig
Muizen ( <i>Mus musculus</i> )	149	0	0	134	15

Wat gebeurt er met de dieren die aan het einde van de procedure in leven worden gehouden?

Soort:	Geraamd aantal te hergebruiken, in het habitat-/houderijsysteem terug te plaatsen of voor adoptie vrij te geven dieren		
	Hergebruikt	Teruggeplaatst	Geadopteerd

Geef de redenen voor het geplande lot van de dieren na de procedure.

De dieren worden aan het einde van het onderzoek (dag 9) pijnloos gedood. Dit is nodig om verschillende redenen.

Ten eerste moeten we stukjes weefsel nemen van de plekken met infectie en het gebied eromheen. Zo kunnen we goed zien of de behandeling heeft gewerkt. We kijken naar hoeveel bacteriën er nog zijn, of het weefsel beschadigd is of juist geneest, en of de bacteriën zich hebben verspreid. Dit kunnen we alleen doen nadat het dier is overleden.

Ten tweede moeten we binnen in het lichaam kijken om te controleren of de infectie zich buiten de wondlocatie heeft verspreid en om te zien waar de behandeling in het lichaam terecht is gekomen. Dit is belangrijk om te weten of de behandeling veilig en effectief is.

Ten derde mogen dieren die zijn gebruikt in infectiestudies niet in leven worden gehouden of opnieuw worden gebruikt in andere studies vanwege veiligheidsregels en dierenwelzijnsbeleid. Dit zijn regels voor veiligheid en dierenwelzijn die altijd gelden bij dit soort onderzoek.

Ten vierde kunnen we de belangrijkste gegevens alleen verzamelen door het weefsel grondig te onderzoeken na het overlijden van het dier. We tellen dan de bacteriën, meten de ontsteking en kijken hoe goed het weefsel is genezen. Deze informatie kunnen we niet krijgen van levende dieren.

Het doden van de dieren gebeurt zonder pijn, onder verdoving, door mensen die hier speciaal voor zijn opgeleid. Dit grondige onderzoek van het weefsel is nodig om te bepalen of de behandeling veilig en effectief is, zodat het misschien later voor patiënten gebruikt kan worden.

## TOEPASSING VAN DE DRIE V'S

### 1. Vervanging

Beschrijf welke diervrije alternatieven op dit gebied voorhanden zijn en waarom zij niet voor het project kunnen worden gebruikt.

In het laboratorium gekweekte cellen en weefsels kunnen ons basisinformatie geven over infecties en immuunreacties. Hoewel deze nuttig zijn voor vroege tests, laten ze niet zien hoe een volledig lichaam een infectie bestrijdt. Ze missen belangrijke aspecten, zoals hoe verschillende organen samenwerken en hoe het lichaam als geheel reageert.

Computerprogramma's kunnen helpen voorspellen hoe infecties zich ontwikkelen en hoe medicijnen kunnen werken. Maar computers kunnen de complexe werking van het immuunsysteem nog niet volledig nabootsen. Ze kunnen niet nabootsen hoe alles in ons lichaam samenwerkt om ons gezond te houden.

Nieuwe uitvindingen zoals "organen-op-chips" zijn interessant, maar ze kunnen nog niet laten zien hoe ons hele lichaam vecht tegen ziektes.

Voor ons onderzoek hebben we een compleet lichaam nodig om te begrijpen hoe ziektes werken en hoe we ze kunnen behandelen. We proberen zo min mogelijk dieren te gebruiken en zoeken steeds naar betere alternatieven, maar soms zijn dierproeven nog nodig om nieuwe medicijnen te ontwikkelen die mensen kunnen helpen.

### 2. Vermindering

Leg uit hoe de aantallen dieren voor dit project zijn bepaald. Beschrijf de stappen die zijn genomen om het aantal te gebruiken dieren te verminderen en de beginselen die zijn gebruikt bij het opzetten van de studies. Beschrijf, waar van toepassing, de praktijken die gedurende het hele project zullen worden toegepast om het aantal dieren die in overeenstemming met de wetenschappelijke doelstellingen werden gebruikt, tot een minimum te beperken. Deze praktijken kunnen bijvoorbeeld bestaan uit proefprojecten, computermodellen, het delen van weefsel en hergebruik.

We hebben zorgvuldig gepland hoeveel dieren we nodig hebben voor dit onderzoek, zodat we zo min mogelijk dieren gebruiken en toch betrouwbare resultaten krijgen.

We hebben samengewerkt met statistiekexperts om het kleinste aantal dieren te berekenen dat nodig is om betekenisvolle resultaten te verkrijgen. Dit helpt ons te voorkomen dat we te veel dieren gebruiken of te weinig, wat zou betekenen dat we het onderzoek moeten herhalen en daardoor onnodig dieren zouden verspillen.

Voor we aan een grote studie beginnen, voeren we eerst kleine teststudies uit. Hiermee controleren we of onze methoden werken en kunnen we de hoofdstudie beter plannen. Zo vermijden we dat dieren worden gebruikt in studies die mogelijk niet werken.

We delen weefsels en monsters waar mogelijk. Wanneer we dierlijke weefsels moeten bestuderen, proberen we meerdere verschillende monsters van elk dier te nemen. Dit betekent dat we minder dieren nodig hebben.

We gebruiken computermodellen en in het laboratorium gekweekte cellen voor vroege tests wanneer dat kan. Dit helpt ons te begrijpen waar we op moeten letten bij het gebruik van dieren, waardoor die studies efficiënter worden.

We houden gedetailleerde dossiers bij van al onze resultaten, delen data met andere onderzoeksteams, gebruiken de modernste testmethoden, trainen al het personeel goed om mislukte experimenten te voorkomen, en plannen experimenten zorgvuldig om zoveel mogelijk informatie uit elk dier te halen.

We evalueren onze methoden regelmatig om nieuwe manieren te vinden om het aantal dieren te verminderen. Zodra betere testmethoden beschikbaar komen, zullen we deze gebruiken om het aantal benodigde dieren verder te verlagen.

### 3. Verfijning

Geef voorbeelden van de specifieke maatregelen (bv. verscherpte monitoring, postoperatieve behandeling,

We zorgen ervoor dat onze dieren tijdens het onderzoek zo min mogelijk pijn ervaren. We houden ze heel goed in de gaten en helpen ze direct als ze tekenen van pijn vertonen, e.e.a. in overleg met ervaren dierversorgers.

Ons getrainde personeel controleert de dieren dagelijks. Ze letten op tekenen dat een dier niet lekker in zijn vel zit. Als ze problemen zien, helpen ze het dier meteen.

<p>pijnbestrijding, training van dieren) die in verband met de procedures moeten worden genomen om de welzijnskosten (schade) voor de dieren tot een minimum te beperken. Beschrijf de mechanismen om gedurende de looptijd van het project nieuwe verfijningstechnieken in gebruik te nemen.</p>	<p>We gaan heel voorzichtig om met de dieren. Ons personeel leert speciale technieken om dieren te hanteren zonder ze bang te maken. We laten de dieren wennen aan hun omgeving en aan de verzorgers voordat we met het onderzoek beginnen.</p> <p>Wanneer we procedures moeten uitvoeren, geven we medicijnen om pijn te voorkomen. We geven pijnstillers vóór, tijdens en na de procedure om de dieren comfortabel te houden. Na een procedure krijgen de dieren extra zorg om ervoor te zorgen dat ze goed herstellen.</p> <p>De dieren leven in schone, comfortabele ruimtes met een goede temperatuur en verlichting. Ze krijgen goed voedsel en schoon water. Ze hebben ruimte om te bewegen en speelgoed om ze actief en gezond te houden.</p> <p>We stoppen elke procedure als een dier te veel ongerief vertoont. We blijven altijd zoeken naar nieuwe, betere manieren om ons werk uit te voeren die minder belastend zijn voor de dieren.</p> <p>We werken samen met dierenartsen om onze zorg voortdurend te verbeteren. We willen goed onderzoek doen terwijl we onze dieren gezond en comfortabel houden.</p>
<p>Licht de keuze van de soorten en de bijbehorende levensstadia toe</p>	<p>We zullen volwassen muizen (acht tot tien weken oud) gebruiken voor deze studie. Muizen zijn uitstekende modellen voor het bestuderen van infecties omdat hun immuunsysteem op een vergelijkbare manier werkt als dat van mensen. Ze worden veel gebruikt in infectieonderzoek, waardoor we beter kunnen begrijpen hoe onze behandeling mogelijk bij mensen werkt. Hun kleine formaat maakt ze ook praktisch voor laboratoriumstudies.</p> <p>We hebben gekozen voor volwassen muizen omdat hun immuunsysteem op deze leeftijd volledig ontwikkeld is en ze in goede gezondheid verkeren. Het gebruik van muizen van acht tot tien weken oud helpt ons betrouwbare resultaten te krijgen, omdat ze volwassen genoeg zijn om de procedures goed te doorstaan, maar niet zo oud dat leeftijdsgebonden veranderingen onze bevindingen beïnvloeden. Deze leeftijdscategorie is standaard in vergelijkbaar onderzoek, wat het eenvoudiger maakt om onze resultaten met andere studies te vergelijken.</p> <p>Deze keuzes helpen ons om duidelijke, betrouwbare resultaten te verkrijgen terwijl we het aantal gebruikte dieren tot een minimum beperken. Onze aanpak zorgt ervoor dat het onderzoek waardevolle informatie oplevert, met behoud van hoge normen voor dierenwelzijn.</p>

## VOOR EEN BEOORDELING ACHTERAF GESELECTEERD PROJECT

Project geselecteerd voor BA?	ja
Termijn voor BA	31-05-2029
<a href="#">Reden voor de beoordeling achteraf</a>	
Bevat ernstige procedures	ja
Maakt gebruik van niet-menselijke primaten	
Andere reden	
Toelichting van de andere reden voor de beoordeling achteraf	

## AANVULLENDE VELDEN

Link naar de eerdere versie van de NTS buiten het EC-systeem	
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## 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.centralecommissiedierproeven.nl](http://www.centralecommissiedierproeven.nl) of in de toelichting op de website.
- Of neem telefonisch contact op (0900-2800028).

#### 1 Gegevens aanvrager

1.1	Heeft u een deelnemernummer van de NVWA? <i>Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.</i>	<input checked="" type="checkbox"/> Ja > Vul uw deelnemernummer in	11500
		<input type="checkbox"/> Nee > U kunt geen aanvraag doen	
1.2	Wat voor aanvraag doet u?	<input checked="" type="checkbox"/> Nieuwe aanvraag > Ga verder met vraag 1.3	
		<input type="checkbox"/> Wijziging > Vul hiernaast het AVD nummer van uw vergunde project in en ga verder met vraag 2.1	
		<input type="checkbox"/> Melding > Vul hiernaast het AVD nummer van uw vergunde project in en ga verder met vraag 2.2	
1.3	Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.	Naam instelling of organisatie	Universiteit Utrecht
		Titel, voorletters en achternaam van de portefeuillehouder	Titel Voorletters Achternaam <input checked="" type="checkbox"/> Dhr <input type="checkbox"/> Mw
		E-mailadres contactpersoon	info@ivd-utrecht.nl
		Titel, voorletters en achternaam van diens gemachtigde (indien van toepassing)	Titel Voorletters Achternaam <input type="checkbox"/> Dhr <input type="checkbox"/> Mw
		E-mailadres gemachtigde	
	Vul de gegevens van het postadres in.	Straat- en huisnummer	Instantie voor Dierenwelzijn Utrecht 50
		Postcode en plaats	3584CJ UTRECHT
		Postbus, postcode en plaats	80.125 3508TC UTRECHT
1.4	Vul de gegevens in van de verantwoordelijke onderzoeker.	(Titel) Naam en voorletters	<input type="checkbox"/> Dhr <input checked="" type="checkbox"/> Mw
		Functie	Assistent Professor
		Afdeling	Orthopedie
		Telefoonnummer	
		E-mailadres	

1.5 (Indien van toepassing) Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.	(Titel) Naam en voorletters		<input type="checkbox"/> Dhr <input type="checkbox"/> Mw
	Functie		
	Afdeling		
	Telefoonnummer		
	E-mailadres		
1.6 (Indien van toepassing) Vul hier de gegevens in van de persoon aan wie de portefeuillehouder de verantwoordelijkheid inzake de algemene uitvoering van het project en de overeenstemming daarvan met de projectvergunning heeft gedelegeerd.	(Titel) Naam en voorletters		<input type="checkbox"/> Dhr <input type="checkbox"/> Mw
	Functie		
	Afdeling		
	Telefoonnummer		
	E-mailadres		
1.7 (Optioneel) Vul hier de gegevens in van de Instantie voor Dierenwelzijn	Telefoonnummer	030-2531569	
	E-mailadres	info@ivd-utrecht.nl	
1.8 Is er voor deze projectaanvraag een gemachtigde?	<input type="checkbox"/> Ja > Stuur dan het ingevulde formulier <i>Melding Machtiging</i> mee met deze aanvraag		
	<input checked="" type="checkbox"/> Nee		

## 2 Over uw aanvraag

2.1 Gaat uw aanvraag over een <i>wijziging</i> op een vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn?	<input checked="" type="checkbox"/> Nee > Ga verder met vraag 3
	<input type="checkbox"/> Ja > Geef hieronder kort de wijziging en de onderbouwing daarvan weer. Geef in de originele formulieren (niet-technische samenvatting, projectvoorstel en bijlage dierproeven) duidelijk aan (bij voorbeeld in een andere kleur) waar de projectaanvraag wijzigt. Ga daarna verder met vraag 6.
2.2 Gaat uw aanvraag over een <i>melding</i> op een vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn?	<input type="checkbox"/> Nee > Ga verder met vraag 3
	<input type="checkbox"/> Ja > Geef hieronder weer wat deze melding inhoudt en ga verder met vraag 6.

## 3 Over uw project

3.1 Wat is de geplande start- en einddatum van het project?	Startdatum	10-03-2025
	Einddatum (t/m)	3 jaar na verlenen vergunning
3.2 Wat is de titel van het project?	Antimicrobial strategies for drug-resistant and biofilm-associated infections	
3.3 Wat is de titel van de niet-technische samenvatting?	Ontwikkeling van nieuwe methoden om hardnekkige bacteriële infecties te bestrijden	
3.4 Wat is de naam van de Dierexperimentencommissie (DEC) van voorkeur?	Naam DEC	DEC Utrecht
	Postadres	Postbus 85500, 3508 GA UTRECHT
	E-mailadres	dec-utrecht@umcutrecht.nl

## 4 Factuurgegevens

4.1 (Indien factuuradres afwijkt van de gegevens uit vraag 1.3) Vul de gegevens van het factuuradres in.

Naam: UU-ASC		Afdeling:	
Straat:			Huisnummer:
Postcode:	Plaats:		
Postbus: 80.011	Postcode: 3508TA	Plaats: UTRECHT	
E-mail: asc.factuur@uu.nl			

4.2 (Optioneel.) Vul hier het ordernummer van de instelling in.

Ordernummer:
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## 5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht	
<input checked="" type="checkbox"/> Projectvoorstel	Aantal bijlage(n) dierproeven 2
<input checked="" type="checkbox"/> Niet-technische samenvatting	
Overige bijlagen, indien van toepassing	
<input type="checkbox"/> Melding machtiging	
<input type="checkbox"/>	

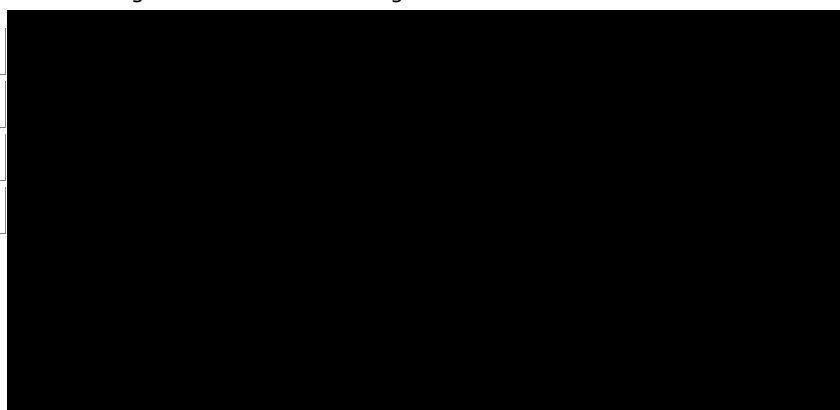
## 6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD en per post naar de Centrale Commissie Dierproeven (voor adresgegevens zie website)

Ondertekening door de portefeuillehouder namens de instellingsvergunninghouder of gemachtigde (zie 1.8). 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 C 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
Functie
Plaats
Datum
Handtekening





## Form Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website ([www.centralecommissiedierproeven.nl](http://www.centralecommissiedierproeven.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'.	11500
1.2 Provide the name of the licenced establishment.	UMC Utrecht
1.3 Provide the title of the project.	Antimicrobial strategies for drug-resistant and biofilm-associated infections

### 2 Categories

2.1 Please tick each of the following boxes that applies to your project.	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational or applied research
	<input type="checkbox"/> Regulatory use or routine production
	<input type="checkbox"/> Research into environmental protection in the interest of human or animal
	<input type="checkbox"/> Research aimed at preserving the species subjected to procedures
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals not used in other animal procedures

### 3 General description of the project

#### 3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.1.

#### Problem

Biofilm-associated infections are a significant global health challenge owing to their inherent resistance to standard treatments. Biofilms, consisting of microorganisms encased in an extracellular matrix, account for 65-80% of chronic bacterial infections and play a substantial role in hospital-acquired infections (Assefa & Amare, 2022; Cangui-Panchi et al., 2022; Jamal et al., 2018). The biofilm matrix functions as a physical barrier that impedes the effective penetration of antibiotics (Shree et al., 2023). Bacteria within the biofilm are often in a

dormant state, making them less susceptible to antibiotics that target actively dividing cells (Lewis, 2007). Additionally, bacteria in biofilms exhibit unique phenotypic characteristics, harbor highly tolerant cells, and exist in spatial and metabolic conditions that facilitate the horizontal transfer of resistance genes (Gebreyohannes et al., 2019; Uruén et al., 2020).

Biofilm-related implant infections are a growing concern in orthopaedic implants and are increasingly recognized in medical research. Periprosthetic joint infections (PJIs) occur in approximately 1–2% of primary joint replacements and 3–5% of revision surgeries (Izakovicova et al., 2019). Once a PJI is established, infection-control procedures such as debridement, antibiotics, and implant retention (DAIR) or revision surgeries achieve success rates of 77–84%. However, biofilm persistence leads to treatment failure in approximately 15–20% of cases, resulting in recurrent infections and further surgical interventions (Maimaiti et al., 2023; Pangaud et al., 2019). These infections transform routine medical interventions into complex clinical challenges, with deep implications for patient recovery and healthcare systems. Studies by Costerton et al. have demonstrated that biofilm-associated implant infections can increase patient hospitalization by an average of 7–10 days and raise treatment costs by 300–400% (Costerton et al., 1999). The economic burden is substantial, with estimated annual healthcare costs in the United States exceeding \$1.5 billion (Kurtz et al., 2012). Notably, a landmark study by Darouiche revealed that prosthetic joint infections lead to a mortality rate of 6–7%, underscoring that each infection represents more than a medical setback—it is a complex interplay of bacterial resilience, host vulnerability, and systemic medical challenges (Darouiche, 2004).

The rise of antimicrobial resistance makes these problems much worse, turning a once-manageable health issue into a possible worldwide crisis. The World Health Organisation warns that by 2050, infections that resist treatment could lead to 10 million deaths each year and cause about \$100 trillion in economic losses. This amount is more than what the world currently spends on healthcare in a year (O'Neill, 2016). These are not speculative predictions but extrapolations based on current infectious disease trends.

Wound infections represent a critical intersection of bacterial pathogenicity and host vulnerability. Unlike brief bacterial exposures, these infections are characterized by complex biofilm formations that create persistent microenvironments resistant to traditional treatments. Research indicates that up to 90% of chronic wounds contain mature biofilms (James et al., 2008), with these bacterial communities capable of increasing antibiotic tolerance by up to 1000 times compared to free-floating bacterial states (Stewart & Costerton, 2001). This medical issue is best illustrated by diabetic foot ulcers. About 15–25% of people with diabetes get ulcers, and 50% of them get infected. These are intricate bacterial ecosystems where multidrug-resistant organisms can persist for months, not just clinical complications. There are serious repercussions, including the possibility of amputation in 15–20% of cases and significant financial burdens due to treatment expenses that can range from \$20,000 to \$50,000 per patient (Naha et al., 2019).

Current diagnostic approaches for biofilm-associated infections, especially in relation to implants, represent a significant obstacle in modern medical treatment, with both technological and methodological limitations creating concerning delays in patient care (Donlan, 2001). Traditional microbiological methods require 48–72 hours for bacterial identification—a critical timeframe during which infections can progress from manageable conditions to severe systemic complications. This diagnostic delay is particularly problematic because biofilms undergo rapid structural changes within hours, while existing imaging techniques often lack the sensitivity to detect early-stage formations (Lewis, 2007). The situation leaves clinicians "blind" until infections reach advanced stages, with current diagnostic modalities missing approximately 60–70% of early biofilm developments, especially in complex medical environments like implant surfaces and chronic wounds (Lewis, 2007). This diagnostic challenge has profound implications for patient outcomes, as each hour of delayed diagnosis can exponentially increase treatment complexity, patient morbidity, and healthcare costs.

These challenges highlight the urgent need to develop innovative antimicrobial strategies and early diagnostic techniques.

### **Possible solutions**

Recent advances in nanotechnology and molecular biology have opened new horizons for addressing biofilm-associated infections through multiple innovative approaches. These emerging solutions show particular promise in overcoming the limitations of conventional treatments while offering new possibilities for both therapeutic and diagnostic applications.

**1. Use of nanoparticle-based antimicrobials to treat infections.** Nanoparticles have emerged as a promising class of antimicrobial agents due to their unique physicochemical properties (Mohanta et al., 2023; Mutalik et al., 2023; Tungare et al., 2024; van de Looij et al., 2022). Nanoparticles establish strong electrostatic interactions with the negatively charged biofilm matrix (Lee et al., 2022; Pinto et al., 2022), significantly improving the localization and retention of the encapsulated antimicrobial agents at the infection site. This electrostatic affinity enables deeper penetration of nanoparticles into structured biofilm layers, optimizing therapeutic effect (Lee et al., 2022). Nanoparticles exhibit multimodal antibacterial mechanisms, including the generation of reactive oxygen species (Kuo et al., 2022; Zhang et al., 2022), which disrupt bacterial membranes, direct membrane interaction leading to leakage of intracellular contents, and ion release mechanisms (Draviana et al., 2023; Zheng et al., 2022). These diverse mechanisms make it significantly more challenging for bacteria to develop resistance, providing a crucial advantage over traditional antibiotics. Furthermore, nanoparticles can be engineered with specific surface modifications to enhance their interaction with bacterial cells while minimizing effects on host tissues, providing an unprecedented level of treatment specificity (Ly et al., 2024).

**2. Use of aptamer technology to improve targeting specificity.** Aptamers are short, single-stranded DNA or RNA molecules that can be engineered to bind with high affinity and specificity to bacterial surface markers or biofilm components (Ye et al., 2024). These molecules are selected through an in vitro process known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (Ellington & Szostak, 1990; Tuerk & Gold, 1990), allowing for the identification of aptamers that specifically recognize *S. aureus* biofilms. The significant advantage of aptamers over conventional antibodies is their chemical synthesis process - they can be produced in vitro with high reproducibility and scalability, eliminating the need for animal usage and avoiding the batch-to-batch differences that often occur in antibody production. This synthetic property can also be subjected to precise chemical modifications to enhance its stability and functionality, providing greater flexibility for design and optimization (Ye et al., 2024). Unlike protein-based antibodies, aptamers are more stable under harsh conditions, including variations in temperature and pH (Ye et al., 2024; Zhou & Rossi, 2017). Additionally, they do not elicit strong immune responses, making them suitable for repeated dosing without the risk of significant immunogenicity (Yasmeen et al., 2020). Furthermore, their high specificity ensures that they can be engineered to bind only to bacterial biofilm components, minimizing interactions with host tissues and reducing potential side effects (Afrasiabi et al., 2020). In diagnostic applications, aptamers can be easily combined with various imaging agents, enabling real-time visualization of bacterial biofilms with high sensitivity and specificity. This capability addresses a critical need for early and accurate detection of biofilms and could revolutionize the current diagnostic paradigm (Ye et al., 2024).

In the context of implant-associated infections, these innovative approaches offer particularly compelling benefits. The local delivery of aptamer-guided nanoparticles can achieve higher therapeutic concentrations at infection sites while minimizing systemic exposure. This targeted approach is especially valuable in treating biofilms on implant surfaces, where traditional systemic antibiotics often fail to achieve effective local concentrations (Flores et al., 2022). For wound care applications, these technologies can be incorporated into advanced delivery systems such as hydrogels or wound dressings, providing sustained release while maintaining an optimal wound healing environment. The ability to monitor infection status through aptamer-based imaging while simultaneously delivering therapeutic agents represents a significant advancement in wound care management. The synergistic combination of these technologies addresses multiple challenges simultaneously: the need for effective non-antibiotic treatments, the requirement for precise targeting and delivery, and the demand for real-time diagnostic capabilities. By integrating these approaches, we can potentially overcome the current limitations in both diagnosis and treatment of biofilm-associated infections, leading to more effective and personalized therapeutic strategies.

### **Research Focus and Approach**

This research proposal aims to develop and validate an **innovative non-antibiotic theranostic platform combining aptamer-guided imaging with antimicrobial nanoparticle therapy for biofilm-associated and antibiotic-resistant infections** through a systematic, three-phase approach. Our strategy uniquely combines two cutting-edge technologies: antimicrobial nanoparticles for treatment and aptamer-based molecular probes for diagnostic imaging and targeted delivery.

**A key focus of this project is addressing methicillin-resistant *Staphylococcus aureus* (MRSA), a major cause of biofilm-associated infections in both hospital and community settings. We have chosen the USA300 LAC strain, a clinically relevant, community-acquired MRSA isolate obtained from the Medical Microbiology Laboratory at UMC Utrecht.**

**Both MRSA and non-resistant *S. aureus* form biofilms, but MRSA presents an additional challenge due to its resistance to common antibiotics such as penicillin. By targeting MRSA, we address both biofilm-mediated tolerance and antibiotic resistance. Using a non-resistant *S. aureus* strain would not fully capture the complexity of real-world infections, where antibiotic resistance often complicates treatment.**

**Our nanoparticles are designed to deliver non-antibiotic agents (e.g., reactive oxygen species) that act independently of traditional antibiotics, ensuring that MRSA's resistance does not limit the selection or efficacy of therapeutic agents. Demonstrating success against MRSA strengthens the therapeutic potential of our approach, as highlighted in the project title.**

**Regarding biosafety, no additional precautions are required. Both MRSA and non-resistant *S. aureus* are classified as ML-2 pathogens (moderate risk) under Dutch RIVM guidelines. All experiments are conducted under strict ML-2 protocols, ensuring full compliance with ethical and regulatory standards.**

In the first phase (Therapeutic Evaluation), we will evaluate the therapeutic efficacy of our newly developed antimicrobial nanoparticles in two clinically relevant mouse models: a subcutaneous implant infection model and a wound infection healing model. These models respectively simulate implant-associated infections and chronic wound infections, allowing us to assess the nanoparticles' effectiveness through both local injection and topical application in gel-based carriers. The non-antibiotic nature of these nanoparticles presents a promising alternative to conventional antibiotics, potentially addressing the pressing challenge of antimicrobial resistance.

The second phase (Diagnostic Imaging) focuses on exploring the diagnostic potential of aptamers that have been successfully selected through in vitro screening. Using the subcutaneous implant infection model, we will investigate the in vivo imaging capabilities of these aptamers through systemic administration. This phase aims to evaluate the aptamers' ability to specifically localize to biofilm sites and provide real-time visualization of biofilm distribution, addressing the critical need for early and accurate infection diagnosis. The high

specificity and stability of aptamers, combined with their synthetic nature and reproducibility, make them particularly suitable for this diagnostic application.

In the **third** phase (Theranostic Approach; Therapy + Diagnostic Imaging), we will develop an innovative theranostic platform that combines diagnostic imaging with therapeutic capabilities. This phase will utilize the subcutaneous implant model to evaluate our dual-function system through systemic administration. The system comprises nanoparticles that serve as both imaging agents for real-time infection monitoring and antimicrobial carriers for therapeutic effects, while aptamers function as molecular navigation systems for precise targeting and biofilm-specific recognition elements. While local administration (as investigated in first phases) can be effective for accessible infections, many clinical biofilm infections occur in deep-seated locations where local treatment is not feasible. Our aptamer-guided nanoparticle system addresses this challenge by enabling targeted delivery through systemic administration. This integrated approach could revolutionize biofilm infection management by offering precise targeting through aptamer-mediated navigation, real-time infection monitoring through nanoparticle imaging capabilities, and effective treatment through nanoparticle-mediated antimicrobial delivery.

MRSA infections represent a subset of biofilm infections, combining biofilm-mediated tolerance with antibiotic resistance. In our project, MRSA is specifically required for therapeutic testing (phases 1 and 3) to validate our approach against both biofilm protection and antibiotic resistance. For diagnostic development (phase 2), MRSA is not strictly necessary, as the tools target universal biofilm features, such as extracellular polymeric substances (EPS), metabolic activity, and structural organization. However, we include MRSA to demonstrate broad applicability—if our approach is effective against MRSA-associated biofilms, it should also be effective against biofilms formed by other bacterial species. While MRSA does not introduce additional technical challenges in diagnostics, its inclusion strengthens our platform's relevance in high-priority, clinically challenging scenarios.

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### **3.2 Purpose**

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

#### **Immediate Goal:**

The immediate goal of this project is to develop and validate an innovative **non-antibiotic** theranostic platform combining aptamer-guided imaging with antimicrobial nanoparticle therapy for biofilm-associated **and antibiotic-resistant** infections. **This platform will use MRSA in** two clinically relevant mouse models: a wound infection model (Appendix 1) and a subcutaneous implant infection model (Appendix 2).

#### **Ultimate Goal:**

**We aim to overcome two critical barriers in infection management: biofilm-mediated tolerance (resistance to immune clearance and drug penetration) and antibiotic resistance (e.g., MRSA's resistance to  $\beta$ -lactams). By integrating non-antibiotic antimicrobial nanoparticles (e.g., reactive oxygen species generators) with biofilm-specific aptamer diagnostics, our platform will provide a dual-action solution to eradicate resistant pathogens while minimizing the risk of further resistance development.**

#### **Importance of using MRSA**

**MRSA is a high-priority multidrug-resistant pathogen that combines biofilm resilience with resistance to frontline antibiotics. Targeting MRSA allows us to validate our platform's ability to address both challenges simultaneously, ensuring clinical relevance in an era of rising antimicrobial resistance.**

#### **Sub-objectives (Sub-goals):**

##### **Phase 1. Therapeutic Evaluation (phase 1)**

- 1.1 Assess antimicrobial nanoparticles in wound infection model (Appendix 1) through topical application, chronic wound treatment.
- 1.2 Assess antimicrobial nanoparticles in subcutaneous implant infection model (Appendix 2) via local injection, mimicking implant-associated infection treatment.

##### **Phase 2. Diagnostic Imaging (phase 2)**

- 2.1 Investigate the in vivo distribution and imaging capabilities of aptamers through systemic administration in the subcutaneous implant infection model (Appendix 2)

##### **Phase 3. Theranostic Approach (Therapy + Diagnostic Imaging)**

- 3.1 Integrate the optimized nanoparticle formulation with aptamer-mediated targeting (identified from Phases 1 and 2) and evaluate the combined system using a subcutaneous implant infection model (Appendix 2) to achieve simultaneous imaging and targeted therapeutic delivery.

The systematic progression through these phases will advance our ultimate goal by first establishing effective treatment strategies for accessible infections (Phase 1), then developing imaging diagnostic capabilities (Phase 2), and finally creating a targeted delivery system for treating and imaging deep-seated infections through systemic administration (Phase 3). This comprehensive approach addresses the full spectrum of clinical challenges in biofilm infection management, from easily accessible wounds to deep-tissue infections requiring systemic treatment.

3.2.2 Provide a justification for the project's feasibility.

The candidate nanoparticle-based antimicrobial agents (like lipid- and metal-based) and targeting aptamers have been previously identified and being validated through in vitro studies. For in vivo evaluation, we will employ a sequential testing strategy, starting with the top-performing candidate from each category. Importantly, if the initial candidate demonstrates satisfactory in vivo efficacy, we will proceed exclusively with that formulation, eliminating the need for further candidate testing. However, should the initial candidate fail to meet our efficacy criteria, we will systematically evaluate subsequent candidates according to our prioritization criteria (selecting a maximum of five nanoparticle formulations and three aptamers). This strategic approach ensures efficient resource utilization while maintaining scientific rigor. Considering the project scope and available resources (including funding and qualified personnel), we anticipate project completion within a three-year timeframe.

**We believe this project is feasible within three years because:**

- Established in vitro data support the transition to animal models – Our group has already identified promising candidates through systematic in vitro evaluation: one aptamer showing high specificity for *S. aureus* biofilms, one lipid-based and one metal-based nanoparticle demonstrating strong antimicrobial efficacy. Additional candidates are available if needed, though we will limit testing to maximum of five nanoparticle formulations and three aptamers.
- Expertise in infection models – [REDACTED]
- Strong Interdisciplinary Collaboration – We have a large and strong interdisciplinary team of researchers working on this project: chemists and engineers working on the synthesis of nanoparticles and identification of aptamers [REDACTED] biologists working on bacteria and animal surgeries [REDACTED] and clinicians with knowledge of the translational aspects [REDACTED]
- Funding – This project is supported by the Dutch Antimicrobial Resistance Technology development and Biofilm Assessment Consortium (DARTBAC), a large-scale initiative under the Netherlands Science Agenda (NWA). DARTBAC brings together an interdisciplinary team of researchers with complementary expertise, ensuring the successful execution of each phase.

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3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

No

Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

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**3.3 Relevance**

3.3.1 What is the scientific and/or social relevance of the objectives described above?

**Social Relevance**

The social impact of this research directly addresses critical healthcare challenges affecting patient outcomes and public health systems. Biofilm-associated infections, particularly in medical device implantation and chronic wounds, represent a significant burden on

healthcare systems, with annual costs exceeding \$1.5 billion in the United States alone (Kurtz et al., 2012). The development of effective non-antibiotic treatments addresses the global crisis of antimicrobial resistance, which is projected to cause up to 10 million deaths annually by 2050 (O'Neill, 2016). Our research aims to reduce both the human burden and economic costs of these infections—studies have shown that biofilm-associated implant infections can increase patient hospitalization by 7-10 days and raise treatment costs by 300-400% (Costerton et al., 1999). Furthermore, by developing rapid diagnostic capabilities, we could significantly reduce the current 48-72 hour diagnostic window (Donlan, 2001), preventing the progression of infections from manageable conditions to severe systemic complications. This is particularly relevant for an aging population, where prosthetic joint infections alone lead to a mortality rate of 6-7% (Darouiche, 2004).

### **Scientific relevance**

From a scientific perspective, our research advances multiple cutting-edge fields while addressing fundamental challenges in infection management. The development of aptamer-guided nanoparticle systems represents a significant innovation in targeted drug delivery, combining the specificity of molecular recognition with the therapeutic potential of nanomaterials (Ye et al., 2024; Zhou & Rossi, 2017). Our approach bridges crucial gaps in current scientific knowledge, particularly in understanding how targeted delivery systems behave in complex biofilm environments, where bacteria exhibit unique phenotypic characteristics and harbor highly resilient persister cells (Gebreyohannes et al., 2019; Uruén et al., 2020). The integration of diagnostic and therapeutic capabilities in a single platform pushes the boundaries of theranostic approaches, advancing beyond traditional diagnostic methods that miss approximately 60-70% of early biofilm developments (Lewis, 2007). The study of different nanoparticles, such as lipid-based and metal-based nanoparticles, contributes to our understanding of different antimicrobial mechanisms (Draviana et al., 2023; Zheng et al., 2022), while the development of aptamer-based imaging techniques advances the field of molecular diagnostics (Ye et al., 2024). Moreover, our research methodology, using two distinct infection models, provides valuable insights into the translation of in vitro findings to in vivo applications, contributing to the broader field of preclinical research in biofilm-associated infections.

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### **3.3.2 Who are the project's stakeholders? Describe their specific interests.**

1. Biomedical Researchers – Scientists working in antimicrobial resistance, nanomedicine, biofilm research, and drug delivery are interested in understanding how aptamer-functionalized nanoparticles improve biofilm targeting and antimicrobial efficacy. This study provides critical data on precision antimicrobial therapies, biofilm eradication mechanisms, and host-pathogen interactions, supporting future research directions.
2. Medical Practitioners & Clinicians – Infectious disease specialists, microbiologists, wound care experts, and surgeons require more effective treatment options for chronic infections, non-healing wounds, and implant-associated infections. This research offers potential new therapeutic approaches that improve patient outcomes, reduce infection recurrence, and minimize surgical interventions.
3. Pharmaceutical & Biotech Industry – Companies developing antibiotics, nanoparticle-based drug delivery systems, and medical coatings have a strong commercial interest in Aptamer-nanoparticle conjugates. The results could lead to patentable innovations, accelerating the development of targeted antimicrobials and supporting product pipelines for anti-biofilm therapies.

4. Regulatory & Ethical Bodies – Organizations such as the CCD, FDA, and institutional ethical committees ensure that research follows animal welfare guidelines, preclinical validation standards, and future clinical trial regulations. This project aligns with 3R principles (Replacement, Reduction, Refinement) and generates preclinical data essential for regulatory approval.
5. Animals: Animals, while not traditional stakeholders due to their lack of agency or decision-making power, occupy a crucial position in research as ethically significant entities. Their welfare is directly impacted by research activities, necessitating moral and ethical considerations to ensure their well-being. Representation through ethical committees, such as IACUCs or DECs, and animal welfare organizations advocates for their protection and inclusion in decision-making processes. Therefore, it is important to integrate animals as passive stakeholders whose interests are safeguarded by proxies. Recognizing animals in this role emphasizes the ethical responsibility of researchers and institutions to minimize harm and uphold humane practices in the pursuit of scientific knowledge.
6. Public Health Organizations & Policymakers – Institutions like the WHO, CDC, and ECDC focus on antimicrobial resistance surveillance, infection control strategies, and antibiotic stewardship programs. The findings of this study could inform new policies on biofilm-associated infections, precision medicine approaches, and antimicrobial resistance containment strategies.
7. Patients & Healthcare Systems – Individuals affected by drug-resistant infections and biofilm-related complications stand to benefit from reduced treatment failures, fewer surgical interventions, shorter hospital stays and improved long-term health outcomes. Healthcare systems could see lower costs associated with recurrent infections and prolonged antibiotic use, making this approach both clinically and economically impactful.

### 3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

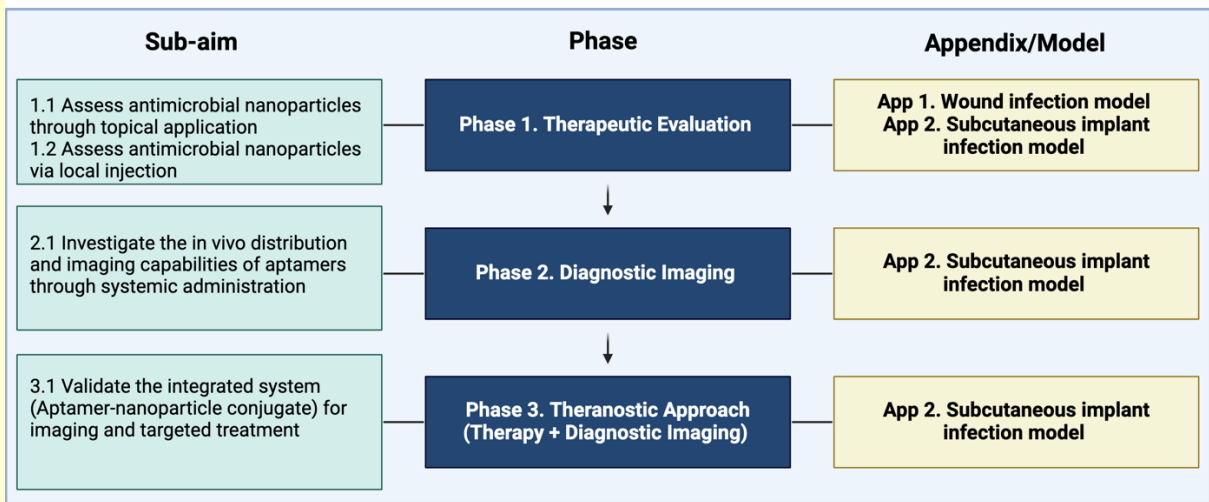
As shown in Figure 1, this study follows a structured, three-phase approach that systematically progresses from investigating therapeutic efficacy in two distinct infection models to developing an integrated theranostic platform. The research utilizes clinically isolated Methicillin-resistant *Staphylococcus aureus* strains, which are leading causes of biofilm-associated infections, particularly in medical implants, chronic wounds, and hospital-acquired infections (Mohamadian et al., 2020).

#### **Choice of aptamer targeting agents and antimicrobials nanoparticles**

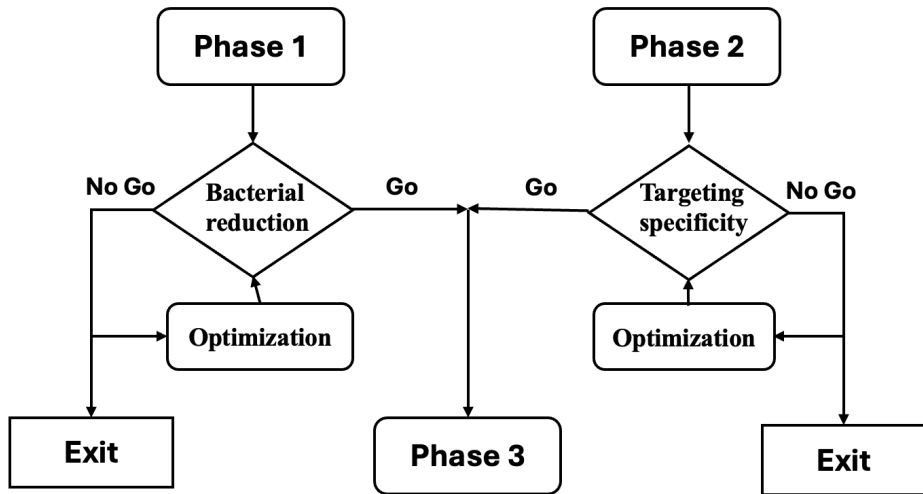
Before conducting in vivo experiments, we have completed systematic in vitro screening to identify optimal aptamer-based targeting agents and antimicrobial nanoparticle formulations for animal studies. Our selection strategy emphasizes efficiency and ethical considerations by limiting the number of candidates for in vivo testing.

For antimicrobial nanoparticles, we have developed two distinct nanoparticle platforms: metal-based nanoparticles and lipid-based nanoparticles, each engineered to exploit unique advantages for biofilm treatment. Through extensive in vitro screening, including minimum inhibitory concentration testing, biofilm penetration assays, and cytotoxicity evaluations, we have identified optimal formulations in both categories. Our metal-based nanoparticles demonstrate intrinsic broad-spectrum antimicrobial activity through multiple mechanisms including oxidative stress induction, membrane disruption, and enzyme inhibition. These properties make them particularly valuable as they operate independently of conventional antibiotic resistance mechanisms. In parallel, our lipid-based nanoparticles showcase superior biocompatibility and enhanced drug loading capacity, facilitating efficient bacterial membrane penetration while protecting encapsulated antimicrobial agents from degradation. We will begin in vivo studies with the most promising candidate from each platform based on their performance in our comprehensive in vitro screening panel. If these initial candidates do not meet our predetermined efficacy criteria, we have additional pre-screened formulations available, though we will limit testing to a maximum of five nanoparticle formulations to ensure ethical use of animal and resources.

For targeting molecules, we have identified novel aptamers specifically targeting Protein A, a well-characterized surface protein present on most *S. aureus* strains that plays a crucial role in immune evasion and host-pathogen interactions (Bear et al., 2023). This targeting strategy leverages the consistent expression of Protein A across *S. aureus* strains and its crucial role in bacterial virulence. Through rigorous in vitro screening based on binding kinetics, specificity assessments against multiple bacterial strains, stability studies in physiological conditions, and binding affinity to *S. aureus* biofilms, we have selected multiple promising aptamer candidates. We will initiate in vivo studies with the top-performing aptamer based on our comprehensive screening criteria. If this candidate demonstrates satisfactory performance in animal studies, meeting our predefined efficacy thresholds, we will proceed with this formulation without testing additional candidates, thereby minimizing animal usage while maintaining scientific rigor.



**Figure 1.** Overview of project design. This study follows a systematic three-phase approach to develop an integrated theranostic platform for biofilm infections. Phase I evaluates therapeutic efficacy through local administration of antimicrobial nanoparticles in two clinically relevant models: a wound infection model (Appendix 1) and a subcutaneous implant infection model (Appendix 2). Phase II investigates aptamer-based diagnostic imaging using bilateral implantation in the subcutaneous model to enable intra-animal comparison. Phase III combines these findings to develop a systemic delivery approach for treating deep-seated infections. The project progresses through clear Go/No-Go decision points to ensure both scientific rigor and efficient resource utilization.



**Figure 2.** This flowchart outlines the decision points for advancing through the project's three phases, specifically for the subcutaneous implant infection model (Appendix 2). In Phase 1, progression requires significant bacterial reduction in biofilm-infected implants. Phase 2 demands high targeting specificity (signal ratio  $\geq 3:1$ ). Phase 1 and Phase 2 are independent and do not influence each other. Phase 3 integrates validated therapeutic and diagnostic components for systemic treatment. The project exits if criteria are unmet.

Go/no-go decision point is both feasible and integral to the experimental design. This checkpoint is strategically placed after the optimization phase to evaluate whether the tested nanoparticle formulations (appendix. 2-phase 1) or aptamers (appendix. 2-phase 2) meet predefined efficacy criteria. If initial candidates fail, a limited number of candidates will be systematically tested, to prevent indefinite progression. Should no candidates achieve the required efficacy, the project terminates (no-go).

### In vivo evaluation

This study utilizes two clinically relevant mouse models: a wound infection model (Appendix 1) and a subcutaneous biofilm implant infection model (Appendix 2). Through a systematic three-phase approach, each with specific sub-goals (see section 3.2.1), we will address critical challenges in biofilm infection management.

### **Phase 1: Therapeutic evaluation**

This initial phase focuses on establishing effective treatment strategies through local administration using two complementary infection models, each representing distinct clinical scenarios that require different therapeutic approaches. Our dual-model strategy is designed to comprehensively evaluate the versatility and effectiveness of our antimicrobial nanoparticles across different infection contexts.

#### **Research Questions:**

1. Wound Infection Model: Can topically administered nanoparticle formulations effectively treat and promote healing in infected wounds?
2. Subcutaneous Biofilm Implant Infection Model: Can locally injected nanoparticle formulations effectively eliminate biofilm infections on implant surfaces?

#### **Model Design:**

The wound infection model follows a well-established and standardized protocol for simulating chronic, biofilm-infected wounds in mice. It is adapted from validated methodologies described in Hou et al. (2024) and Lei et al. (2024). This model involves creating full-thickness excisional wounds (~8 mm in diameter) on the dorsal surface of mice, followed by bacterial inoculation to establish a biofilm infection. After 48 hours, infected wounds receive daily topical application of nanoparticle formulations for 7 days. Wound closure is monitored through digital photography and image analysis software, while therapeutic efficacy is assessed by evaluating wound healing progression and quantifying bacterial load (CFU counts from wound tissue homogenates). Tissue response is further analysed through histological assessments, including H&E staining and immunohistochemistry, to evaluate inflammation, granulation tissue formation, and re-epithelialization. The level of discomfort for animals in this model is classified as Moderate under EU Directive 2010/63/EU. Transient pain during wound creation is mitigated with perioperative analgesia. Mice typically maintain normal activity and body weight ( $\pm 10\%$ ), with no signs of systemic illness (Hou et al., 2024; Lei et al., 2024).

For the subcutaneous biofilm implant infection model, implants will be pre-colonized with bacterial biofilm by incubating with *S. aureus* under appropriate conditions to establish mature biofilm on the surface. These biofilm-colonized implants will then be subcutaneously inserted into flank of mice. After 48 hours of in vivo infection establishment, nanoparticle formulations will be locally injected around the implant site. The bacterial burden will be determined through two methods: (1) CFU counts from the implant surface following sonication to dislodge adherent bacteria, and (2) CFU counts from surrounding tissue homogenates. Tissue integration and inflammatory response will be evaluated through histological examination of the peri-implant tissue, including assessment of inflammatory cell infiltration, fibrosis, and vascularization.

#### **Go/No-Go Criteria for Advancing from Phase 1 to Phase 3:**

For the Wound Model. Outcomes do not influence the continuation of the studies in this application.

For Implant Model (Advancing from Phase 1 to Phase 3):

Go Criteria:

- Bacterial reduction: Minimum 2-log reduction in bacterial load (both tissue homogenate and implant surface)

No-Go Criteria:

- Bacterial reduction:  $< 2$ -log reduction in bacterial load

### **Phase 2: Diagnostic Imaging**

#### **Research Question:**

Can aptamer-based molecular imaging effectively distinguish infected implants from sterile implants in the same animal?

#### **Model Design:**

The bilateral implant model involves subcutaneous insertion of two identical implants in the same animal - one pre-colonized with bacterial biofilm and one sterile. The infected implant will be prepared by pre-incubating with *S. aureus* under appropriate conditions to establish

biofilm, while the contralateral implant remains sterile. After 48 hours of in vivo infection establishment, aptamer-based imaging probes will be administered systemically via tail vein injection.

In vivo imaging will be performed at five key timepoints over 7 days: such as, baseline (0h), early distribution (4h), Day 1, Day 3, and Day 7. Each imaging session will be limited to 15 minutes under carefully controlled anesthesia, with animals maintained on a heating pad to prevent hypothermia. A minimum 24-hour recovery period will be ensured between imaging sessions to minimize animal stress. Bacterial burden will be confirmed through CFU counts from both implant surfaces (following sonication) and surrounding tissue homogenates at study endpoint. This paired design enables direct comparison of targeting specificity while controlling for individual variations in biodistribution, thereby reducing biological variability and total animal numbers required.

In addition to analyzing aptamer binding at the two implant sites (infected and sterile), we will assess their distribution in non-target organs such as the liver, spleen, and kidneys. This step is essential for evaluating aptamer specificity, biodistribution, and safety.

We expect initial accumulation in highly vascularized organs, which should decrease over time, while signals at target sites (infected implants) should increase or remain stable, indicating effective delivery and retention. This analysis not only validates targeting specificity but also supports safety assessments. If significant off-target accumulation occurs, we will optimize aptamer design (e.g., PEGylation) to improve precision, ensuring the platform's safety and readiness for clinical translation.

### **Go/No-Go Criteria Advancing from Phase 2 to Phase 3:**

Go Criteria:

- Targeting specificity: Signal ratio  $\geq 3:1$  (infected implant vs. sterile implant)
- Background signal:  $< 20\%$  accumulation at sterile implant site compared to infected implant

No-Go Criteria:

- Targeting specificity: Signal ratio  $< 3:1$  (infected vs. sterile implant)
- Background signal:  $> 20\%$  accumulation at sterile implant site

### **Phase 3: Theranostic Approach**

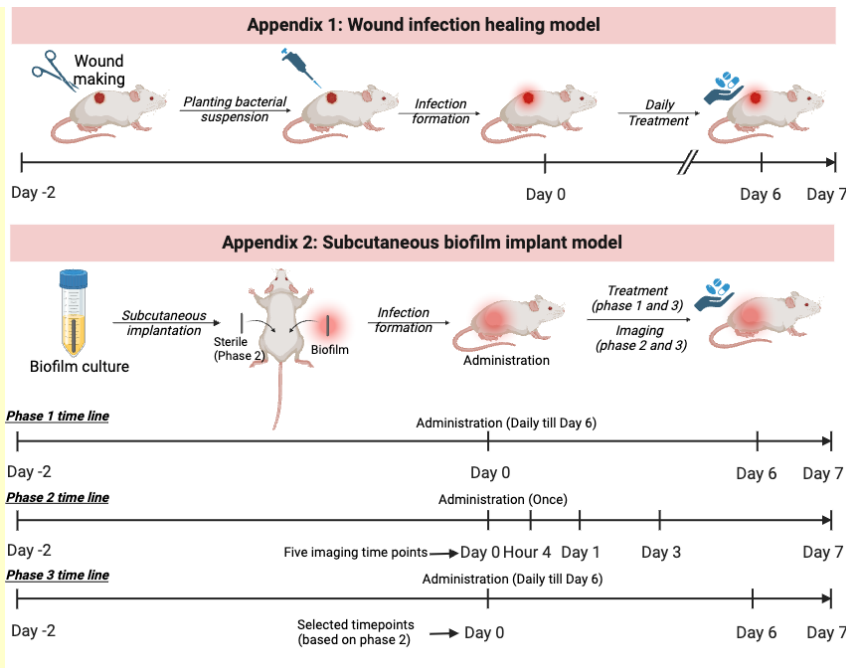
#### **Research Question:**

Can systemically administered aptamer-guided nanoparticle conjugates effectively target and treat deep-seated biofilm infections?

#### **Model Design:**

This phase utilizes a single implant model with aptamer-functionalized dual-functional nanoparticles designed for both therapeutic and diagnostic purposes. The nanoparticles will be conjugated with specific aptamers targeting bacterial biofilm components and labeled with near-infrared fluorescent dyes to enable real-time tracking of their biodistribution. An implant pre-colonized with bacterial biofilm will be inserted subcutaneously. After 48 hours of in vivo infection establishment, aptamer-guided theranostic nanoparticles will be administered systemically via tail vein injection.

In vivo near infra red imaging will be performed at five key timepoints (based on Phase 2): baseline (prior aptamer administration), early distribution (such as 4h after administration), and 3 additional time points, such as Day 1, Day 3, and Day 7. Each imaging session will be conducted under carefully controlled anesthesia with temperature maintenance. Each imaging session will be limited to 15 minutes under carefully controlled anesthesia, with animals maintained on a heating pad to prevent hypothermia. A minimum 24-hour recovery period will be ensured between imaging sessions to minimize animal distress. Bacterial burden will later be confirmed post mortem through CFU counts from implant surfaces (following sonication) and surrounding tissue homogenates at study endpoint. Comprehensive post mortem biodistribution studies will analyze nanoparticle accumulation in major organs through fluorescence imaging and quantification. Safety assessments will include blood analysis, histopathological examination of major organs, and monitoring of clinical parameters.



**Figure 3.** Schematic representation of infection models used in this study. (Appendix 1) In the wound infection healing model, a full-thickness wound is created, followed by the application of a bacterial suspension to induce infection and assess wound healing dynamics. (Appendix 2) In the subcutaneous biofilm implant model, biofilm-coated implant rods are inserted subcutaneously to establish localized biofilm infections. For Phase II diagnostic imaging evaluation, the model incorporates bilateral implantation with infected and sterile implants, enabling each animal to serve as its own control and thereby reducing the total number of animals required for statistical validation.

### 3.4.2 Provide a justification for the strategy described above.

The strategy described above has been carefully designed to maximize scientific insights while adhering to ethical principles of animal research. Our approach is justified through several key considerations:

#### Systematic Progression and Model Selection

Our three-phase design follows a logical progression from therapeutic validation to diagnostic imaging development and finally the integration of therapy and diagnosis (theranostic approach). The wound infection model tests topical treatment for accessible infections, while the subcutaneous biofilm implant model simulates device-associated infections, allowing evaluation of both local and systemic therapies. The selection of two complementary infection models allows us to address distinct clinical scenarios while minimizing the total number of animals needed. Both models are well-described in the literature and the subcutaneous biofilm implant model has been successfully implemented in our laboratory, and the wound infection healing model has been successfully implemented in our laboratory's collaborators in Hong Kong, demonstrating our team's established expertise in these experimental systems. Each model serves a specific purpose: the wound model represents accessible infections requiring topical treatment, while the implant model simulates device-associated infections and enables evaluation of both local and systemic administration approaches.

Both models demonstrate strong validity in preclinical infection research. Face validity is supported by their similarity to human infections. The wound infection model mimics chronic wounds, such as diabetic ulcers, while the subcutaneous biofilm implant model replicates implant-associated infections, both of which are major clinical challenges (Kwiecinski et al., 2023). Construct validity is evident as both models accurately reflect biofilm formation, immune responses, and bacterial persistence, which are critical in chronic infections. Biofilm-associated bacteria in these models exhibit increased antibiotic resistance and immune evasion, similar to human infections (de Vor et al., 2022). Translational validity is demonstrated by their widespread use in biofilm-targeting research. The subcutaneous implant model has informed antimicrobial coating development, while the wound infection model has contributed to novel wound care strategies (van Dijk et al., 2024). Their ability to generate clinically relevant data makes them ideal for testing innovative therapeutic and diagnostic approaches.

#### Optimization of Animal Usage

We have incorporated several strategies to minimize animal numbers while maintaining statistical power. Most notably, in Phase II, we utilize a bilateral implantation approach where each animal receives both infected and sterile implants, enabling intra-animal comparison and reducing the number of animals needed by up to 50% and even more compared to traditional single-implant designs. This refinement aligns with the 3Rs principle (Reduction, Refinement, Replacement) in animal research.

### Decision Point Strategy and Resource Optimization

Our strategy employs a rigorous, stepwise approach with clearly defined decision points to maximize efficiency and minimize resource usage. The implementation of clear Go/No-Go criteria at each phase ensures that animals are only used when justified by prior results. Our approach of testing the most promising candidates first, with a maximum of five nanoparticle formulations and three aptamers, prevents unnecessary animal use while maintaining scientific rigor. This strategic limitation is based on extensive in vitro characterization and optimization, ensuring that only the most promising formulations proceed to in vivo testing. Regular data review points are scheduled to enable timely go/no-go decisions based on predetermined efficacy thresholds, including reduction in bacterial burden, time to infection clearance, and safety parameters.

### Animal Welfare Considerations

The study design incorporates multiple safeguards for animal welfare. We have established clear monitoring protocols and humane endpoints based on weight progression, behavioral changes, and clinical signs. Animals will be monitored daily for signs of pain or distress using a comprehensive scoring system that includes assessment of mobility, grooming behavior, food and water intake, and wound appearance. Appropriate analgesia will be administered throughout the study period, and all surgical procedures will be performed under adequate anesthesia by trained personnel. Environmental enrichment, including nesting materials and social housing where appropriate, will be provided to promote natural behaviors and reduce stress. The use of imaging in Phase II and III reduces the need for terminal sampling timepoints, as treatment progress can be monitored non-invasively in the same animal over time. Additionally, we have implemented a detailed post-operative care protocol (see check list for humane endpoint in Appendix 1 and 2). All procedures will be conducted in accordance with institutional guidelines and experienced staff, with emergency protocols in place for unexpected adverse events.

### Clinical Relevance

Our strategy progresses from readily accessible infections to addressing deep-seated infections, reflecting the spectrum of clinical challenges. The transition from local to systemic administration in Phase III is particularly important for developing treatments for anatomically challenging infections, where direct access is limited. This progression ensures that our findings will have meaningful clinical applications while justifying the use of animal models.

This strategy reflects a carefully balanced approach between scientific objectives and ethical considerations, ensuring that each experiment contributes meaningfully to our understanding while maintaining the highest standards of animal welfare.

### References

- de Vor, L., van Dijk, B., van Kessel, K., Kavanaugh, J. S., de Haas, C., Aerts, P. C., Viveen, M. C., Boel, E. C., Fluit, A. C., Kwiecinski, J. M., et al. (2022). Human monoclonal antibodies against *Staphylococcus aureus* surface antigens recognize in vitro and in vivo biofilm. *eLife*, 11eLife, 11, ehttps://doi.org/10.7554/eLife.67301
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3.4.3 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Wound infection healing model
2	Subcutaneous biofilm implant model
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4	Click or tap here to enter text.
5	Click or tap here to enter text.
6	Click or tap here to enter text.
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## 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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website ([www.centralecommissiedierproeven.nl](http://www.centralecommissiedierproeven.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’.	11500	
1.2 Provide the name of the licenced establishment.	UMC Utrecht	
1.3 List the serial number and type of animal procedure  <i>Use the numbers provided at 3.4.3 of the project proposal.</i>	Serial number	Type of animal procedure
	1	Wound infection healing model

### 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.

##### Experimental Design

The wound infection healing model has been selected to evaluate topical antimicrobial treatments in a clinically relevant context that closely mirrors chronic wound infections, particularly those complicated by biofilm formation. This model (Figure 1) is especially valuable for our research as it provides direct access to the infection site, enabling evaluation of topical antimicrobial nanoparticle formulations delivered through biocompatible carrier systems. The direct accessibility makes this model ideal for studying local treatment strategies that could be readily translated to clinical wound management, where topical interventions are the primary treatment approach for chronic wounds such as diabetic ulcers, pressure sores, and non-healing surgical wounds.

The study encompasses a 9-day experimental period:

- Day -2: Creation of standardized full-thickness wounds and bacterial inoculation.
- Day 0: Biofilm infection establishment
- Day 0-6: Treatment phase with daily monitoring
- Day 7: Kill animals and collect samples

## Appendix 1: Wound infection healing model



**Figure 1.** Surgical procedure of wound infection healing model. A full-thickness wound is created at day -2 alongside bacterial inoculation in the wound site. At day 0, infection is established, and treatment phase starts. Last day of treatment is at day 6, and at day 7 mice are killed and samples collected. Rodent in the image represents a mouse.

### Research Questions

This model will be used for sub-aims 1.1 of the project (see section 3.2.1 of project proposal).

- Can topically administered nanoparticle formulations effectively treat and promote healing in infected wounds?

### Overview of Studies

The experimental design follows a 9-day timeline, spanning from wound creation to tissue harvesting. On Day -2, standardized full-thickness wounds are created using a biopsy punch and a standardized *S. aureus* suspension is introduced to the wound site, followed by a 48-hour biofilm formation period. Biofilm infection establishment occurs on Day 0, followed by treatment phase running from Day 0 to 6, during which daily treatment is administered and wound parameters are regularly monitored. The animals will be killed on Day 7 and tissues will be sampled.

The study includes four groups: (1) an untreated infection control group, (2) a treatment group receiving nanoparticle 1 formulations in carrier systems, (3) a treatment group receiving nanoparticle 2 formulations in carrier system, and (4) a standard treatment group (positive control) receiving antibiotics/iodine. Sample size is determined based on power analysis for wound healing studies, with a 5 animals per group required per group. The final group sizes include a 10% attrition rate to ensure adequate statistical power is maintained even if some animals need to be excluded from the study.

### Outcome Parameters and Justification

Our evaluation strategy focuses on two complementary primary outcome parameters that together provide comprehensive assessment of treatment efficacy while minimizing invasive procedures.

1. Wound healing progression: we evaluate wound healing through macroscopic assessment: macroscopic evaluation utilizes standardized photography and digital planimetry performed daily throughout the study period. This non-invasive approach enables continuous monitoring of wound closure rates and overall healing progression without any disruption to the wound environment. Wound healing rate (%) =  $(A_0 - A_n)/A_0 \times 100\%$ , where  $A_0$  represents the initial wound area on day 0 and  $A_n$  denotes the remaining wound area on postoperative day  $n$ .
2. Bacterial burden assessment: quantification of viable bacteria through standard culture-based methods (CFU counts) serves as our primary indicator of antimicrobial efficacy. Tissue biopsies from the wound bed are collected only at the terminal timepoint (Day 7), which coincides with the end of the study and tissue collection for histological analysis. This approach reduces animal distress by avoiding intermediate invasive sampling while still providing definitive evidence of treatment effectiveness.

Microscopic assessment through histological analysis at the terminal timepoint (Day 7) provides detailed insight into tissue repair quality. Key parameters include granulation tissue formation as an indicator of the inflammatory response, and re-epithelialization as a measure of local tissue reaction to treatment.

The selection of these parameters is justified by several key factors. First, they align directly with standard clinical wound assessment methods (Schultz et al., 2003), enhancing the translational relevance of our findings. Second, they provide complementary information while minimizing animal distress through non-invasive daily monitoring and single terminal sampling. Third, these methods are well-established (Fila et al., 2016; Hou et al., 2024; Lei et al., 2024) and technically feasible, ensuring reproducible data collection.

### Reference

- Fila, G., Kasimova, K., Arenas, Y., Nakonieczna, J., Grinholc, M., Bielawski, K. P., & Lilge, L. (2016). Murine Model Imitating Chronic Wound Infections for Evaluation of Antimicrobial Photodynamic Therapy Efficacy. *Frontiers in Microbiology*, 7, 1258. <https://doi.org/10.3389/fmicb.2016.01258>
- Hou, J., Wu, Q., Xiong, R., Malakar, P. K., Zhu, Y., Zhao, Y., & Zhang, Z. (2024). A Standardized Mouse Model for Wound Infection with *Pseudomonas aeruginosa*. *International Journal of Molecular Sciences*, 25(21), 11773. <https://doi.org/10.3390/ijms252111773>
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Schultz, G. S., Sibbald, R. G., Falanga, V., Ayello, E. A., Dowsett, C., Harding, K., Romanelli, M., Stacey, M. C., Teot, L., & Vanscheidt, W. (2003). Wound bed preparation: A systematic approach to wound management. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, 11 Suppl 1, S1-28. <https://doi.org/10.1046/j.1524-475x.11.s2.1.x>

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

#### **Animal Housing Conditions**

Mice will be housed in individually ventilated cages (IVC) under standard laboratory conditions, including a 12-hour light/dark cycle and a maintained ambient temperature of 21–23°C. Animals will be housed individually to prevent cross-contamination and interference caused by mutual grooming or licking of wounds. The wounds will be created on the dorsal midline, between the scapulae, to avoid self-licking. Rodent chow diet and water will be provided ad libitum, along with environmental enrichment such as nesting material and chew blocks to reduce stress-induced variability. **Group housing is not feasible due to the risk of wound interference between animals (e.g., scratching or biting), a factor also reported in Rhea et al. (2020). However, the short experimental duration (9 days total) minimizes the impact of individual housing on animal welfare.**

Upon delivery, mice will be acclimatized to the new facility for at least 1 week prior the start of the experiment to minimize distress and physiological variations (housed in groups during acclimation week).

#### **Anesthesia Protocol**

All surgical procedures will be performed under isoflurane anesthesia to ensure proper sedation and prevent pain. Anesthesia will be induced at 4-5% isoflurane and maintained at 1.5-3% via inhalation using a nose cone system. The depth of anesthesia will be continuously monitored throughout the procedure, with adjustments made as necessary to assure proper anesthesia and prevent further discomfort. This method provides a rapid induction and recovery while ensuring consistent anesthesia levels throughout the procedure.

#### **Pain Management**

To ensure adequate analgesia, buprenorphine (0.1 mg/kg SC) will be administered at least 30 minutes before surgery and 10-12 hours prior the first administration, and continued twice daily for up to 48 hours postoperatively. If signs of pain or distress persist, additional doses of painkiller, such as buprenorphine or meloxicam (5 mg/kg SC), will be administered. The inclusion of pre-emptive analgesia minimizes perioperative pain and improves postoperative recovery. This approach ensures effective perioperative pain management while allowing for extended analgesia when clinically necessary, aligning with standard clinical practices and ethical animal research practice.

#### **Antiseptic Techniques**

All surgical procedures will be conducted under aseptic conditions except for the infection establishment step. The surgical area will be shaved and disinfected with 70% ethanol and povidone-iodine solution before making an incision. The surgical team will follow strict aseptic protocols, including wearing sterile gloves, surgical masks, and head covers. All surgical instruments will be sterilized before the procedure, and a sterile surgical field will be maintained throughout.

#### **Surgical Technique: Wound Creation and Infection Establishment**

Full-thickness excisional wounds (~8 mm in diameter) will be created on the dorsal midline, between the scapulae, using a biopsy punch, following strict aseptic protocols. The wound will be inoculated with 100 µL of a *S. aureus* suspension to establish a localized infection (Lei et al., 2024). **Wounds will remain open and undressed to simulate natural healing conditions. This approach has been used in previous studies, such as Hou et al. (2024) and Lei et al. (2024), which demonstrated that leaving wounds uncovered provides a more physiologically relevant infection environment.**



**Figure 2.** Visual appearance of the wound at day 0. Image from (Lei et al., 2024).

#### **Treatment Protocol**

After infection establishment (48 hours post-inoculation, Day 0 in the experimental protocol), treatments will commence. Mice will be divided into groups to receive one of the following interventions: nanoparticle 1, nanoparticle 2, positive control (iodine or antibiotics) or

negative control (PBS). Treatments will be topically applied daily for seven days (Day 0 to Day 6 in the experiment protocol). This frequency ensures consistent drug exposure to the infected area while minimizing handling stress.

#### **Postoperative Care and Monitoring**

Mice will be closely monitored for 72 hours post-surgery to assess pain levels, wound healing, and potential complications. For the first three days postoperatively, animals will be checked at least twice daily, and their body weight, activity levels, hydration status, and signs of infection (redness, swelling, discharge) will be recorded. After this period, daily monitoring will continue until the end of the experiment. Prior severe discomfort is reached; including persistent weight loss exceeding 20%, lethargy, or wound dehiscence; the animal will be removed from the study and humanely killed.

#### **Duration of Treatment**

The total duration of the experiment for all the animals will be 9 days, from wound creation to killing. **Therefore, individual housing will be 9 days for all the animals.**

#### **Killing Protocol**

At the conclusion of the study, animals will be killed using CO<sub>2</sub> asphyxiation followed by cervical dislocation, in compliance with Annex IV of Directive 2010/63/EU. Wound tissue will be collected for bacterial CFU quantification, histological evaluation of wound healing, and cytokine analysis.

#### **Justification for the Selected Approach**

The selected wound infection model represents an optimal approach for evaluating our targeted nanoparticle therapy, offering direct clinical relevance by closely mimicking human wound infections and treatment conditions. The topical administration route reflects real-world clinical practice, while the complex biological environment incorporates key elements including immune responses and natural wound healing processes, ensuring therapeutic efficacy is assessed under physiologically relevant conditions (“Wound Infection in Clinical Practice,” 2008).

The model provides robust scientific validation through multiple outcome measures, including bacterial burden quantification, wound closure rate measurements, and histological evaluation of tissue regeneration. The easily accessible wound site allows for precise treatment application and detailed observation of healing progression, while the controlled environment enables accurate assessment of both local and systemic effects of the treatment.

Animal welfare is carefully taken into account in our experimental design. All procedures will be conducted under appropriate anesthesia and analgesia, with comprehensive post-operative care protocols including pain management and infection prevention. Animals will be housed individually in temperature-controlled, pathogen-free environments with enrichment materials to promote natural behaviours and reduce stress. Daily health monitoring will include assessment of wound healing, behavioural changes, and physiological parameters. Clear humane endpoints have been established, including criteria for early intervention or study removal if signs of distress, excessive inflammation, or systemic infection are observed. The surgical procedures will be performed by trained personnel using sterile techniques to minimize complications and ensure consistent wound creation.

The selected approach strongly adheres to ethical considerations and the 3R principles of animal research. We have carefully designed the study to use the minimum number of animals required for statistical significance (Reduction), implemented refined procedures to minimize distress and optimize welfare (Refinement), and conducted preliminary in vitro studies to validate our approach before proceeding to animal studies (Replacement). The comprehensive monitoring protocols ensure early detection of any adverse effects, allowing for prompt intervention when necessary. This careful attention to animal welfare not only fulfils our ethical obligations but also enhances the quality and reliability of our research outcomes.

#### **Reference**

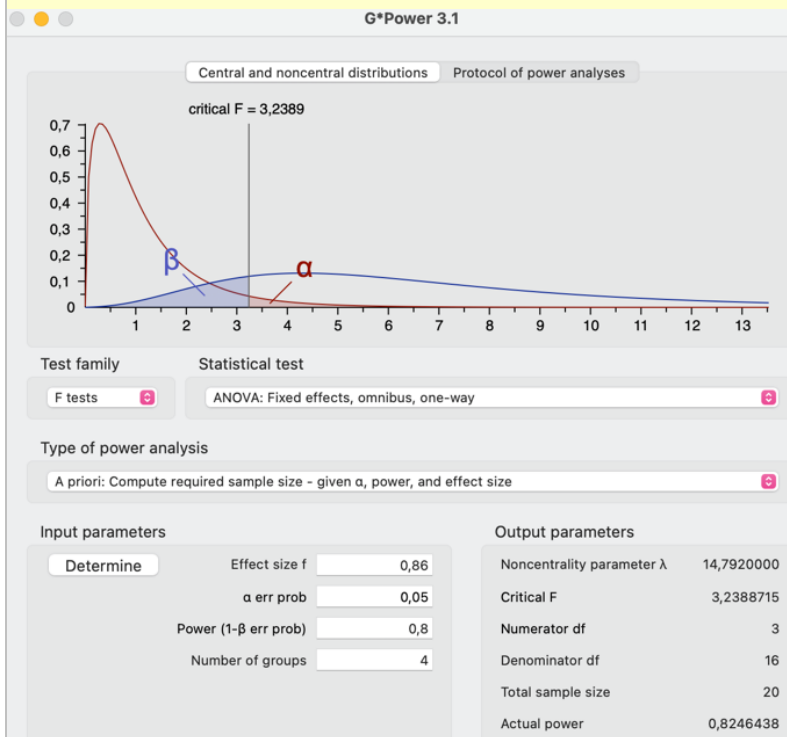
- Lei, J., Zhang, T., Wang, L., Jiang, H., Wu, J., & Zheng, Y. (2024). Chirality-influenced antibacterial behavior of gold nanoclusters. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 683, 133033. <https://doi.org/10.1016/j.colsurfa.2023.133033>
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Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

#### **Statistical Design and Sample Size Calculation**

The statistical design follows a two-phase approach, beginning with a pilot study to validate our model and experimental procedures. This initial validation phase will use a minimum number of animals (2 per group) to confirm infection establishment, treatment feasibility, and measurement reliability before proceeding with the full study.

For the full study phase, based on the results of Lei and colleagues (Hou et al., 2024)(Lei et al., 2024), power analysis using G\*Power software (Version 3.1) was performed to select the group sizes. Wound healing rate was used as the primary outcome, with expected effect size of 0.86, power of 0.8, and significance level of 0.05, which indicated that a minimum of 5 animals per group is required to detect a statistically significant difference in this study. To account for a 10% dropout rate, the final group size was adjusted to n=6 per condition.



**Figure 3.** Print screen from samples size calculation for the full study phase, based on the results of Lei and colleagues (Hou et al., 2024).

### **Number of Animals (up to 58 mice)**

#### **Initial Model Validation (up to 6 mice):**

All personnel involved in this study have prior training in relevant surgical and experimental procedures. Where additional training is required for specific techniques, it will be conducted ex vivo to minimize the use of live animals. Furthermore, whenever feasible, cadavers from animals that have been humanely killed at the end of experiments will be utilized for training purposes, ensuring compliance with the 3Rs principle by reducing the number of additional animals needed for training.

A small-scale validation study will be conducted to confirm the effectiveness of the wound infection model in our laboratory and provide hands-on training before proceeding with the full experiment. This preliminary phase will help us to optimize protocols while minimizing animal usage.

#### **Group allocation:**

1. Infection control (bacteria inoculation + PBS treatment): n=2 mice
2. Non-infected control (PBS treatment): n=2 mice
- If the initial infection protocol proves suboptimal based on predetermined criteria (e.g., inconsistent bacterial colonization or excessive/insufficient infection severity), the protocol will be refined and validation repeated using up to 2 additional mice. The maximum number of mice used in this validation phase will not exceed 6.
- To maximize efficiency and reduce total animal numbers, successful validation data will be incorporated into the full experimental phase, serving as part of the control groups. This approach aligns with the 3Rs principle of reduction in animal research while ensuring scientific validity.

#### **Full Study Phase (up to 52 mice):**

Building upon the validation phase data, the full study will evaluate the therapeutic efficacy of our nanoparticle formulations:

#### **Group allocation:**

1. Control group (bacteria + PBS): n=4\*
2. Nanoparticle 1: n=6
3. Nanoparticle 2: n=6
4. Standard treatment (antibiotics/iodine, as positive control): n=6

\*Note: The control group requires only 4 additional animals as data from 2 mice in the validation phase will be incorporated in the full study phase, achieving minimum n=5 for statistical analysis while adhering to the 3Rs principle of reduction.

Optimization Strategy (n= up to 30 mice):

- We have developed 5 different nanoparticle formulations
- Initial testing will begin with 2 most promising formulations (nanoparticle 1 and nanoparticle 2)
- If these prove suboptimal, alternative formulations will be evaluated
- Each nanoparticle formulation requires 6 mice (accounting for 10% dropout rate)
- Maximum additional animals for nanoparticle optimization: up to 30 mice (sufficient for testing all 3 additional formulations (n=6 each), and if needed 4 additional animals for positive/negative control will be included per extra nanoparticle formulation tested, up to a total of 12 mice). To further reduce animal numbers while maintaining statistical rigor, we will assess inter-experiment variation. If variability between experiments remains low, historical control data will be incorporated, reducing the need for the 4 additional positive and negative control animals per nanoparticle formulation. However, if significant variability is observed, the 4 extra animals will be included to ensure robust statistical comparisons. This adaptive approach aligns with the 3Rs principle while preserving scientific integrity. That will be carried out after discussion with statisticians on work protocol.
- **Justification: The allocation of up to 30 mice is exclusively for testing the efficacy of nanoparticle formulations, not for optimizing the infection models. These animals will be used only if the initial two nanoparticle formulations fail to meet efficacy criteria. The breakdown is as follows:**
  - 18 experimental animals (6 mice per formulation, testing up to three additional candidates).
  - 12 control animals (4 mice per formulation, including positive and negative controls).

The choice of 6 mice per group is based on statistical power analysis ( $\alpha = 0.05$ ,  $\beta = 0.2$ ), ensuring robust conclusions.

The number of control animals is sufficient through data integration: historical control data (6 mice) will be combined with 2 newly included controls per formulation, achieving n = 8, which is validated for statistical robustness. Historical control data from previous experiments under identical conditions will be used, provided inter-experiment variability remains low.

This sample size calculation was reviewed and discussed with the biostatistician in the presence of the IVD, confirming its validity.

This staged approach design allows for systematic evaluation of different formulations while maintaining clear limits on animal usage, ensuring both scientific rigor and ethical considerations.

### **Strategies to Minimize Animal Numbers**

We have implemented several key strategies to minimize animal use while maintaining scientific validity. Our staged approach begins with model validation using only 4 animals, proceeding to subsequent stages only after confirming model reliability. Our experimental design maximizes data obtained from each animal through multiple outcome measures. We collect bacterial counts, wound closure progression, and histological analysis from the same animals, reducing the total number needed. Non-invasive daily monitoring through standardized photography enables longitudinal data collection without requiring additional animals for intermediate timepoints. Standardization procedures play a crucial role in reducing variability and thus animal numbers. We maintain strict control of animal age and weight ranges, consistent housing conditions, and validated procedures for wound creation, infection establishment, and treatment. Technical procedures are performed by experienced personnel using calibrated instruments and standardized protocols to minimize variation.

### **Statistical Analysis Plan**

Statistical analysis will follow a comprehensive plan to ensure robust evaluation of treatment efficacy. Primary outcome measures will use log-transformed CFU counts and wound healing rate analyzed by one-way ANOVA. Secondary endpoints include wound closure assessment using repeated measures ANOVA with time as a within-subject factor, and histological scores evaluated using non-parametric Kruskal-Wallis tests followed by Mann-Whitney U tests. Data from any animals reaching humane endpoints will be included up to the point of withdrawal to maximize information gained. Quality control measures include regular validation of measurement techniques, double-checking of data entry, and blinded assessment of wound measurements and histological scoring. This comprehensive statistical approach ensures that each animal contributes maximum scientific value while maintaining robust and reliable results.

### **Reference**

Hou, J., Wu, Q., Xiong, R., Malakar, P. K., Zhu, Y., Zhao, Y., & Zhang, Z. (2024). A Standardized Mouse Model for Wound Infection with *Pseudomonas aeruginosa*. *International Journal of Molecular Sciences*, 25(21), 11773. <https://doi.org/10.3390/ijms252111773>

## **B. The animals**

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Mouse	Charles River Laboratories	Adult (>20 g)	58	Male	No	C57BL/6

Provide justifications for these choices

Species	Mice are widely used in preclinical infection and wound healing studies due to their well-characterized immune system, cost-effectiveness, and the availability of established wound infection models. Their small size and short wound healing time allow for efficient assessment of therapeutic interventions.
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Origin	Mice will be obtained from Charles River Laboratories, a well-established supplier known for providing standardized research animals. Using animals from a consistent source helps reduce variability in genetics, microbiome composition, and immune function.
Life stages	Adult (>20 g) are selected because they have a fully developed immune system while still being in a stage of active tissue regeneration, making them ideal for evaluating both infection progression and wound healing.
Number	A maximum of 58 is estimated based on power analysis, with group sizes determined to ensure statistical validity while minimizing the number of animals used. Extra animals are included to account for potential losses due to surgical complications or secondary infections.
Gender	<p>We fully acknowledge the historical bias in preclinical research towards male subjects and recognise that post-orthopaedic and wound infections affect both sexes in human medicine. Addressing this issue is important, and we are committed to ensuring that our findings are translationally relevant.</p> <p>The decision to use exclusively male mice in this project is based on scientific and methodological considerations that prioritise experimental consistency and statistical robustness. Male mice exhibit more stable physiological characteristics in terms of bone healing and immune responses, as they are not subject to cyclical hormonal fluctuations. This reduces variability, which is particularly important when studying subtle differences in infection progression and treatment efficacy.</p> <p>Female mice undergo oestrous cycles, causing periodic variations in oestrogen levels, which can influence immune responses and wound healing processes. Such fluctuations could introduce additional variability, potentially confounding our results. This approach aligns with common practice in early mechanistic validation studies, allowing us to focus on core experimental variables (such as nanoparticle targeting) without the complexities introduced by sex-related differences.</p> <p>Furthermore, using a single-sex cohort at this stage minimises within-group variation and increases statistical power, enabling us to obtain reliable results with smaller sample sizes, in accordance with the "Reduction" principle of the 3Rs. Additionally, the orthopaedic infection model we employ has well-established parameters and baseline data in male mice, allowing us to directly compare our findings with existing literature, thereby enhancing the reliability and reproducibility of our research.</p> <p>Our approach follows a strategic experimental sequence rather than perpetuating bias. We begin with male mice to establish foundational mechanisms before incorporating sex as a biological variable in future studies. The antimicrobial mechanisms we study operate at the cellular and molecular levels, which are largely conserved across sexes. Since our focus is on pathogen-targeting strategies rather than host-response modulation, the core principles of our findings are expected to be broadly applicable.</p> <p>To ensure sex-inclusive research, <b>our plan includes subsequent studies with female animals once core mechanisms are validated (outside the scope of this application)</b>. This stepwise approach balances scientific rigour and reproducibility, ensuring that sex differences can be properly assessed in later phases without unnecessary variability in early mechanistic studies.</p> <p>This sequential approach aligns with best practices in translational research, allowing us to first establish proof-of-concept before expanding into more complex, sex-inclusive models.</p>
Genetic alterations	Wild-type mice will be used. No genetic modifications are necessary for this study since the focus is on infection targeting rather than host genetic factors.
Strain	<p>The C57BL/6 strain is selected for this wound healing model due to its well-characterized wound healing responses. This strain exhibits standardized tissue repair processes and demonstrates consistent inflammatory responses during wound healing, making it ideal for evaluating novel therapeutic interventions. The extensive literature on C57BL/6 in wound healing studies provides valuable reference data for result interpretation and comparison with previous findings (Nippe et al., 2011; Pal-Ghosh et al., 2008).</p> <p><b>Reference</b></p> <p>Nippe, N., Varga, G., Holzinger, D., Löffler, B., Medina, E., Becker, K., Roth, J., Ehrchen, J. M., &amp; Sunderkötter, C. (2011). Subcutaneous infection with <i>S. aureus</i> in mice reveals association of resistance with influx of neutrophils and Th2 response. <i>The Journal of Investigative Dermatology</i>, 131(1), 125–132. <a href="https://doi.org/10.1038/jid.2010.282">https://doi.org/10.1038/jid.2010.282</a></p> <p>Pal-Ghosh, S., Tadvalkar, G., Jurjus, R. A., Zieske, J. D., &amp; Stepp, M. A. (2008). BALB/c and C57BL6 mouse strains vary in their ability to heal corneal epithelial debridement wounds. <i>Experimental Eye Research</i>, 87(5), 478–486. <a href="https://doi.org/10.1016/j.exer.2008.08.013">https://doi.org/10.1016/j.exer.2008.08.013</a></p>

## C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

Yes

No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

The animals will be housed individually during the experimental period due to the nature of the wound infection model. Group housing is not feasible, as mice may groom or bite each other's wounds, potentially disrupting the infection model and affecting treatment outcomes. Individual housing minimizes these risks, ensuring consistent infection establishment and wound healing assessment. **Group housing is not feasible due to the risk of wound interference between animals (e.g., scratching or biting), a factor also reported in Rhea et al. (2020). However, the short experimental duration (9 days total) minimizes the impact of individual housing on animal welfare.**

Despite individual housing, efforts will be made to reduce stress and support animal welfare. Each cage will be equipped with environmental enrichment, including nesting materials and shelters, to encourage natural behaviors. Mice will be housed in individually ventilated cages (IVC) under standard laboratory conditions, including a 12-hour light/dark cycle and a maintained temperature of 21–23°C. They will have access to rodent chow and water ad libitum throughout the study.

Animals will be closely monitored (twice per day, daily, for 72 hours post-surgery, and daily for the remaining time of the experiment) for signs of pain, distress, or complications, and appropriate interventions (e.g., analgesia adjustments) will be made as needed. After the study is completed, animals will be killed following Annex IV of Directive 2010/63/EU.

#### D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

No

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

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

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Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

All surgical procedures will be performed under isoflurane anaesthesia with an induction concentration of 4-5% and maintenance at 1.5-3%. To minimize postoperative discomfort, pre-emptive and postoperative analgesia will be administered. For example, Buprenorphine (0.1 mg/kg SC) will be administered at least 30 minutes before surgery and 10-12 hours prior the first administration, and continued twice daily for up to 48 hours postoperatively. If additional pain relief is required, analgesic, such as meloxicam (5 mg/kg SC) will be provided. Mice will be observed twice daily for 72 hours post-surgery to assess pain, wound healing, and general condition. If any animal shows signs of excessive pain, distress, or complications, it will be removed from the study and killed humanely.

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

Animals may experience temporary discomfort at the wound site, including mild swelling, redness, or localized irritation due to the surgical procedure and bacterial infection. Post-surgical stress may occur due to handling during daily monitoring and treatment applications. Additionally, inflammatory responses at the wound site are expected as part of the immune reaction to infection and nanoparticle treatment. If any signs of severe distress, excessive inflammation, delayed wound healing, or systemic illness are observed, appropriate interventions will be taken, including analgesia adjustments or, if necessary, humane endpoint reached, animal will be killed.

Explain why these effects may emerge.

These effects may emerge due to the nature of the wound infection model and the treatments being tested. Temporary mild-moderate discomfort at the wound site is expected as a result of the surgical procedure required to create the full-thickness wound and the subsequent bacterial infection. Post-surgical stress may arise from handling, daily monitoring, and treatment administration, as animals will undergo repeated interventions, including wound assessment and drug application. Inflammatory responses occur naturally as part of the body's immune reaction to bacterial infection and the therapeutic agents. While some level of inflammation is necessary for proper wound healing, excessive inflammation could indicate an adverse reaction to the nanoparticles or an uncontrolled infection.

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

- Adequate use of analgesics
- Adequate use of anaesthesia
- Observation of vital signs during surgery. Animals will be regularly checked both during and after the surgery and every time anaesthesia is induced. Depth or narcosis will be assessed by monitoring reflex responses, including the pedal withdrawal reflex (toe pinch test), palpebral reflex, and response to tail pinch. Additionally, respiration rate and pattern will be observed to ensure appropriate anaesthesia depth.
- Scoring of the animals twice a day, daily, for 72 hours after surgery. If an unexpected adverse event will occur, the adequate actions will be taken.

- Strict aseptic surgical techniques will be followed, including the sterilization of all surgical tools and proper wound irrigation with betadine to prevent unintended infections.
- Animals will be housed individually for the duration of the study to allow wound closure and prevent cross-contamination through wound licking.
- Killing will be performed according to one of the methods listed in Appendix IV of Directive 2010/63/EU.

## E. 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 F

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

According to the humane endpoint checklist we developed, mice will be euthanized with a total score of 15 or higher:

### 1. Welfare Scoring System

#### A. Coat Condition (0-3 points)

- 0 = Smooth, glossy
- 1 = Slightly ruffled
- 2 = Dull, ruffled
- 3 = Very rough, unkempt

#### B. Posture (0-3 points)

- 0 = Normal, active
- 1 = Mildly hunched
- 2 = Moderately hunched
- 3 = Severely hunched, reluctant to move

#### C. Activity (0-3 points)

- 0 = Normal, exploring
- 1 = Reduced activity, less exploration
- 2 = Slow movement, hesitant to move
- 3 = Minimal or no movement

#### D. Weight Loss (0-15 points)

- 0 = None (from pre-surgery measurement)
- 2 = >5% (from pre-surgery measurement)
- 3 = >10% (from pre-surgery measurement)
- 5 = >15% (from pre-surgery measurement)
- 15 = >20% (from pre-surgery measurement)

#### E. Body Temperature (0-15 points)

- 0 = Normal (within 35.5°C to 38.0°C)
- 1 = Mildly elevated (1-2°C above normal)
- 3 = Moderately elevated (2-4°C above normal)
- 15 = Severely elevated (>4°C above normal) or hypothermic (<35.5°C)

#### F. Wound Assessment (0-5 points)

- 0 = Clean, healing well
- 3 = Severe inflammation (marked redness, significant swelling, purulent discharge)
- 4 = Necrotic tissue (black or brown discoloration, tissue death)
- 5 = Spreading infection (red streaks extending from the wound, cellulitis)

#### G. Clinical Condition (0 or 15 points)

- 0 = Normal
- 15 = Moribund (unresponsive, gasping, near death)

#### H. Sepsis Indicators (Any one triggers immediate humane endpoint - 15 points)

- Labored breathing
- Significantly increased respiratory rate
- Pale gums/mucous membranes
- Prolonged capillary refill time
- Cold extremities

### 2. Scoring Guidelines

- Total Score = Sum of all parameters
- Intervention Threshold: Total score  $\geq$  10 (requires veterinary assessment and potential intervention)
- Humane Endpoints:

- Total score  $\geq 15$  OR
- Any single parameter scoring 15 points OR
- Presence of any sepsis indicators

Indicate the likely incidence.

10% based on previously published studies

## F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

- 1) Animal discomfort due to handling: Mild
- 2) Animal discomfort due to individual housing: Mild
- 3) Animal discomfort due to wound creation: Moderate
- 4) Animal discomfort due to bacterial infection: Moderate
- 5) Animal discomfort due to anaesthetic induction with isoflurane: Mild
- 6) Animal discomfort and pain postoperatively due to infection and treatment application, with adequate pain management: Mild to moderate
- 7) Animal discomfort due to wound assessment and dressing changes: Mild
- 8) Animal discomfort due to euthanasia under anaesthesia: Mild

The **cumulative discomfort is classified as Moderate in 90% of the animals (n=52)**, as severe pain, prolonged moderate pain, or mortality are not expected. The study includes appropriate pain management, wound monitoring, and humane endpoints to prevent excessive distress. However, sepsis cannot be ruled out, therefore, we have accounted for the possibility that **up to 10% of the animals (n=6) in this study may experience severe discomfort.**

Severe pain or distress will be mitigated by predefined humane endpoints, including non-healing wounds, excessive weight loss ( $>20\%$ ), signs of systemic infection (sepsis), or severe inflammatory responses. If any of these conditions arise, animals will be removed from the study and humanely killed before experiencing severe discomfort, in accordance with ethical guidelines.

## G. 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 complex interactions between host immune response, bacterial infection, and wound healing in our study necessitate the use of animal models, as these processes cannot be adequately replicated in in vitro or computational models. However, we have implemented extensive in vitro screening of our nanoparticle formulations prior to animal studies to reduce the number of candidates requiring in vivo testing, partially addressing the principle of Replacement within the constraints of our research objectives.
Reduction	To achieve reduction, our experimental design maximizes data obtained from each animal through multiple outcome measures, including bacterial counts, wound healing progression, and histological analysis. Non-invasive monitoring enables longitudinal data collection without requiring additional animals for intermediate timepoints. Through careful standardization of procedures, we prevent unnecessary animal use while maintaining statistical validity. Moreover, all personnel have already received comprehensive training in relevant surgical and experimental procedures, so no additional animals are required for training.
Refinement	Our protocols incorporate several measures to minimize animal distress while improving data quality. We implement a comprehensive pain management protocol and house animals individually to prevent wound interference. Daily monitoring follows standardized protocols, with clear humane endpoints established. Environmental enrichment is provided throughout the study, and all procedures are performed by experienced personnel following standardized protocols. These refinements ensure both animal welfare and scientific rigor, enhancing the quality of our research outcomes while minimizing potential distress to the animals.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

No

Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

Click or tap here to enter text.

## H. Re-use

Will animals be used that have already been used in other animal procedures ?

No > Continue with question I.

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.

Click or tap here to enter text.

## I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

## J. 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 K.

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.

Click or tap here to enter text.

## 3. End of experiment

## K. Destination of the animals

Will the animals be killed during or after the procedures?

No > Provide information on the destination of the animals.

Click or tap here to enter text.

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

Killing is necessary at the end of the experiment to allow for comprehensive analysis of bacterial burden, wound healing progression, and systemic toxicity. Tissue collection from the wound site, major organs, and blood is required for histological analysis, cytokine profiling, and bacterial quantification, all of which cannot be performed in living animals.

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.

Click or tap here to enter text.

Yes > Will a method of killing be used for which specific requirements apply?

No > Describe the method of killing.

The animals will be killed using CO<sub>2</sub> asphyxiation followed by cervical dislocation to ensure death.

Yes > Describe the method of killing that will be used and provide justifications for this choice.

Click or tap here to enter text.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Click or tap here to enter text.



## 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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website ([www.centralecommissiedierproeven.nl](http://www.centralecommissiedierproeven.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'.	11500	
1.2 Provide the name of the licenced establishment.	UMC Utrecht	
1.3 List the serial number and type of animal procedure  <i>Use the numbers provided at 3.4.3 of the project proposal.</i>	Serial number	Type of animal procedure
	2	Subcutaneous biofilm implant model

### 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.

##### Experimental Design

This study utilizes a subcutaneous biofilm implant model in mice (Figure 1) to evaluate antimicrobial nanoparticle therapy via local administration, aptamer-based imaging (diagnosis) and targeted drug delivery (theranostic approach) through systemic administration. The study is divided into three phases:

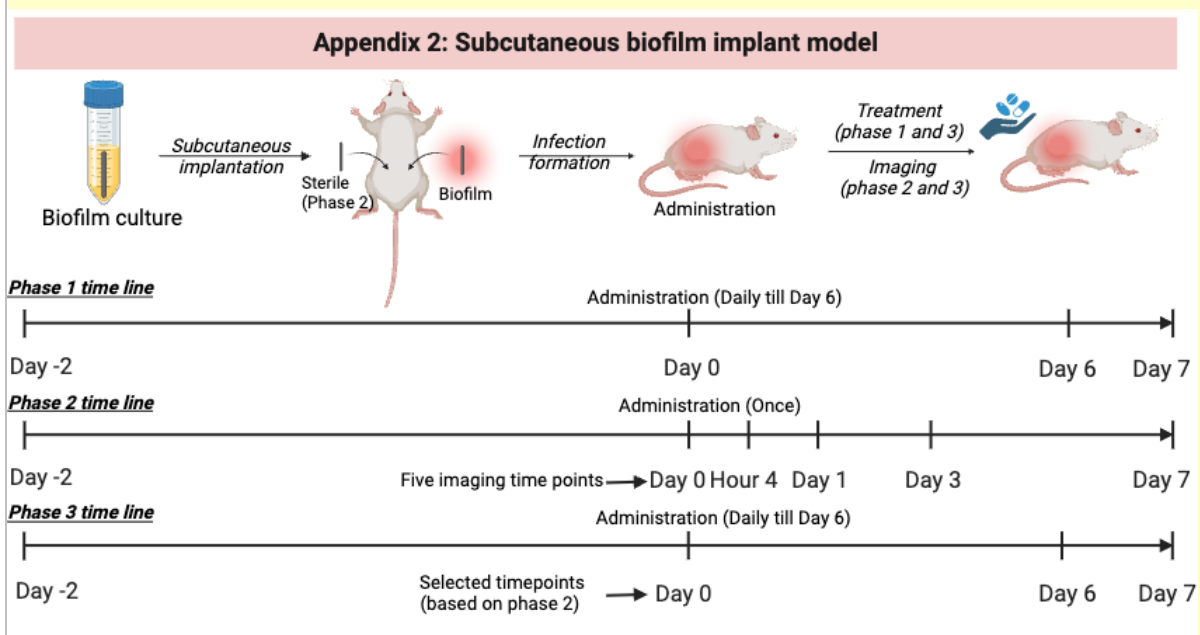
- Phase 1 – Therapeutic Evaluation:
  - Assess antimicrobial efficacy of nanoparticles in treating biofilm-associated infections.
  - Local administration of nanoparticles at the infection site.
- Phase 2 – Diagnostic Imaging:
  - Evaluate aptamer-based imaging probes for detecting biofilm infections.
  - Systemic administration of aptamers and real-time imaging.
- Phase 3 – Theranostic Approach (Therapy + Diagnostic Imaging):
  - Test aptamer-guided nanoparticle delivery for targeted therapy and imaging.
  - Systemic administration of aptamer-nanoparticle conjugates.

The study encompasses a 9-days experimental period:

- Day -2: Biofilm/infected Rob implantation
- Day 0: Biofilm infection formation and:
  - Phase 1

- Day 0-6: Treatment
  - Phase 2
    - Day 0-7: In vivo imaging
  - Phase 3
    - Day 0-6: Treatment
    - Day 0-7: In vivo imaging
- Day 7: Kill animals and collect samples

Each phase includes Go/No-Go decision points to determine the continuation of the study, ensuring ethical use of animals.



**Figure 1.** Surgical procedure of subcutaneous biofilm implantation. In the subcutaneous biofilm implant model, biofilm-coated implant rods are inserted subcutaneously to establish localized biofilm infections. For Phase II diagnostic imaging evaluation, the model incorporates bilateral implantation with infected and sterile implants, enabling each animal to serve as its own control and thereby reducing the total number of animals required for statistical validation.

### Research Questions

This model will be used for sub-aims 1.2-3.1 of the project (see section 3.2.1 of project proposal):

Phase 1 (Therapeutic Evaluation):

- Can locally injected nanoparticle formulations effectively eliminate biofilm infections on implant surfaces?

Phase 2 (Diagnostic Imaging):

- Can aptamer-based molecular imaging effectively distinguish infected implants from sterile implants in the same animal?

Phase 3 (Theranostic Approach (Therapy + Diagnostic Imaging)):

- Can systemically administered aptamer-guided nanoparticle conjugates effectively target and treat deep-seated biofilm infections?

### Overview of Studies

The study is divided into three phases, each with distinct objectives and methodologies to evaluate the efficacy of antimicrobial nanoparticles and aptamer-based imaging for biofilm-associated infections.

In Phase 1 (Therapeutic Evaluation), antimicrobial NPs will be administered via local injection at the site of infection to determine their ability to reduce bacterial burden in the subcutaneous biofilm implant model. The primary outcome of this phase is the reduction of bacterial load, which will be assessed post-mortem through colony-forming unit (CFU) counts. Secondary outcome measures include histological analysis of infected tissues (post mortem).

In Phase 2 (Diagnostic Imaging), aptamer-based imaging probes will be administered systemically via tail vein to evaluate their specificity in detecting biofilm infections in vivo. The primary outcome of this phase is biofilm-specific imaging, which will be measured in vivo through near infra read and bioluminescence imaging techniques to assess aptamer-biofilm co-localization. **Our diagnostic strategy is specifically designed for implant-associated infections, which pose unique challenges for early detection. Unlike superficial wound infections, which are easily accessible and monitored through visual inspection or routine swabs, orthopaedic implants are often embedded deep within tissues or bone, creating a physical barrier that conceals early signs of infection. To address this, we use systemically administered aptamer-based probes conjugated with imaging agents. These probes circulate through the bloodstream, bind to biofilm-specific markers, and accumulate at infection sites. In small-animal models, we utilize near-infrared fluorescence (NIR) imaging, which provides high contrast but is limited to tissue penetration of 1–3 cm. For human applications, the NIR label can be replaced with radionuclides, enabling PET/SPECT imaging, which offers deeper tissue penetration and higher sensitivity for detecting infections in orthopaedic implants.**

In Phase 3 (Theranostic Approach), aptamer-functionalized nanoparticles will be administered systemically via tail vein to investigate their ability to selectively target biofilm sites while providing antimicrobial effects. The primary outcome of this phase is the combined effect of targeted delivery and bacterial reduction, which will be assessed in vivo through near infra read and bioluminescence imaging techniques, and secondary outcome measures include post mortem through CFU counts, imaging data, and histological analysis.

### **Outcome Parameters and Justification**

To evaluate the effectiveness of antimicrobial nanoparticles and aptamer-based imaging strategies in the subcutaneous biofilm implant model, four primary outcome parameters have been selected. These parameters are assigned to specific experimental phases based on their relevance, ensuring a comprehensive yet targeted assessment while minimizing invasive procedures and animal distress.

In Phase 1 (Therapeutic Evaluation), the primary focus is on bacterial reduction and tissue response following treatment with antimicrobial nanoparticles. The primary outcome parameter, bacterial burden assessment, serves as a direct and quantitative measure of antimicrobial efficacy. This is determined post mortem through colony-forming unit (CFU) counts obtained from tissue biopsies by sonication at the terminal timepoint (Day 7). By limiting bacterial sampling to a single collection point, unnecessary distress from repeated invasive procedures is avoided while still obtaining definitive evidence of treatment effectiveness.

The secondary outcome parameter in Phase 1 is histological analysis, which provides critical insight into tissue response following infection and treatment. This includes assessing granulation tissue formation as an indicator of the inflammatory response, and re-epithelialization as a measure of local tissue reaction to treatment. Evaluating these parameters helps determine whether the antimicrobial nanoparticle treatment effectively reduces biofilm burden while also ensuring it does not negatively impact tissue healing.

In Phase 2 (Diagnostic Imaging), the primary goal is to evaluate the ability of aptamer-based imaging probes to selectively detect biofilm infections. The primary outcome parameter, in vivo imaging using fluorescence techniques, is used to assess aptamer binding specificity and biofilm co-localization. Imaging is performed in vivo non-invasively at multiple timepoints, allowing dynamic monitoring of aptamer biodistribution and biofilm targeting efficiency.

In Phase 3 (Theranostic Approach: Diagnosis + Therapy), where aptamer-functionalized antimicrobial nanoparticles are systemically administered, to investigate their ability to selectively target biofilm sites while providing antimicrobial effects. The two co-primary outcome of this phase is the effect of targeted delivery and bacterial reduction, which will be assessed in vivo through near infra read and bioluminescence imaging techniques, and secondary outcome measures include post mortem measurements through CFU counts, imaging data, and histological analysis.

For all phases, survival and clinical scoring is measured to monitor animal health, weight progression, and overall condition. These parameters ensure that the treatment is safe for both local and systemic administration. Humane endpoints will be strictly followed, with predefined criteria for euthanasia if animals exhibit excessive distress (see check list for humane endpoint in both Appendix 1 and 2).

These outcome parameters were selected for their scientific validity, clinical relevance, and ethical considerations. CFU counts and histological analysis in Phase 1 and Phase 3 provide robust, quantifiable data on bacterial reduction and tissue response. In vivo imaging in Phase 2 and Phase 3 enables real-time tracking of biofilm localization and treatment effects, minimizing the need for excessive animal sampling. Clinical scoring across all phases ensures animal welfare while also serving as an additional measure of treatment impact. Together, these parameters offer a comprehensive yet phase-specific evaluation of the proposed antimicrobial and imaging strategies, supporting both scientific rigor and ethical animal research practices.

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

#### **Animal Housing Conditions**

Mice will be group-housed (2–4 per cage) in individually ventilated cages (IVC) under standard laboratory conditions, including a 12-hour light/dark cycle and a maintained ambient temperature of 21–23°C. Mice will be provided with rodent chow diet and water ad libitum. Environmental enrichment, such as nesting material and chew blocks, will be included to promote natural behaviors and reduce stress-induced variability.

To prevent wound-related complications, mice will be housed individually for the first 48 hours post-surgery to minimize the risk of wound disruption due to social interactions. After this period, mice will be returned to their original groups by default, as previously cohoused individuals are less likely to show territorial behavior. Caretaker monitoring will be implemented to assess any signs of aggression upon reintroduction. If persistent aggression or bite wounds are observed, alternative housing strategies will be applied, such as continued individual housing.

Upon delivery, mice will be acclimatized to the new facility for at least 1 week prior the start of the experiment to minimize distress and physiological variations.

#### **Anesthesia Protocol**

All surgical procedures will be performed under isoflurane anesthesia to ensure proper sedation and prevent pain. Anesthesia will be induced at 4-5% isoflurane and maintained at 1.5-3% via inhalation using a nose cone system. The depth of anesthesia will be continuously monitored throughout the procedure, with adjustments made as necessary to assure proper anesthesia and prevent further discomfort. This method provides a rapid induction and recovery while ensuring consistent anesthesia levels throughout the procedure.

#### **Pain Management**

To ensure adequate analgesia, buprenorphine (0.1 mg/kg SC) will be administered at least 30 minutes before surgery and 10-12 hours prior the first administration, and continued twice daily for up to 48 hours postoperatively. If signs of pain or distress persist, additional doses of painkiller, such as buprenorphine or meloxicam (5 mg/kg SC) will be administered. The inclusion of pre-emptive analgesia minimizes perioperative pain and improves postoperative recovery. This approach ensures effective perioperative pain management while allowing for extended analgesia when clinically necessary, aligning with standard clinical practices and ethical animal research practice.

#### **Antiseptic Techniques**

All surgical procedures will be conducted under aseptic conditions except for the infection establishment step. The surgical area will be shaved and disinfected with 70% ethanol and povidone-iodine solution before making an incision. The surgical team will follow strict aseptic protocols, including wearing sterile gloves, surgical masks, and head covers. All surgical instruments will be sterilized before the procedure, and a sterile surgical field will be maintained throughout.

#### **Surgical Technique: Subcutaneous Biofilm Implantation**

A subcutaneous biofilm implantation procedure will be performed to establish a controlled infection model. Each mouse will undergo a 5 mm skin incision on the dorsal flank, followed by blunt dissection to create a subcutaneous pocket for implant placement. **In phase 1** (therapeutic evaluation) and **phase 3** (theranostic approach), a single implant pre-colonized with staphylococcus aureus MRSA strain will be inserted to establish a biofilm infection. **In phase 2** (Diagnostic imaging), a bilateral implantation approach will be used, where each mouse receives two implants—one pre-colonized with staphylococcus aureus to form a biofilm (infected implant) and one sterile implant (negative control) to enable within-animal comparisons of aptamer targeting specificity, thereby reducing the number of animals required. The implants will be carefully positioned within the subcutaneous pockets, ensuring uniform placement across all subjects. The incisions will be closed using absorbable sutures, and the surgical site will be disinfected post-closure to minimize the risk of secondary infection. Post-surgical monitoring will be conducted to ensure proper healing and to detect any signs of distress, with analgesia provided pre- and postoperatively.

#### **Postoperative Care and Monitoring**

Mice will be closely monitored for 72 hours post-surgery to assess pain levels, wound healing, and potential complications. For the first three days postoperatively, animals will be checked twice daily, and their body weight, activity levels, hydration status, and signs of infection (redness, swelling, discharge) will be recorded. After this period, daily monitoring will continue until euthanasia. If severe distress is observed—including persistent weight loss exceeding 20%, lethargy, or wound dehiscence—the animal will be removed from the study and killed (see humane end point).

#### **Nature, Frequency, and Duration of Treatment**

The treatment/diagnosing phase spans over seven days (Figure 1) following a 48-hour biofilm infection establishment period (Day 0).

Treatments will be administered once daily for phase 1 and 3, with variations depending on the experimental phase and intervention type. In **Phase 1** (Therapeutic Evaluation), antimicrobial nanoparticles will be delivered locally via injection at the implant site once per day for seven

consecutive days (Day 0- 6), allowing for the assessment of bacterial reduction over time. In **Phase 2** (Diagnostic Imaging), aptamer-based imaging formulation will be administered systemically via intravenous injection (tail vein) once, followed by imaging at multiple timepoints (baseline (0h), early distribution (4h), Day 1, Day 3, and Day 7) to track aptamer distribution and biofilm targeting efficiency. Imaging sessions will occur at predefined intervals within 24 hours post-injection, based on the pharmacokinetics of the aptamer probe. In **Phase 3** (Theranostic Approach (Therapy + Diagnostic Imaging)), aptamer-functionalized antimicrobial nanoparticles will be administered systemically via intravenous injection once per day for seven consecutive days (Day 0 – 6), with imaging performed at selected timepoints to evaluate both therapeutic efficacy and targeted biofilm localization. The frequency and duration of treatments across all phases are designed to mimic clinical therapeutic regimens, ensure sufficient exposure to antimicrobial and imaging agents, and align with the natural progression of biofilm infections.

#### **Killing Protocol**

Mice will be killed using CO<sub>2</sub> asphyxiation followed by cervical dislocation, in compliance with Annex IV of Directive 2010/63/EU. Following euthanasia, the biofilm-infected and sterile implants will be retrieved for further microbiological and histopathological analysis.

#### **Justification for the Selected Approach**

The selected approach is justified based on scientific validity, clinical relevance, and ethical considerations, ensuring a robust, translational, and ethically sound evaluation of antimicrobial nanoparticles and aptamer-based imaging for biofilm-associated infections. The subcutaneous biofilm implant model was chosen because it accurately mimics implant-associated infections in clinical settings while allowing for controlled assessment of antimicrobial and imaging interventions. The 48-hour infection establishment period ensures that a mature biofilm is formed before initiating treatment, reflecting the chronic nature of biofilm infections seen in human patients.

The seven-day treatment period was selected to align with standard antimicrobial therapy durations used in clinical practice, ensuring that treatment efficacy is assessed over a realistic therapeutic timeframe. The daily treatment schedule allows for sustained antimicrobial exposure and mirrors conventional dosing regimens for infection management. The systemic administration of aptamer-based imaging probes and theranostic nanoparticles in Phase 2 and Phase 3 provides a clinically relevant method for biofilm-targeted diagnostics and therapy, while local administration in Phase 1 enables direct assessment of antimicrobial efficacy at the infection site.

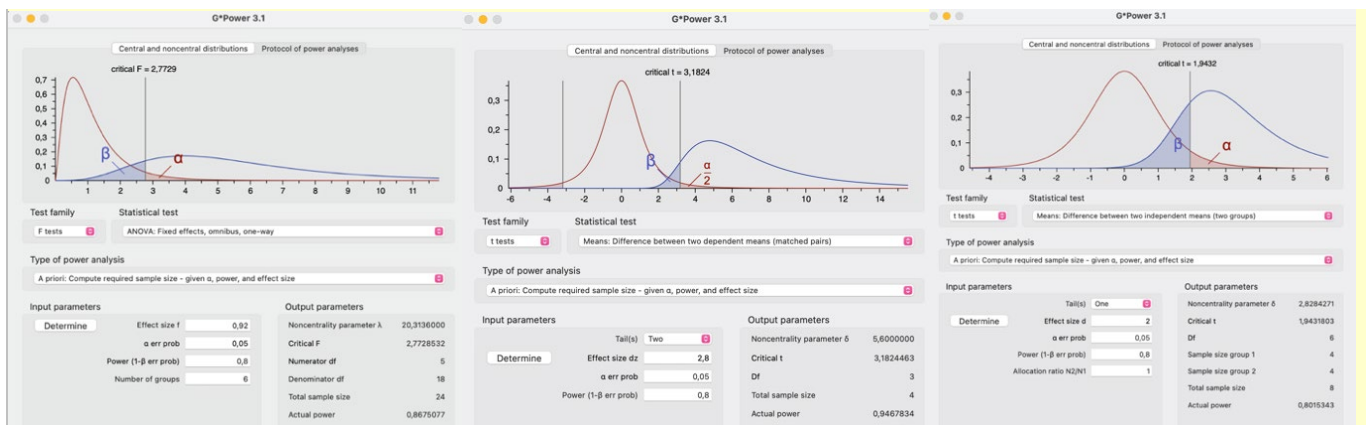
Additionally, the bilateral implantation approach in Phase 2 reduces inter-animal variability by allowing each animal to serve as its own control, minimizing the total number of animals required and aligning with the 3R principles (Replacement, Reduction, and Refinement). The selected outcome parameters—bacterial burden assessment, histological analysis, in vivo imaging, and clinical scoring—ensure a comprehensive evaluation of both therapeutic efficacy and diagnostic accuracy while minimizing unnecessary animal distress through non-invasive monitoring and single terminal sampling. This integrated approach enhances the scientific robustness and translational potential of the findings while maintaining compliance with ethical guidelines for animal research.

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

#### **Statistical Design and Sample Size Calculation**

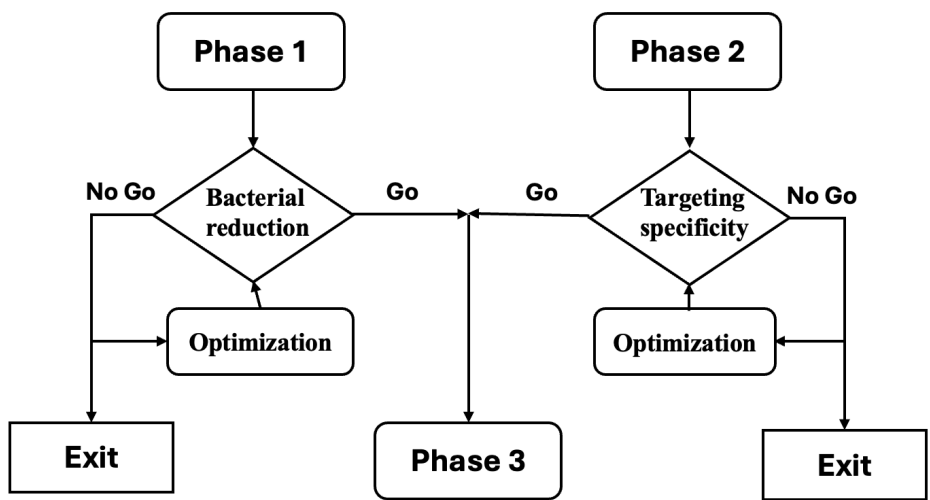
The statistical design follows a single-phase approach, directly implementing the full experimental design without the need of a separate pilot study for the subcutaneous biofilm implant model validation.

power analysis using G\*Power software (Version 3.1), the final group sizes were determined using bacterial burden reduction (colony-forming unit counts) in Phase 1 and Phase 3, and ROI-based near infra red signal intensity in Phase 2 as primary outcome measures. For Phase 1, the analysis assumed an expected effect size of 0.92, a statistical power of 0.8, and a significance level of 0.05, indicating that a minimum of four animals per group would be required to detect a statistically significant difference. To account for a 10% dropout rate, the final group size was adjusted to  $n = 5$  per condition. For Phase 2, the analysis assumed an expected effect size of 2.8, a statistical power of 0.8, and a significance level of 0.05, indicating that a minimum of four animals per group would be required to detect a statistically significant difference. To account for a 10% dropout rate, the final group size was adjusted to  $n = 5$  per condition. For Phase 3, the analysis assumed an expected effect size of 2, a statistical power of 0.8, and a significance level of 0.05, indicating that a minimum of four animals per group would be required to detect a statistically significant difference. To account for a 10% dropout rate, the final group size was adjusted to  $n = 5$  per condition.



**Figure 2.** Print screen from samples size calculation for the phase 1 (left), 2 (middle) and 3 (right).

The statistical analysis plan has been tailored to each experimental phase. For Phase 1 (Therapeutic Evaluation), we will analyse bacterial burden data using log-transformed one-way ANOVA for multiple comparisons, as this phase involves six different treatment groups. This transformation ensures normality and homoscedasticity of the data, improving the reliability of our statistical conclusions. For Phase 2 (Diagnostic Imaging), paired t-tests will be used to compare imaging signal intensities between infected and sterile implants within the same animal. For Phase 3 (Theranostic Approach (Therapy + Diagnostic Imaging)), Student's t-test will be used to compare the two independent groups.



**Figure 2.** This flowchart outlines the decision points for advancing through the project's three phases in the subcutaneous implant infection model (Appendix 2). Phase 1 requires a significant reduction in bacterial load within biofilm-infected implants. Phase 2 focuses on achieving high targeting specificity (signal ratio  $\geq 3:1$ ). These phases are independent and do not influence each other. Phase 3 integrates the validated therapeutic and diagnostic components for systemic treatment. The project is terminated if the predefined criteria are not met.

A go/no-go decision point is both feasible and integral to the experimental design. This checkpoint is strategically placed after the formulation efficacy testing phase to assess whether the tested nanoparticle formulations (Appendix 2 – Phase 1) or aptamers (Appendix 2 – Phase 2) meet predefined efficacy criteria. If the initial candidates fail, a limited number of alternatives will be systematically tested to prevent indefinite progression. If no candidates achieve the required efficacy, the project will be terminated (no-go).

### **Number of Animals (up to 91 mice)**

All personnel involved in this study have prior training in relevant surgical and experimental procedures. Where additional training is required for specific techniques, it will be conducted ex vivo to minimize the use of live animals. Furthermore, whenever feasible, animals that have been humanely euthanized at the end of experiments will be utilized for training purposes, ensuring compliance with the 3Rs principle by reducing the number of additional animals needed for training.

### **Phase 1: Therapeutic Evaluation (up to 57 mice)**

- Research question: Can locally injected nanoparticle formulations effectively eliminate biofilm infections on implant surfaces?

- **Model Design:**  
Implants will be pre-colonized with bacterial biofilm by incubating with *S. aureus* under appropriate conditions to establish mature biofilm on the surface. These biofilm-colonized implants will then be subcutaneously inserted into mice. After 48 hours of in vivo infection establishment, nanoparticle formulations will be locally injected around the implant site. The bacterial burden will be determined through two methods: (1) CFU counts from the implant surface following sonication to dislodge adherent bacteria, and (2) CFU counts from surrounding tissue homogenates. Tissue integration and inflammatory response will be evaluated through histological examination of the peri-implant tissue, including assessment of inflammatory cell infiltration, fibrosis, and vascularization. All measurements will be done post-mortem.
- **Group allocation:**
  1. Untreated infection control (n = 5)
  2. Nanoparticle formulation 1 – low concentration (n = 5)
  3. Nanoparticle formulation 1 – high concentration (n = 5)
  4. Nanoparticle formulation 2 – low concentration (n = 5)
  5. Nanoparticle formulation 2 – high concentration (n = 5)
  6. Antibiotics (positive control, n=5)
- If formulations 1 and 2 do not meet efficacy criteria, formulations 3, 4, and 5 will be evaluated sequentially
- Each new formulation will be tested at both high and low concentrations (n=5 per group, up to 15 mice)

To further reduce animal numbers while maintaining statistical rigor, we will assess inter-experiment variation. If variability between experiments remains low, historical control data will be incorporated, reducing the need for additional positive and negative control animals. However, if significant variability is observed, extra animals will be included to ensure robust statistical comparisons (up to 12 mice: 4 mice per formulation, 2 as negative and 2 as positive control). This adaptive approach aligns with the 3Rs principle while preserving scientific integrity. That will be carried out after discussion with statisticians on work protocol.

#### **Go/No-Go Criteria for Advancing from Phase 1 to Phase 3:**

Go Criteria:

- Bacterial reduction: Minimum 2-log reduction in bacterial load (both tissue homogenate and implant surface)

No-Go Criteria:

- Bacterial reduction: <2-log reduction in bacterial load

#### **Phase 2: Diagnostic Imaging (up to 24 mice, Dual-Implantation Model)**

- Research question: Can aptamer-based molecular imaging effectively distinguish infected implants from sterile implants in the same animal?
- **Model Design:**  
The bilateral implant model involves subcutaneous insertion of two identical implants in the same animal - one pre-colonized with bacterial biofilm and one sterile. The infected implant will be prepared by pre-incubating with *S. aureus* under appropriate conditions to establish biofilm, while the contralateral implant remains sterile. After 48 hours of rob implantation (Day 0) the biofilm model will be established, and aptamer-based imaging probes will be administered systemically via tail vein injection. In vivo imaging will be performed at five timepoints over 7 days: baseline (prior aptamer administration), early distribution (4h after administration), Day 1, Day 3, and Day 7 after administration. Each imaging session will be limited to 15 minutes under carefully controlled anesthesia, with animals maintained on a heating pad to prevent hypothermia. A minimum 24-hour recovery period will be ensured between imaging sessions to minimize animal distress. Bacterial burden will later be confirmed post mortem through CFU counts from both implant surfaces (following sonication) and surrounding tissue homogenates at study endpoint. This paired design enables direct comparison of targeting specificity while controlling for individual variations in biodistribution, thereby reducing biological variability and total animal numbers required.
- **Group allocation (Each mouse receives both implants for internal control):**
  1. Aptamer 1 (n = 5)
  2. Non-specific aptamer (n = 5)
- If Aptamer 1 does not achieve desired imaging outcomes, we have two additional aptamer candidates (Aptamer 2 and 3) ready for evaluation, each requiring 5 mice for testing (n= 5 per group, up to 10 mice). To reduce uncertainty, when we test new candidates, we would add two new control animals (non-specific aptamer) per test (up to 4 mice, 2 per each additional aptamer).

#### **Go/No-Go Criteria Advancing from Phase 2 to Phase 3:**

Go Criteria:

- Targeting specificity: Signal ratio  $\geq 3:1$  (infected implant vs. sterile implant)
- Background signal: <20% accumulation at sterile implant site compared to infected implant

#### No-Go Criteria:

- Targeting specificity: Signal ratio <3:1 (infected vs. sterile implant)
- Background signal: >20% accumulation at sterile implant site

#### **Phase 3: Theranostic Approach (10 mice)**

- Research question: Can systemically administered aptamer-guided nanoparticle conjugates effectively target and treat deep-seated biofilm infections?
- Model Design:

This phase utilizes a single implant model with aptamer-functionalized dual-functional nanoparticles designed for both therapeutic and diagnostic purposes. The nanoparticles will be conjugated with specific aptamers targeting bacterial biofilm components and labeled with near-infrared fluorescent dyes to enable real-time tracking of their biodistribution. An implant pre-colonized with bacterial biofilm will be inserted subcutaneously. After 48 hours of in vivo infection establishment, aptamer-guided theranostic nanoparticles will be administered systemically via tail vein injection.

In vivo near infra red imaging will be performed at five key timepoints (based on Phase 2): baseline (prior aptamer administration), early distribution (such as 4h after administration), and 3 additional time points, such as Day 1, Day 3, and Day 7 after administration. Each imaging session will be limited to 15 minutes under carefully controlled anesthesia, with animals maintained on a heating pad to prevent hypothermia. A minimum 24-hour recovery period will be ensured between imaging sessions to minimize animal distress. Bacterial burden will later be confirmed post mortem through CFU counts from implant surfaces (following sonication) and surrounding tissue homogenates at study endpoint. Comprehensive post mortem biodistribution studies will analyze nanoparticle accumulation in major organs through fluorescence imaging and quantification. Safety assessments will include blood analysis, histopathological examination of major organs, and monitoring of clinical parameters.
- Group allocation:
  1. Untreated infection control (n = 5)
  2. Aptamer- nanoparticle conjugate (n = 5)

#### **Expected Outcomes:**

Positive (and research with current formulations continues – out of the scope of this application):

- Therapeutic efficacy:  $\geq 90\%$  reduction in bacterial burden
- Imaging sensitivity: Signal-to-noise ratio  $\geq 3:1$  at infection site
- Safety:  $< 20\%$  change in blood chemistry/cell counts; no significant organ toxicity

Negative (re-evaluation of formulations and back to the drawing board):

- Therapeutic efficacy:  $< 90\%$  bacterial reduction after 72h
- Imaging sensitivity: Signal-to-noise ratio  $< 3:1$
- Safety:  $> 20\%$  change in blood parameters such as white blood cell count (WBC) and C-reactive protein (CRP), or significant organ toxicity

#### **Considerations for Minimizing Animal Use**

This study is designed to minimize the number of animals used while maintaining scientific validity, adhering to the 3R principles (Replacement, Reduction, and Refinement). Several key strategies have been implemented to achieve this. First, a bilateral implantation model is used in Phase 2 (Diagnostic Imaging), where each mouse receives two subcutaneous implants—one infected implant pre-colonized with *Staphylococcus aureus* to form a biofilm and one sterile implant as a negative control. This within-animal control design reduces inter-animal variability, allowing for paired statistical comparisons (refinement) and significantly decreasing the number of animals required for statistical significance.

Second, each animal contributes to multiple outcome measures, eliminating the need for separate animal cohorts for different endpoints. In Phase 1 (Therapeutic Evaluation) and Phase 3 (Theranostic Approach (Therapy + Diagnostic Imaging)), bacterial burden (colony-forming unit counts) and histological analysis are performed on the same samples at the terminal timepoint (Day 7) to avoid additional animals for intermediate sampling. In Phase 2, non-invasive fluorescence/bioluminescence imaging enables longitudinal monitoring of aptamer targeting over time, eliminating the need for multiple groups of animals at different timepoints.

Third, pre-study pilot data and literature-based optimization reduce unnecessary animal use. Instead of conducting a separate pilot study, the experimental approach is based on previously validated biofilm models and treatment protocols, ensuring that all procedures, including bacterial inoculation, nanoparticle administration, and imaging settings, are already optimized. Dosing regimens and imaging timepoints have been refined through prior in vitro and small-scale in vivo studies, ensuring efficient use of resources and preventing unnecessary replication of validation experiments.

Lastly, strict standardization protocols help reduce biological variability, allowing for a lower required sample size. All animals are controlled for age, sex, body weight, and housing conditions, ensuring that treatment effects are not confounded by extraneous factors. Technical procedures, including surgical implantation, infection establishment, treatment administration, and imaging protocols, are performed by experienced personnel using calibrated instruments, further minimizing variability and ensuring that fewer animals are needed to achieve statistically robust results. These combined strategies ensure that the study is conducted with maximum efficiency, scientific rigor, and ethical responsibility while adhering to animal welfare guidelines.

## Reference

Dijk, B. van, Oliveira, S., Hooning van Duyvenbode, J. F. F., Nurmohamed, F. R. H. A., Mashayekhi, V., Hernández, I. B., van Strijp, J., de Vor, L., Aerts, P. C., Vogely, H. C., Weinans, H., & van der Wal, B. C. H. (2024). Photoimmuno-antimicrobial therapy for Staphylococcus aureus implant infection. *PloS one*, 19(3), e0300069. <https://doi.org/10.1371/journal.pone.0300069>

van Dijk, B., Hooning van Duyvenbode, J. F. F., de Vor, L., Nurmohamed, F. R. H. A., Lam, M. G. E. H., Poot, A. J., Ramakers, R. M., Koustoulidou, S., Beekman, F. J., van Strijp, J., Rooijackers, S. H. M., Dadachova, E., Vogely, H. C., Weinans, H., & van der Wal, B. C. H. (2023). Evaluating the Targeting of a Staphylococcus-aureus-Infected Implant with a Radiolabeled Antibody In Vivo. *International Journal of Molecular Sciences*, 24(5), 4374. <https://doi.org/10.3390/ijms24054374>

## B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Mouse	Charles River Laboratories	Adult (>20 g)	91	Male	No	Balb/cAnNCrl

Provide justifications for these choices

Species	Mice are selected because they are widely used in biofilm infection models. They provide a reliable and reproducible system for studying bacterial colonization, immune response, and aptamer targeting. Their small size makes them suitable for near infra red imaging, allowing real-time tracking of aptamer biodistribution. Murine models of S. aureus chronic biofilm infections are widely used to evaluate antimicrobial therapies, providing translational insights into human infections.
Origin	Mice will be obtained from Charles River Laboratories, a well-established supplier known for providing standardized research animals. Using animals from a consistent source helps reduce variability in genetics, microbiome composition, and immune function.
Life stages	Adult mice weighing over 20 g will be used. This weight ensures that they are fully developed and can tolerate implantation without major physiological variations. Younger mice (juveniles) have an immature immune system, which may lead to different inflammatory and healing responses, potentially affecting study outcomes (Jackson et al., 2017).  <b>Reference</b> Jackson, S. J., Andrews, N., Ball, D., Bellantuono, I., Gray, J., Hachoumi, L., Holmes, A., Latcham, J., Petrie, A., Potter, P., Rice, A., Ritchie, A., Stewart, M., Strepka, C., Yeoman, M., & Chapman, K. (2017). Does age matter? The impact of rodent age on study outcomes. <i>Laboratory Animals</i> , 51(2), 160–169. <a href="https://doi.org/10.1177/0023677216653984">https://doi.org/10.1177/0023677216653984</a>
Number	A maximum of 91 mice is estimated based on power analysis, with group sizes determined to ensure statistical validity while minimizing the number of animals used. Extra animals are included to account for potential losses due to surgical complications, or secondary infections or injection failures. Each mouse in Phase 2 will receive both an infected biofilm implant and a sterile control implant, reducing variability and minimizing the number of animals needed.
Gender	<b>We fully acknowledge the historical bias in preclinical research towards male subjects and recognise that post-orthopaedic and wound infections affect both sexes in human medicine. Addressing this issue is important, and we are committed to ensuring that our findings are translationally relevant.</b>  <b>The decision to use exclusively male mice in this project is based on scientific and methodological considerations that prioritise experimental consistency and statistical robustness. Male mice exhibit more stable physiological characteristics in terms of bone healing and immune responses, as they are not subject to cyclical hormonal</b>

	<p>fluctuations. This reduces variability, which is particularly important when studying subtle differences in infection progression and treatment efficacy.</p> <p>Female mice undergo oestrous cycles, causing periodic variations in oestrogen levels, which can influence immune responses and wound healing processes. Such fluctuations could introduce additional variability, potentially confounding our results. This approach aligns with common practice in early mechanistic validation studies, allowing us to focus on core experimental variables (such as nanoparticle targeting) without the complexities introduced by sex-related differences.</p> <p>Furthermore, using a single-sex cohort at this stage minimises within-group variation and increases statistical power, enabling us to obtain reliable results with smaller sample sizes, in accordance with the "Reduction" principle of the 3Rs. Additionally, the orthopaedic infection model we employ has well-established parameters and baseline data in male mice, allowing us to directly compare our findings with existing literature, thereby enhancing the reliability and reproducibility of our research.</p> <p>Our approach follows a strategic experimental sequence rather than perpetuating bias. We begin with male mice to establish foundational mechanisms before incorporating sex as a biological variable in future studies. The antimicrobial mechanisms we study operate at the cellular and molecular levels, which are largely conserved across sexes. Since our focus is on pathogen-targeting strategies rather than host-response modulation, the core principles of our findings are expected to be broadly applicable.</p> <p>To ensure sex-inclusive research, <b>our plan includes subsequent studies with female animals once core mechanisms are validated (outside the scope of this application)</b>. This stepwise approach balances scientific rigour and reproducibility, ensuring that sex differences can be properly assessed in later phases without unnecessary variability in early mechanistic studies.</p> <p>This sequential approach aligns with best practices in translational research, allowing us to first establish proof-of-concept before expanding into more complex, sex-inclusive models.</p>
Genetic alterations	Wild-type mice will be used. No genetic modifications are necessary for this study since the focus is on aptamer targeting rather than host genetic factors.
Strain	<p>The Balb/cAnNCrI strain is selected for this implant infection model due to its well-documented immune responses to biomaterial-associated infections. This strain shows reproducible host-pathogen interactions that closely mirror human responses to implant infections. The extensive validation data available for Balb/cAnNCrI mice in similar infection models enhances the translational relevance of our findings and facilitates comparison with established literature (Kim et al., 2014).</p> <p><b>Reference</b> Kim, H. K., Missiakas, D., &amp; Schneewind, O. (2014). Mouse models for infectious diseases caused by <i>Staphylococcus aureus</i>. <i>Journal of Immunological Methods</i>, 410, 88–99. <a href="https://doi.org/10.1016/j.jim.2014.04.007">https://doi.org/10.1016/j.jim.2014.04.007</a></p>

### C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

Yes

No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

Due to the specific needs of post-surgical recovery, animals will be individually housed during the initial recovery period of 2 days (maximum of three days, depending on incision recovery), to ensure proper healing of surgical sites and to minimize the risk of complications from interactions that could disturb the wounds. After the surgical wounds have sufficiently healed, the animals will be returned to group housing, with 2–4 mice per cage, to allow social interaction while maintaining their well-being. To minimize aggression, male mice will be rehoused in their original groups, as previously cohoused individuals are less likely to show territorial behavior. Caretaker monitoring will be implemented to assess any signs of aggression upon reintroduction. If persistent aggression or bite wounds are observed, alternative housing strategies will be applied, such as continued individual housing for maximum of nine days. For infection models, the initial individual housing

also reduces the risk of cross-contamination between animals during the critical postoperative phase. Despite the short time individual housing, efforts will be made to reduce stress and support animal welfare.

#### **D. Pain and compromised animal welfare**

Will the animals experience pain during or after the procedures?

No

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

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

Click or tap here to enter text.

Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

All surgical procedures will be performed under isoflurane anesthesia with an induction concentration of 4-5% and maintenance at 1.5-3%. To minimize postoperative discomfort, pre-emptive and postoperative analgesia will be administered. For example, Buprenorphine (0.1 mg/kg SC) will be administered at least 30 minutes before surgery and 10-12 hours prior the first administration, and continued twice daily for up to 48 hours postoperatively. If additional pain relief is required, analgesic, such as meloxicam (5 mg/kg SC) will be provided. Mice will be observed twice daily for 72 hours post-surgery to assess pain, wound healing, and general condition. If any animal shows signs of excessive pain, distress, or complications, it will be removed from the study and killed humanely.

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

Animals may experience temporary discomfort at the wound site, including mild swelling, redness, or localized irritation due to the surgical procedure and bacterial infection. Post-surgical stress may occur due to handling during daily monitoring and treatment applications. If any signs of severe distress, excessive inflammation, delayed wound healing, or systemic illness are observed, appropriate interventions will be taken, including analgesia adjustments or, if necessary, humane endpoint reached, animal will be killed

Explain why these effects may emerge.

These effects are associated with the implantation procedure and infection establishment. Mild inflammation and localized discomfort are expected due to the presence of *S. aureus* biofilms at the implantation site.

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

- Adequate use of analgesics
- Adequate use of anaesthesia
- Observation of vital signs during surgery. Animals will be regularly checked both during and after the surgery and every time anaesthesia is induced. Depth or narcosis will be assessed by monitoring reflex responses, including the pedal withdrawal reflex (toe pinch test), palpebral reflex, and response to tail pinch. Additionally, respiration rate and pattern will be observed to ensure appropriate anesthesia depth.
- Scoring of the animals twice a day, daily, for 72 hours after surgery. If an unexpected adverse event will occur, the adequate actions will be taken.
- Strict aseptic surgical techniques will be followed, including the sterilization of all surgical tools and proper wound irrigation with betadine to prevent unintended infections.
- Animals will be housed individually for up to 3 days after the surgery to allow the wounds closure.
- Killing will be performed according to one of the methods listed in Appendix IV of Directive

#### **E. 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 F

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

According to the humane endpoint checklist, mice will be removed from the study (killed) with a total score of 15 or higher:

##### **1. Welfare Scoring System**

**A. Coat Condition (0-3 points)**

- 0 = Smooth, glossy
- 1 = Slightly ruffled
- 2 = Dull, ruffled
- 3 = Very rough, unkempt/hair loss

**B. Posture (0-3 points)**

- 0 = Normal, active
- 1 = Mildly hunched
- 2 = Moderately hunched
- 3 = Severely hunched, immobile

**C. Activity (0-3 points)**

- 0 = Normal, exploring
- 1 = Reduced activity
- 2 = Slow movement
- 3 = Minimal/no movement

**D. Weight Loss (0-15 points)**

- 0 = None (from pre-surgery measurement)
- 2 = >5% (from pre-surgery measurement)
- 3 = >10% (from pre-surgery measurement)
- 5 = >15% (from pre-surgery measurement)
- 15 = >20% (from pre-surgery measurement)

**E. Body Temperature (0-15 points)**

- 0 = Normal (within 35.5°C to 38.0°C)
- 1 = Mildly elevated (1-2°C above normal)
- 3 = Moderately elevated (2-4°C above normal)
- 15 = Severely elevated (>4°C above normal) or hypothermic (<35.5°C)

**F. Food/Water Intake (0-15 points)**

- 0 = Normal
- 1 = Slightly reduced
- 3 = Markedly reduced
- 5 = Minimal intake
- 15 = No intake >24h

**G. Implant Site Assessment (0-5 points)**

Local Swelling:

- 0 = None
- 1 = Mild (<5mm)
- 2 = Moderate (5-10mm)
- 3 = Severe (>10mm)

Exudate:

- 0 = None
- 1 = Mild serous
- 2 = Moderate serous
- 3 = Severe serous
- 5 = Purulent

Tissue Necrosis:

- 0 = None
- 2 = Limited
- 3 = Moderate
- 5 = Extensive

Infection Spread:

- 0 = None
- 1 = Limited to implant site
- 2 = Within 1cm
- 3 = 1-2cm
- 5 = >2cm
- 15 = Systemic spread

**H. Clinical Condition (0-15 points)**

- 0 = Normal
- 3 = Mildly depressed
- 5 = Markedly depressed
- 15 = Moribund

### I. Sepsis Indicators (Any one triggers immediate endpoint - 15 points)

- Labored breathing
- Significantly increased respiratory rate
- Pale mucous membranes
- Prolonged capillary refill time (>3 sec)
- Cold extremities

### 2. Implementation Guidelines

- Total Score = Sum of all parameters
- Intervention Threshold:  $\geq 10$  points (requires veterinary assessment)
- Humane Endpoints:
  - Total score  $\geq 15$  OR
  - Any single score of 15 points OR
  - Weight loss  $>20\%$  OR
  - Any sepsis indicator OR
  - Systemic infection spread OR
  - Extensive tissue necrosis OR
  - No food intake  $>24$ h

Indicate the likely incidence.

10% based on our groups experience with the model.

### F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

- 1) Animal discomfort due to the handling: mild
- 2) Animal discomfort due to individual housing: mild
- 3) Animal discomfort due to the surgery: moderate
- 4) Animal discomfort due to the infection: moderate
- 5) Animal discomfort due to the anaesthetic induction with isoflurane: mild
- 6) Animal discomfort and pain postoperatively due to the implantation with adequate pain medication: mild to moderate
- 7) Animal discomfort due to imaging under anaesthesia: mild to moderate
- 8) Animal discomfort due to the euthanasia under anaesthesia: mild

The **cumulative discomfort is classified as Moderate in 90% of the animals (n=82)**, as severe pain, prolonged moderate pain, or mortality are not expected. The study includes appropriate pain management, wound monitoring, and humane endpoints to prevent excessive distress. However, sepsis cannot be ruled out, therefore, we have accounted for the possibility that **up to 10% of the animals (n=9) in this study may experience severe discomfort.**

### G. 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	While biofilm-associated infections are complex conditions that cannot be fully replicated in vitro, this study incorporates alternative non-animal approaches wherever possible to minimize reliance on live animals. Extensive in vitro screening is conducted before proceeding to animal experiments. Bacterial biofilm models, microfluidic platforms, and co-culture systems are used to evaluate the efficacy of antimicrobial nanoparticles and aptamer-based imaging probes under controlled conditions. This ensures that only the most promising candidates are selected for in vivo testing, preventing unnecessary animal use. Additionally, computational modeling and simulations help refine experimental conditions, predicting optimal nanoparticle formulations and aptamer
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	targeting efficiency before live testing. However, since biofilm infections involve complex host-pathogen interactions, which cannot be fully replicated in vitro, animal models remain necessary to evaluate treatment efficacy and in vivo distribution in a physiologically relevant environment.
Reduction	Reduction focuses on using the minimum number of animals necessary to obtain statistically significant and reproducible results. In this study, several strategies have been implemented to achieve this goal. First, a bilateral implantation model is used in Phase 2 (Diagnostic Imaging), where each mouse receives both an infected biofilm implant and a sterile control implant, allowing within-animal comparisons. This design reduces inter-animal variability and halves the number of animals required for imaging studies. Additionally, each animal contributes to multiple outcome measures, including bacterial burden, histological analysis, and imaging, eliminating the need for separate cohorts for different readouts. The statistical design is also optimized through power analysis, ensuring that the sample size is large enough for statistical robustness but not excessive, preventing unnecessary animal use. Moreover, all personnel have already received comprehensive training in relevant surgical and experimental procedures, so no additional animals are required for training.
Refinement	Refinement ensures that procedures are designed to minimize pain, distress, and discomfort while improving animal welfare. In this study, all surgical procedures are performed under strict aseptic conditions with pre-, intra-, and post-operative analgesia to ensure minimal pain. Absorbable sutures are used to eliminate the need for post-procedural removal, reducing handling stress. Animals are housed in enriched environments with proper bedding, nesting materials, and social housing whenever possible to promote natural behaviors and reduce stress. Additionally, non-invasive imaging techniques (near infra red and bioluminescence imaging) allow real-time tracking of biofilm infections without requiring repeated invasive sampling, further improving animal welfare. Humane endpoints are strictly followed, with daily health monitoring and predefined euthanasia criteria to prevent unnecessary suffering.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

No

Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

Click or tap here to enter text.

#### H. Re-use

Will animals be used that have already been used in other animal procedures ?

No > Continue with question I.

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.

Click or tap here to enter text.

#### I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

#### J. 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 K.

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.

Click or tap here to enter text.

### 3. End of experiment

#### K. Destination of the animals

Will the animals be killed during or after the procedures?

No > Provide information on the destination of the animals.

Click or tap here to enter text.

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

Killing is necessary at the end of the experiment to allow for comprehensive analysis of bacterial burden, implanted constructs analysis, and systemic toxicity. Tissue collection from the wound site, major organs, and blood is required for histological analysis, cytokine profiling, and bacterial quantification, all of which cannot be performed in living animals.

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.

Click or tap here to enter text.

Yes > Will a method of killing be used for which specific requirements apply?

No > Describe the method of killing.

The animals will be killed using CO<sub>2</sub> asphyxiation followed by cervical dislocation to ensure death.

Yes > Describe the method of killing that will be used and provide justifications for this choice.

Click or tap here to enter text.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Click or tap here to enter text.

**A. Algemene gegevens over de procedure**

1. Aanvraagnummer : AVD11500202518775
2. Titel van het project : Antimicrobial strategies for drug-resistant and biofilm-associated infections
3. Titel van de NTS : Ontwikkeling van nieuwe methoden om hardnekkige bacteriële infecties te bestrijden

## 4. Type aanvraag:

- nieuwe aanvraag projectvergunning  
 wijziging van vergunning met nummer :

## 5. Contactgegevens DEC

Naam DEC : DEC Utrecht  
Telefoonnummer contactpersoon : 06-31118069  
Emailadres contactpersoon : dec-utrecht@umcutrecht.nl

## 6. Adviestraject (data dd-mm-jjjj):

- ontvangen door DEC: 28-02-2025  
 aanvraag compleet:  
 in vergadering besproken: 05-03-2025  
 anderszins behandeld:  
 termijnonderbreking(en) van / tot: 11-03-2025 / 25-03-2025  
 besluit van CCD tot verlenging van de totale adviestermijn met max. 15 werkdagen:  
 aanpassing aanvraag:  
 advies aan CCD: 07-04-2025

## 7. De aanvraag is afgestemd met de IvD en deze is hiermee akkoord.

## 8. Eventueel horen van aanvrager

- Datum: 05-03-2025
- Plaats: Online via teams
- Aantal aanwezige DEC-leden: 6
- Aanwezige (namens) aanvrager: Verantwoordelijk onderzoeker en collega
- Gestelde vragen en verstrekte antwoorden: De DEC heeft de onderzoekers o.a. gehoord over de achtergrond, de doelstellingen, de te gebruiken bacteriën (MRSA / stafylokokken aureus), de argumentatie voor mannelijke of vrouwelijke dieren en de individuele huisvesting. Hieruit zijn onderstaande vragen, zoals vermeld bij punt A9, voortgekomen, die schriftelijk aan de onderzoekers werden voorgelegd.
- Het horen van de aanvrager heeft geleid tot aanpassing van de aanvraag.

## 9. Correspondentie met de aanvrager

- Datum vragen: 11-03-2025
- Datum antwoord: 25-03-2025
- Strekking gestelde vragen en antwoorden:

### *Project proposal*

#### 3.1 Background

The project is primarily directed to a therapeutic approach to combat of biofilm infections of wounds and implants. Could you please explain why you want to use an MRSA strain and not a non-resistant *Staphylococcus aureus* strain. Using an MRSA clinical isolate would increase an additional challenge in the selection of therapeutic agents (inside the nanoparticles). Moreover using a clinical MRSA isolate require an additional microbiological safety level in the experimental setting?

*A key focus of this project is addressing methicillin-resistant *Staphylococcus aureus* (MRSA), a major cause of biofilm-associated infections in both hospital and community settings. We have chosen the USA300 LAC strain, a clinically relevant, community-acquired MRSA isolate obtained from the Medical Microbiology Laboratory at UMC Utrecht.*

*Both MRSA and non-resistant *S. aureus* form biofilms, but MRSA presents an additional challenge due to its resistance to common antibiotics such as penicillin. By targeting MRSA, we address both biofilm-mediated tolerance and antibiotic resistance. Using a non-resistant *S. aureus* strain would not fully capture the complexity of real-world infections, where antibiotic resistance often complicates treatment.*

*Our nanoparticles are designed to deliver non-antibiotic agents (e.g., reactive oxygen species) that act independently of traditional antibiotics, ensuring that MRSA's resistance does not limit the selection or efficacy of therapeutic agents. Demonstrating success against MRSA strengthens the therapeutic potential of our approach, as highlighted in the project title. Regarding biosafety, no additional precautions are required. Both MRSA and non-resistant *S. aureus* are classified as ML-2 pathogens (moderate risk) under Dutch RIVM guidelines. All experiments are conducted under strict ML-2 protocols, ensuring full compliance with ethical and regulatory standards.*

Could you please consistently distinguish between biofilm infections and MRSA infections in your description? For which goals do you need MRSA and for which goals this is not necessary?

*We have clarified this distinction in the text.*

*MRSA infections represent a subset of biofilm infections, combining biofilm-mediated tolerance with antibiotic resistance. In our project, MRSA is specifically required for therapeutic testing (phases 1 and 3) to validate our approach against both biofilm protection and antibiotic resistance.*

*For diagnostic development (phase 2), MRSA is not strictly necessary, as the tools target universal biofilm features, such as extracellular polymeric substances (EPS), metabolic activity, and structural organization. However, we include MRSA to demonstrate broad applicability—if our approach is effective against MRSA-associated biofilms, it should also be effective against biofilms formed by other bacterial species. While MRSA does not introduce additional technical*

*challenges in diagnostics, its inclusion strengthens our platform's relevance in high-priority, clinically challenging scenarios.*

Please give also a short explanation on the origins of the MRSA strains you want to use. *The MRSA strain used is USA300 LAC, a clinically relevant, community-acquired strain obtained from the Medical Microbiology Laboratory at UMC Utrecht.*

Could you please focus in the description of the clinical relevance on data (examples) which are directly linked to this research? For example, you mention in the background that 15-20% of all orthopaedic surgeries lead to infections. This figure seems to be very high. Please rephrase or give a recent reference justifying such a statement.

*We acknowledge that the original statement may have been misleading. The 15–20% figure refers to the re-failure rate after complete infection-control procedures, not the overall infection rate in orthopaedic surgeries. We have re-written for clarity (below).*

*Biofilm-related implant infections are a growing concern in orthopaedic implants and are increasingly recognized in medical research. Periprosthetic joint infections (PJIs) occur in approximately 1–2% of primary joint replacements and 3–5% of revision surgeries (Izakovicova et al., 2019). Once a PJI is established, infection-control procedures such as debridement, antibiotics, and implant retention (DAIR) or revision surgeries achieve success rates of 77–84%. However, biofilm persistence leads to treatment failure in approximately 15–20% of cases, resulting in recurrent infections and further surgical interventions (Maimaiti et al., 2023; Pangaud et al., 2019).*

#### *Reference*

*Izakovicova, P., Borens, O., & Trampuz, A. (2019). Periprosthetic joint infection: Current concepts and outlook. EFORT Open Reviews, 4(7), 482–494. <https://doi.org/10.1302/2058-5241.4.180092>*

*Maimaiti, Z., Li, Z., Xu, C., Fu, J., Hao, L.-B., Chen, J.-Y., & Chai, W. (2023). Host Immune Regulation in Implant-Associated Infection (IAI): What Does the Current Evidence Provide Us to Prevent or Treat IAI? Bioengineering, 10(3), 356. <https://doi.org/10.3390/bioengineering10030356>*

*Pangaud, C., Ollivier, M., & Argenson, J.-N. (2019). Outcome of single-stage versus two-stage exchange for revision knee arthroplasty for chronic periprosthetic infection. EFORT Open Reviews, 4(8), 495–502. <https://doi.org/10.1302/2058-5241.4.190003>*

#### *Purpose*

In the conversation with the DEC members, you indicated that combating MRSA is also an important goal of your research. Could you include this purpose in the project description and clarify the importance of using MRSA in your project (see also question 1)?

*We have revised and re-written the description to explicitly incorporate combating MRSA as a goal and clarified the rationale for using MRSA. Below is the updated text: **Immediate Goal:** The immediate goal of this project is to develop and validate an innovative non-antibiotic theranostic platform combining aptamer-guided imaging with antimicrobial nanoparticle therapy for biofilm-associated and antibiotic-resistant infections. This platform will use MRSA in two clinically relevant mouse models: a wound infection model (Appendix 1) and a subcutaneous implant infection model (Appendix 2).*

#### **Ultimate Goal:**

We aim to overcome two critical barriers in infection management: biofilm-mediated tolerance (resistance to immune clearance and drug penetration) and antibiotic resistance (e.g., MRSA's resistance to  $\beta$ -lactams). By integrating non-antibiotic antimicrobial nanoparticles (e.g., reactive oxygen species generators) with biofilm-specific aptamer diagnostics, our platform will provide a dual-action solution to eradicate resistant pathogens while minimizing the risk of further resistance development.

**Importance of using MRSA:**

MRSA is a high-priority multidrug-resistant pathogen that combines biofilm resilience with resistance to frontline antibiotics. Targeting MRSA ensures our platform is tested under the most clinically relevant and challenging conditions. Demonstrating efficacy against MRSA validates our approach for broader application against biofilm-associated infections and antimicrobial resistance.

**Strategy**

Could you please add in the figures or in an additional flow diagram the decision point more prominently (go/no go approach)? For example, phase 2 seems to be independent of phase 1, but Phase 3 would require a positive outcome of both phase 1 and phase 2.

We have added into the project a workflow diagram to explicitly highlight the go/no-go decision points and clarify interdependencies between phase 1 and phase 2.

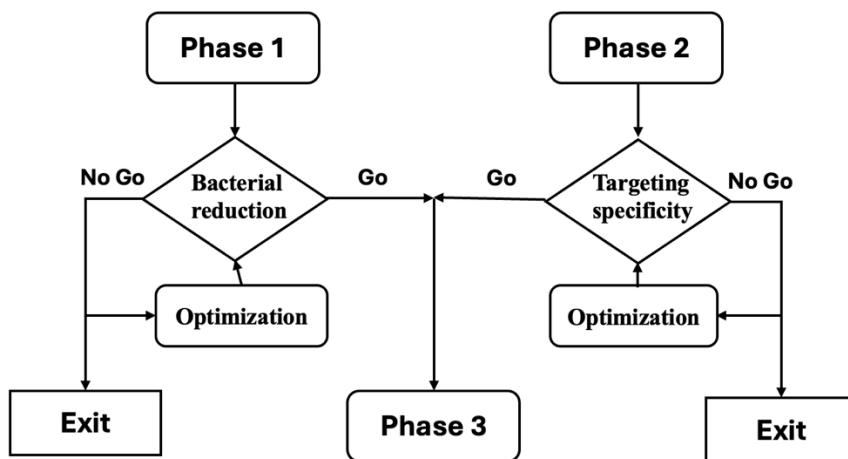


Figure 2. This flowchart outlines the decision points for advancing through the project's three phases in the subcutaneous implant infection model (Appendix 2). Phase 1 requires a significant reduction in bacterial load within biofilm-infected implants. Phase 2 focuses on achieving high targeting specificity (signal ratio  $\geq 3:1$ ). These phases are independent and do not influence each other. Phase 3 integrates the validated therapeutic and diagnostic components for systemic treatment. The project is terminated if the predefined criteria are not met.

Could you please explain a bit more about the wound infection healing model? Is this a standard model and do you have a reference indicating this and indicating the level of discomfort for the animal.

We have revised the project to include references that validating the standardization of the wound infection model and indicating the level of animal discomfort.

The wound infection model follows a well-established and standardized protocol for simulating chronic, biofilm-infected wounds in mice. It is adapted from validated methodologies described

in Hou et al. (2024) and Lei et al. (2024). This model involves creating full-thickness excisional wounds (~8 mm in diameter) on the dorsal surface of mice, followed by bacterial inoculation to establish a biofilm infection.

After 48 hours, infected wounds receive daily topical application of nanoparticle formulations for 7 days. Wound closure is monitored through digital photography and image analysis software, while therapeutic efficacy is assessed by evaluating wound healing progression and quantifying bacterial load (CFU counts from wound tissue homogenates). Tissue response is further analysed through histological assessments, including H&E staining and immunohistochemistry, to evaluate inflammation, granulation tissue formation, and re-epithelialization.

The level of discomfort for animals in this model is classified as Moderate under EU Directive 2010/63/EU. Transient pain during wound creation is mitigated with perioperative analgesia. Mice typically maintain normal activity and body weight ( $\pm 10\%$ ), with no signs of systemic illness (Hou et al., 2024; Lei et al., 2024).

#### References

Hou, J., Wu, Q., Xiong, R., Malakar, P. K., Zhu, Y., Zhao, Y., & Zhang, Z. (2024). A Standardized Mouse Model for Wound Infection with *Pseudomonas aeruginosa*. *International Journal of Molecular Sciences*, 25(21), 11773. <https://doi.org/10.3390/ijms252111773>

Lei, J., Zhang, T., Wang, L., Jiang, H., Wu, J., & Zheng, Y. (2024). Chirality-influenced antibacterial behavior of gold nanoclusters. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 683, 133033. <https://doi.org/10.1016/j.colsurfa.2023.133033>

You will also examine the binding of aptamers to other (non-target) organs. Could you please explain the relevance of this step and indicate what would be the expected outcome?

In addition to analyzing aptamer binding at the two implant sites (infected and sterile), we will assess their distribution in non-target organs such as the liver, spleen, and kidneys. This step is essential for evaluating aptamer specificity, biodistribution, and safety.

We expect initial accumulation in highly vascularized organs, which should decrease over time, while signals at target sites (infected implants) should increase or remain stable, indicating effective delivery and retention. This analysis not only validates targeting specificity but also supports safety assessments. If significant off-target accumulation occurs, we will optimize aptamer design (e.g., PEGylation) to improve precision, ensuring the platform's safety and readiness for clinical translation.

#### Appendices 1 and 2

##### A. Experimental approach and primary outcome parameters

Could you please substantiate why 30 animals are needed for optimization of the model? Will the number of control animals be sufficient for this set-up?

The allocation of up to 30 mice is exclusively for testing the efficacy of nanoparticle formulations, not for optimizing the infection models. These animals will be used only if the initial two nanoparticle formulations fail to meet efficacy criteria. The breakdown is as follows:

- 18 experimental animals (6 mice per formulation, testing up to three additional candidates).
- 12 control animals (4 mice per formulation, including positive and negative controls).

*The choice of 6 mice per group is based on statistical power analysis ( $\alpha = 0.05$ ,  $\beta = 0.2$ ), ensuring robust conclusions.*

*The number of control animals is sufficient through data integration: historical control data (6 mice) will be combined with 2 newly included controls per formulation, achieving  $n = 8$ , which is validated for statistical robustness. Historical control data from previous experiments under identical conditions will be used, provided inter-experiment variability remains low.*

*This sample size calculation was reviewed and discussed with the biostatistician in the presence of the IVD, confirming its validity.*

Would a go/no go decision point be feasible between the optimization experiment(s) and the following part?

*A go/no-go decision point is both feasible and integral to the experimental design. This checkpoint is strategically placed after the formulation efficacy testing phase to assess whether the tested nanoparticle formulations (Appendix 2 – Phase 1) or aptamers (Appendix 2 – Phase 2) meet predefined efficacy criteria.*

*If the initial candidates fail, a limited number of alternatives will be systematically tested to prevent indefinite progression. If no candidates achieve the required efficacy, the project will be terminated (no-go).*

You have indicated that imaging will provide a means for a quick diagnosis procedure in the clinic. Could you please explain this in some more detail, as certainly in orthopaedic surgery the implant is “hidden” in bone tissue, and this is likely to form a barrier for imaging? In contrast, in wound infection imaging is expected to give good read-outs as in experiments with small animals (mice) where the implant is placed subcutaneously.

*Our diagnostic strategy is specifically designed for implant-associated infections, which pose unique challenges for early detection. Unlike superficial wound infections, which are easily accessible and monitored through visual inspection or routine swabs, orthopedic implants are often embedded deep within tissues or bone, creating a physical barrier that conceals early signs of infection.*

*To address this, we use systemically administered aptamer-based probes conjugated with imaging agents. These probes circulate through the bloodstream, bind to biofilm-specific markers, and accumulate at infection sites.*

*In small-animal models, we utilize near-infrared fluorescence (NIR) imaging, which provides high contrast but is limited to tissue penetration of 1–3 cm. For human applications, the NIR label can be replaced with radionuclides, enabling PET/SPECT imaging, which offers deeper tissue penetration and higher sensitivity for detecting infections in orthopaedic implants.*

Could you please clarify whether the wound will remain open or will be dressed with gauze or any other dressing material? If the wound is dressed / occluded, would this make group housing of the animals possible?

*In the wound infection model, wounds will remain open and undressed to simulate natural healing conditions. This approach has been used in previous studies, such as Hou et al. (2024) and Lei et al. (2024), which demonstrated that leaving wounds uncovered provides a more physiologically relevant infection environment.*

*Group housing is not feasible due to the risk of wound interference between animals (e.g.,*

scratching or biting), a factor also reported in Rhea et al. (2020). However, the short experimental duration (9 days total) minimizes the impact of individual housing on animal welfare.

#### References

Hou, J., Wu, Q., Xiong, R., Malakar, P. K., Zhu, Y., Zhao, Y., & Zhang, Z. (2024). A Standardized Mouse Model for Wound Infection with *Pseudomonas aeruginosa*. *International Journal of Molecular Sciences*, 25(21), 11773. <https://doi.org/10.3390/ijms252111773>

Lei, J., Zhang, T., Wang, L., Jiang, H., Wu, J., & Zheng, Y. (2024). Chirality-influenced antibacterial behavior of gold nanoclusters. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 683, 133033. <https://doi.org/10.1016/j.colsurfa.2023.133033>

Rhea, L., & Dunnwald, M. (2020). Murine Excisional Wound Healing Model and Histological Morphometric Wound Analysis. *Journal of visualized experiments : JoVE*, (162), 10.3791/61616. <https://doi.org/10.3791/61616>

#### B. The animals

Could you please discuss in more detail why the experiments are only carried out with male animals?

*We also acknowledge the historical bias towards male subjects in preclinical research and recognise the importance of validating findings in female models. While this is beyond the scope of the current project, due to feasibility\*, **we plan to validate our results in female mice in future studies to ensure broader translational relevance.***

*The decision to use exclusively male mice in this project is based on scientific and methodological considerations that prioritise experimental consistency and statistical robustness. Male mice exhibit more stable physiological characteristics in terms of bone healing and immune responses, as they are not subject to cyclical hormonal fluctuations. This reduces variability, which is particularly important when studying subtle differences in infection progression and treatment efficacy.*

*Female mice undergo oestrous cycles, causing periodic variations in oestrogen levels, which can influence immune responses and wound healing processes. Such fluctuations could introduce additional variability, potentially confounding our results. This approach aligns with common practice in early mechanistic validation studies, allowing us to focus on core experimental variables (such as nanoparticle targeting) without the complexities introduced by sex-related differences.*

*Furthermore, using a single-sex cohort at this stage minimises within-group variation and increases statistical power, enabling us to obtain reliable results with smaller sample sizes, in accordance with the "Reduction" principle of the 3Rs. Additionally, the orthopaedic infection model we employ has well-established parameters and baseline data in male mice, allowing us to directly compare our findings with existing literature, thereby enhancing the reliability and reproducibility of our research.*

*\* time length of the license, funding availability, personnel availability.*

Please describe why this does (not) lead to male biased-science and how you would compensate for that within your project or in future work? We all agree that post-orthopaedic surgery infections and wound infections are not limited to males in human

medicine, and hence this question needs to be addressed in the project proposal.

**We fully acknowledge the historical bias in preclinical research towards male subjects and recognise that post-orthopaedic and wound infections affect both sexes in human medicine.** Addressing this issue is important, and we are committed to ensuring that our findings are translationally relevant.

*Our approach follows a strategic experimental sequence rather than perpetuating bias. We begin with male mice to establish foundational mechanisms before incorporating sex as a biological variable in future studies. The antimicrobial mechanisms we study operate at the cellular and molecular levels, which are largely conserved across sexes. Since our focus is on pathogen-targeting strategies rather than host-response modulation, the core principles of our findings are expected to be broadly applicable.*

**To ensure sex-inclusive research, our plan includes subsequent studies with female animals once core mechanisms are validated (outside the scope of this application).** This stepwise approach balances scientific rigour and reproducibility, ensuring that sex differences can be properly assessed in later phases without introducing unnecessary variability in early mechanistic studies.

*This sequential approach aligns with best practices in translational research, allowing us to first establish proof-of-concept before expanding into more complex, sex-inclusive models.*

#### C. Housing and care

Could you please clearly indicate the maximum number of days the animals will be housed individually for each appendix? This potentially influences the degree of discomfort for the animals, which the DEC needs to take into consideration in the advice to the CCD.

*The maximum duration of individual housing is now clearly specified in the appendices:*

- Appendix 1 (wound infection healing model): 9 days (full experimental duration)
- Appendix 2 (subcutaneous biofilm implant model): 2 days (post-surgery recovery)

#### Non-Technical Summary

Could you please check on the numbers of animals (sepsis 15 animals or 14 animals?) so the NTS matches the appendices?

*We have reviewed and corrected the animal numbers to ensure full consistency between the NTS and appendices. The correct number is 15.*

The NTS is for the general public. Could you please refrain from using technical jargon and replace it by plain language that is generally understood?

*We have revised the NTS to ensure it is accessible to the general public by replacing technical jargon with clear, plain language. The updated version explains the project's goals and methods in a way that is easy to understand while maintaining accuracy.*

- De antwoorden hebben geleid tot aanpassing van de aanvraag.

10. Eventuele adviezen door experts (niet lid van de DEC): n.v.t.

## **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. De aanvraag is toetsbaar en heeft voldoende samenhang. Met deze kortlopende pilotstudie wil men onderzoek doen in muizen naar wondgenezing en naar biofilms op orthopedische implantaten en daarop de behandeling, targeting en gecombineerde behandeling (targeting en imaging) nagaan. Tevens is het doel om specifieke MRSA bacteriële infecties te bestrijden. Bacteriële biofilms kunnen hevige ontstekingsreacties veroorzaken die lastig te behandelen zijn met antibiotica. Het falen van de behandeling kan leiden tot terugkerende of chronische infectie of kan (herhaalde) chirurgische ingrepen in de orthopedie noodzakelijk maken. Zowel MRSA als methicilline/penicilline sensitieve Stafylokokken aureus stammen kunnen biofilms vormen, waarbij MRSA in de kliniek als een bijzonder hoog risico wordt gezien. In dit project worden twee muismodellen ingezet: het genezingsmodel voor wondinfectie (bijlage 1) en het subcutaan biofilm-implantaatmodel (bijlage 2).
2. Voor zover de DEC bekend, is er geen mogelijk tegenstrijdige wetgeving die het uitvoeren van de dierexperimenten in de weg zou kunnen staan.
3. De in de aanvraag aangekruiste doelcategorie(ën), te weten fundamenteel en toegepast onderzoek, sluiten aan bij de hoofddoelstelling(en).

#### *Belangen en waarden*

4. Het directe doel van het project is het targeten van bacteriële biofilms met metaal- en lipide-nanopartikels en daarnaast het bestrijden van MRSA, en in de toekomst mogelijkerwijze ook andere biofilm-infecties. Het uiteindelijke doel van het project is meer inzicht in antimicrobiële strategieën voor biofilm-geassocieerde infecties. De DEC is van mening dat er een duidelijke relatie is tussen het directe en het uiteindelijke doel, en dat het doel gerechtvaardigd is in de context van preventie van (chronische) infecties en specifiek de behoeften vanuit de (orthopedische) geneeskunde.
5. De belangrijkste belanghebbenden in dit onderzoeksproject zijn: proefdieren, onderzoekers en patiënten / geneeskundige zorg. De proefdieren, de muizen, hebben een groot belang gevrijwaard te blijven van de experimenten en het daaruit voortkomende (ernstige) ongerief en de vroegtijdige dood. Voor de individuele onderzoeker kan het van belang zijn om aansprekende resultaten te boeken na hun onderzoek voor een kwetsbare patiëntengroep, maar in de uiteindelijke afweging kent de DEC daar weinig gewicht aan toe. De patiënten met een biofilm-infectie en de geneeskundige zorg hebben een groot belang bij meer kennis ten aanzien van biofilm-infecties bij orthopedische implantanten en MRSA omdat die regelmatig tot chronische, therapie-resistente infecties en zelfs tot sepsis kunnen leiden.

6. De aanvrager geeft niet aan nadelige effecten op het milieu te verwachten. De DEC ziet geen aanleiding om aan te nemen dat zich toch nadelige effecten zullen voordoen. De behandeling vindt immers in Nederland plaats en betreft geen radioactief materiaal meer. Ten aanzien van het gebruik van MRSA bacteriën heeft de onderzoeker aangegeven de geldende richtlijnen en protocollen te volgen.

#### *Proefopzet en haalbaarheid*

7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd en dragen eraan bij dat de doelstellingen behaald kunnen worden, dat aan de 3V-beginselen voldaan kan worden en dat voorkomen kan worden dat mens, dier en milieu negatieve effecten ondervinden als gevolg van de dierproeven.
8. Het project is goed opgezet en ingedeeld in drie fases met go-no go beslismomenten, waarbij volgens de DEC in figuur 2 (bijlage 2) nog duidelijker weergegeven had kunnen worden dat fase 3 doorgaat bij een 'go' van fase 1 én fase 2 (in plaats van een 'go' van fase 1 óf fase 2). De voorgestelde experimentele opzet en uitkomstparameters sluiten logisch en helder aan bij de aangegeven doelstellingen. De DEC heeft gediscussieerd over de keuze voor de bacterieresistente MRSA-stam in plaats van de niet-resistente Staphylokokken aureus-stam. Voor onderzoek naar alleen biofilms maakt de soort bacterie geen verschil terwijl het gebruik van klinisch MRSA-isolaat voldoet aan de directe klinische vraag, omdat MRSA vaak zowel in chronische wondinfecties alsook in de orthopedie vaker aangetroffen wordt.. De onderzoeker heeft deze keuze voldoende toegelicht. Uiteindelijk wil men beeldvorming toepasbaar maken voor snelle klinische diagnostiek, zodat gevolgd kan worden wat er met een infectie gebeurt. Dit is volgens de DEC klinisch relevant.
- De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project.

#### *Welzijn dieren*

9. 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)
  - Niet gefokt voor dierproeven (11, bijlage I EU richtlijn)
  - Zwerfdieren (10h)
  - Hergebruik (1e lid 2)
  - Locatie: buiten instelling vergunninghouder (10g)
  - Geen toepassing verdoving/pijnbestrijding (13)
  - Dodingsmethode niet volgens bijlage IV EU richtlijn (13c lid 3)

10. De dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de EU-richtlijn. Een kanttekening hierbij betreft de huisvesting van de dieren, allen mannelijk (zie C18). De muizen in bijlage 1 worden namelijk voor de duur van het experiment (negen dagen) gehuisvest in individueel geventileerde kooien (IVC) om te voorkomen dat de dieren het wondgebied van een ander dier aanraken of erin bijten. In het gekozen wondinfectiemodel, blijven de wonden open en worden niet afgeplakt. Gezien de mogelijke nadelige effecten van groeps-huisvestingen op de onderzoeksvraagstelling en het onderzoeksresultaat ziet de DEC de noodzakelijkheid van individuele huisvesting in. Het inzetten van vrouwelijke buddies is volgens de DEC hierdoor ook niet mogelijk. De muizen in bijlage 2 worden eveneens individueel gehuisvest (maximaal drie dagen) in verband met de postoperatieve herstelperiode en om kruisbesmetting tegen te gaan. Daarna worden deze dieren wel weer teruggeplaatst in de groep. Als de mannelijke dieren onderling te agressief reageren kunnen de dieren maximaal negen dagen individueel gehuisvest worden. In beide experimenten wordt het welzijn van de dieren zorgvuldig gemonitord en de kooi verrijkt.
11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Er is sprake van cumulatief matig ongerief voor alle dieren door met name de chirurgische ingreep, de wond, de bacteriële infectie, de ontstekingsreacties, individuele huisvesting en postoperatieve stress. In dit type onderzoek kan niet worden uitgesloten dat incidenteel sepsis optreedt, waardoor ernstig ongerief wordt veroorzaakt (de verwachting is dat niet meer dan 15 muizen (10%) sepsis zullen ontwikkelen). De dieren worden zorgvuldig op ongerief gemonitord aan de hand van een uitgebreide scorelijst. De chirurgische ingrepen vinden onder algehele anesthesie plaats. Tevens wordt pre- en postoperatieve pijnstilling toegediend. De dieren krijgen waar nodig pijnbestrijding, maar dit interfereert niet met het project.  
De wondgenezing wordt met een wetenschappelijk geaccepteerd, standaard wondinfectiemodel onderzocht. Er wordt met een soort perforator een gat in de huid van de muis gemaakt van 8mm diameter. Doordat de wond tussen de schouders wordt aangebracht, kan zelflikken voorkomen worden. Dit is volgens de DEC de beste optie om ongerief bij de dieren voor dit experiment te beperken.
12. De integriteit van de dieren wordt met name fysiek en mentaal aangetast door het operatief aanbrengen van een geïnfecteerde wond (en de kleine kans op sepsis met ernstig ongerief als gevolg) en door de noodzakelijke individuele huisvesting van meerdere dagen in verband met het wondinfectie genezingsmodel.
13. De humane eindpunten zijn voor iedere bijlage dierproeven goed gedefinieerd en het percentage dieren (10%) dat naar verwachting een humaan eindpunt bereikt is goed ingeschat. De DEC waardeert de welzijnsscorelijst, waar ook sepsisindicatoren in opgenomen zijn. De DEC realiseert zich dat de kans op sepsis beperkt is, maar niet uitgesloten kan worden, en ondanks de zorgvuldige aseptische procedures en humane eindpunten is daardoor ernstig ongerief niet geheel te voorkomen.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, zoals volledige inzet van computationele modellen, om de interactie tussen immuunrespons, biofilm infectie en wondgenezing te onderzoeken. De onderzoekers hebben wel de testmaterialen zover mogelijk *in vitro* in biofilmmodellen getest.
15. Het aantal te gebruiken dieren is realistisch ingeschat, al had de DEC graag een consequente vermelding van de aantallen dieren voor de controlegroep gezien. In bijlage 1 geeft men aan vier extra dieren voor de controlegroep benodigd te hebben per proef, terwijl in de beantwoording van de vraag twee controledieren, aangevuld met historische controlegegevens, vermeld worden.

Er is een heldere strategie om ervoor te zorgen dat tijdens het project met het kleinst mogelijke aantal dieren wordt gewerkt waarmee nog een betrouwbaar resultaat kan worden verkregen. Zo worden de geselecteerde kandidaten voorafgaand aan de diermodellen via uitgebreide *in vitro* screening geëvalueerd. Verder wordt longitudinale data verzameld door middel van non-invasieve monitoring en non-invasieve beeldvormingstechnieken. Ook krijgen muizen zowel een geïnfecteerd biofilm-implantaat als een steriel controle-implantaat waardoor geen extra (controle-)dieren nodig zijn. Tot slot hebben betrokkenen een vooropleiding gevolgd in de benodigde chirurgische en experimentele procedures, en eventuele aanvullende trainingen zullen *ex vivo* worden uitgevoerd.

16. Het project is in overeenstemming met de vereiste van verfijning van dierproeven, zoals kooiverrijking, voer en water *ad libitum* en resorbeerbare hechtingen waardoor minder handelingen aan de muizen nodig zijn, en het project is zodanig opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd.
17. Er is geen sprake van wettelijk vereist onderzoek.

#### *Dieren in voorraad gedood en bestemming dieren na afloop proef*

18. Er zullen in zowel bijlage I als in bijlage II alleen mannelijke dieren gebruikt worden. Door alleen mannelijke muizen te gebruiken wordt de kans op hormonale variaties, veroorzaakt door cycli bij vrouwelijke muizen, verminderd en wordt de immuunrespons en wondgenezing hierdoor niet beïnvloed. De DEC heeft gediscussieerd over het inzetten van alleen mannelijke dieren. Bij het gebruik van zowel vrouwelijke als mannelijke dieren zou het aantal dieren hoger worden maar wordt *male bias science* voorkomen. Tevens is er tussen de beide seksen een licht verschil in wondgenezing en dit vindt de DEC ook een relevant argument. Het gekozen 3V's argument werkt volgens de DEC wél *male bias science* in de hand. De DEC is er desondanks van overtuigd dat de aanvrager in voldoende mate wetenschappelijk heeft onderbouwd dat het, om de

doelstellingen te bereiken, noodzakelijk is om de proeven in deze pilotstudie met alleen mannelijke dieren uit te voeren. De DEC vindt het echter, evenals de onderzoeker, van groot belang dat in de noodzakelijke vervolgstudies ook vrouwelijke dieren opgenomen worden zodat uiteindelijk voor deze gender-bias wordt gecorrigeerd. Het is echter niet zeker dat er een vervolgstudie komt en dat er dan inderdaad ook effecten in vrouwelijke dieren zullen worden bestudeerd.

19. De dieren worden in het kader van het project gedood. Postmortem worden wondweefsel, organen, bloed en biofilm-geïnfekteerde en steriele implantaten verzameld voor verdere histologische en microbiologische analyse. De dieren worden op een passende wijze, in overeenstemming met bijlage IV van de EU richtlijn, gedood.
20. De vraag over hergebruik is niet van toepassing omdat de dieren gedood worden in het kader van het experiment.

*NTS*

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

#### **D. Ethische afweging**

1. De morele vraag die de DEC dient te beantwoorden is of het belang van dit onderzoek, namelijk onderzoek doen in muizen naar wondgenezing en naar bacteriële biofilm-infecties op orthopedische implantaten en de bestudering van gerichte behandelmethoden, en gecombineerde behandeling (targeting en imaging), de onvermijdelijke aantasting van het welzijn en de integriteit van de gebruikte proefdieren rechtvaardigt.
2. Er vindt een aanzienlijke aantasting van welzijn en integriteit van de maximaal 149 volwassen proefdieren plaats, met cumulatief matig en mogelijk ernstig ongerief voor resp. 134 en 15 muizen. Indien de hierboven genoemde doelstellingen behaald worden, dan zal deze pilotstudie er toe bijdragen dat meer kennis verkregen wordt over veelvoorkomende biofilm-gemedieerde antibioticatolerantie en -resistentie en dat er in de toekomst mogelijk minder risicovolle chronische infecties bij patiënten en herhaalde ziekenhuisopnames en operaties noodzakelijk zullen zijn. Het is aannemelijk dat de fundamentele en toegepaste doelstelling behaald zal worden. Daarvoor is de inzet van proefdieren noodzakelijk, maar de onderzoekers doen al het mogelijke om het ongerief voor de dieren en het aantal dieren tot een minimum te beperken.
3. Op grond van het bovenstaande is de DEC van oordeel dat het onderzoek naar de toepassing van behandelingen van infecties veroorzaakt door biofilms op (orthopedische) implantaten, waarbij tevens wordt gekeken naar mogelijkheden MRSA geassocieerde infecties te behandelen,

een substantieel belang vertegenwoordigt en van groot wetenschappelijk belang is en een belangrijke zorgvraag betreft. Dit belang voor humane patiënten weegt op tegen de aanzienlijke aantasting van het welzijn en de integriteit van de proefdieren. De relatie tussen het directe en het uiteindelijk doel is voldoende helder. Het is aannemelijk dat de directe doelstelling behaald zal worden. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat er geen sprake zal zijn van onbedoelde negatieve effecten voor mens, dier en milieu als gevolg van de dierproeven. Het gebruik van de proefdieren zoals beschreven in de aanvraag is daarmee gerechtvaardigd. Aangezien een aantal dieren (maximaal 10% ofwel 15 muizen), ondanks de toepassing van humane eindpunten, mogelijk ernstig ongerief kunnen ervaren, zal een reflectie op het project en het ongerief door middel van een Beoordeling Achteraf noodzakelijk zijn.

### **E. Advies**

#### 1. Advies aan de CCD

De DEC adviseert de vergunning te verlenen.

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

Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.

Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist

Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...

De DEC adviseert de vergunning niet te verlenen vanwege:

De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:...

De volgende doorslaggevende ethische bezwaren:...

De volgende tekortkomingen in de aanvraag:...

#### 2. Het uitgebrachte advies is gebaseerd op consensus.

#### 3. Er zijn geen knelpunten/dilemma's naar voren gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies.



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**Centrale Commissie  
Dierproeven**

Postbus 93118  
2509 AC Den Haag  
centralecommissiedierproeven.nl  
0800 789 0789  
info@zbo-ccd.nl

**Onze referentie**

Aanvraagnummer  
AVD11500202518775

**Bijlagen**

2

Datum 28 februari 2025

Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [REDACTED]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 28 februari 2025. Het gaat om uw project "Antimicrobial strategies for drug-resistant and biofilm-associated infections". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD11500202518775. 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](http://www.centralecommissiedierproeven.nl), stuur een e-mail naar [info@zbo-ccd.nl](mailto:info@zbo-ccd.nl) of neem telefonisch contact met ons op: 0800 789 0789.

**Datum:**

28 februari 2025

**Aanvraagnummer:**

AVD11500202518775

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: 11500  
Naam instelling of organisatie: UMC Utrecht  
Naam portefeuillehouder of diens gemachtigde: [REDACTED]  
Postbus: 12007  
Postcode en plaats: 3508 GA UTRECHT

Gegevens verantwoordelijke onderzoeker

Naam: [REDACTED]  
Functie: Assistant Professor  
Afdeling: Orthopedie  
Telefoonnummer: [REDACTED]  
E-mailadres: [REDACTED]

### Over uw aanvraag

Wat voor aanvraag doet u?  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: 10 maart 2025  
Geplande einddatum: 10 maart 2028  
Titel project: Antimicrobial strategies for drug-resistant and biofilm-associated infections  
Titel niet-technische samenvatting: Ontwikkeling van nieuwe methoden om hardnekkige bacteriële infecties te bestrijden  
Naam DEC: DEC Utrecht  
Postadres DEC: Postbus 85500, 3508 GA UTRECHT  
E-mailadres DEC: dec-utrecht@umcutrecht.nl

### Betaalgegevens

De leges bedragen: € 2.034,-  
De leges voldoet u: na ontvangst van de factuur

### Checklist bijlagen

Verplichte bijlagen:  Projectvoorstel  
 Beschrijving Dierproeven  
 Niet-technische samenvatting

**Ondertekening**

Naam:

[REDACTED]

Functie:

[REDACTED]

Plaats:

Utrecht

Datum:

28 februari 2025



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UU-ASC  
Postbus 80.011  
3508 TA UTRECHT  


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**Onze referentie**  
Aanvraagnummer  
AVD11500202518775  
**Bijlagen**  
2

Datum 28 februari 2025  
Betreft Factuur aanvraag projectvergunning Dierproeven

**Factuur**  
Factuurdatum: 28 februari 2025  
Vervaldatum: 30 maart 2025  
Factuurnummer: 2518775  
Ordernummer: CB.841910.3.01.011

Omschrijving	Bedrag
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD11500202518775	€ 2.034,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven te 's Gravenhage.



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UMC Utrecht

[Redacted]

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**Centrale Commissie  
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0800 789 0789  
info@zbo-ccd.nl

**Onze referentie**

Aanvraagnummer  
AVD11500202518775

**Bijlagen**

3

Datum 19 mei 2025  
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [Redacted]

Op 28 februari 2025 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Antimicrobial strategies for drug-resistant and biofilm-associated infections" met aanvraagnummer AVD11500202518775. Wij hebben uw aanvraag beoordeeld.

**Beslissing**

Wij keuren uw aanvraag goed. Uit artikel 10a, eerste lid van de Wet op de dierproeven (hierna: de wet) volgt daarom dat het is toegestaan om uw project uit te voeren binnen de gestelde vergunningsperiode. Deze vergunning wordt afgegeven voor de periode van 12 mei 2025 tot en met 11 mei 2028.

Aan de vergunning hebben wij de volgende voorwaarde verbonden op grond van artikel 10a1, tweede lid van de wet.

*Beoordeling achteraf*

In dit project worden dierproeven toegepast die vallen in de categorie ernstig volgens artikel 10b van de wet. Daarom bent u verplicht om na afloop van de vergunning in een Beoordeling achteraf over uw project te rapporteren. Deze beoordeling zal uiterlijk mei 2029 plaatsvinden. Er zal dan conform artikel 10a2, derde lid van de wet, beoordeeld worden of de doelstellingen van het project werden bereikt.

De onderbouwing van deze beslissing vindt u onder 'Overwegingen'.

## **Procedure**

**Datum:**

19 mei 2025

**Aanvraagnummer:**

AVD11500202518775

### *Advies dierexperimentencommissie*

Wij hebben advies gevraagd bij de dierexperimentencommissie DEC Utrecht (hierna: DEC). Dit advies is ontvangen op 8 april 2025. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, derde lid van de wet.

### *Nadere vragen aanvrager*

Op 28 april 2025 hebben wij u om aanvullingen gevraagd. U heeft tijdig antwoord gegeven. Het verzoek om aanvullingen had betrekking op de Niet Technische Samenvatting. Uw reactie is betrokken bij de behandeling van uw aanvraag.

## **Overwegingen**

Wij kunnen ons vinden in de inhoud van het advies van de DEC, inclusief de daaraan ten grondslag liggende motivering.

### *Beoordeling achteraf*

Na afloop van het project moet er een beoordeling plaatsvinden zoals bedoeld in artikel 10a1, eerste lid, onder d en artikel 10a1, derde lid van de wet. De reden van deze beoordeling achteraf is dat in dit project dieren ernstig ongerief ondergaan. Deze beoordeling zal uiterlijk mei 2029 plaatsvinden. Meer informatie over de eisen die gesteld worden bij de beoordeling achteraf vindt u in de bijlage 'Weergave wet- en regelgeving'.

## **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 93118, 2509 AC 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 nummers 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. Nadat u een bezwaarschrift heeft ingediend kunt u een voorlopige voorziening vragen bij de voorzieningenrechter van de rechtbank in de vestigingsplaats van de vergunninghouder. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisende situatie.

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 vergunninghouder valt.

**Datum:**

19 mei 2025

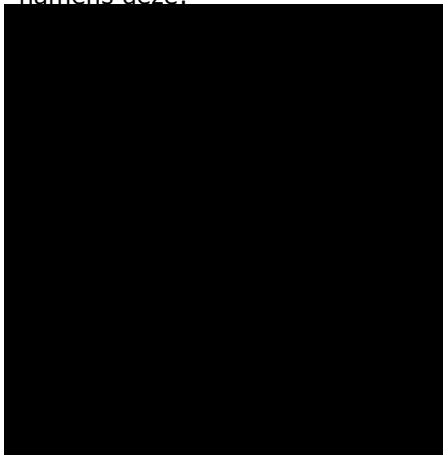
**Aanvraagnummer:**

AVD11500202518775

**Meer informatie**

Heeft u vragen, kijk dan op [www.centralecommissiedierproeven.nl](http://www.centralecommissiedierproeven.nl), stuur een e-mail naar [info@zbo-ccd.nl](mailto:info@zbo-ccd.nl) of neem telefonisch contact met ons op: 0800 789 0789.

Centrale Commissie Dierproeven  
namens deze:



Bijlagen:

- Projectvergunning
- DEC-advies
- Weergave wet- en regelgeving



# Projectvergunning

## gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam: UMC Utrecht  
Adres: Postbus 12007  
Postcode en plaats: 3508 GA UTRECHT  
Deelnemersnummer: 11500

deze projectvergunning voor het tijdvak 12 mei 2025 tot en met 11 mei 2028, voor het project "Antimicrobial strategies for drug-resistant and biofilm-associated infections" met aanvraagnummer AVD11500202518775, na advies van dierexperimentencommissie DEC Utrecht. De functie van de verantwoordelijk onderzoeker is Assistant Professor. Het besluit is gebaseerd op de volgende (aangepaste) stukken:

- 1 een aanvraagformulier projectvergunning dierproeven, zoals ontvangen op 28 februari 2025
- 2 de bij het aanvraagformulier behorende bijlagen:
  - a Projectvoorstel, zoals ontvangen op 8 april 2025;
  - b Bijlagen dierproeven
    - 3.4.3.1. Wound infection healing model, zoals ontvangen op 8 april 2025;
    - 3.4.3.2. Subcutaneous biofilm implant model, zoals ontvangen op 8 april 2025;
  - c Niet-technische Samenvatting van het project, zoals ontvangen op 8 mei 2025;
  - d Advies van dierexperimentencommissie, zoals ontvangen op 8 april 2025
  - e De aanvullingen op uw aanvraag, zoals ontvangen op 8 mei 2025.

Naam proef	Diersoort/ Stam	Aantal dieren	Ongerief
<b>3.4.3.1. Wound infection healing model</b>			
	Muizen (Mus musculus)	58	10,3% Ernstig 89,7% Matig
<b>3.4.3.2. Subcutaneous biofilm implant model</b>			
	Muizen (Mus musculus)	91	8,9% Ernstig 91,1% Matig

### Voorwaarden

#### *Beoordeling achteraf*

In dit project worden dierproeven toegepast die vallen in de categorie ernstig volgens artikel 10b van de wet. Daarom bent u verplicht om na afloop van de vergunning in een Beoordeling achteraf over uw project te rapporteren. Deze beoordeling zal uiterlijk mei 2029 plaatsvinden. Er zal dan conform artikel 10a2, derde lid van de wet, beoordeeld worden of de doelstellingen van het project werden bereikt.

**Aanvraagnummer:** AVD11500202518775

**Geldende voorschriften**

Wij wijzen u op onderstaande geldende voorschriften, die volgen uit artikel 1d, vierde lid, artikel 10, eerste lid en/of artikel 10a3 van de wet.

- Go/ no go momenten worden voor aanvang van elk experiment afgestemd met de IvD.
- Het is verboden 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.
- Het is verboden dierproeven te verrichten voor een doel waarvan het belang niet opweegt tegen het ongerief dat aan het proefdier wordt berokkend.
- Overige wettelijke bepalingen blijven van kracht.



**Aanvraagnummer:**

AVD11500202518775

## 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, derde lid van de wet. Uit artikel 10b, eerste lid van de wet 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, eerste lid van de wet de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven. Artikel 10b, tweede en derde lid van de wet schrijven 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 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 van de wet 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

**Aanvraagnummer:**  
AVD11500202518775

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 van de wet 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 van de wet 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 van de wet 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.

### **Beoordeling achteraf**

Volgens artikel 10a1, eerste lid onder d en derde lid van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld.