SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Animal models of retinal pigment epithelium transplantation: a systematic review	
2.	Authors (names, affiliations, contributions)	MSc. Céline Koster (AMC) Prof. Dr. Arthur A. Bergen (AMC) Dr. A.L.M.A. ten Asbroek (AMC) Dr. K.E. Wever (Radboudumc)	
3.	Other contributors (names, affiliations, contributions)	René Spijker (AMC)	
4.	Contact person + e-mail address	Céline Koster; c.koster@amc.uva.nl	
5.	Funding sources/sponsors	Uitzicht, ZonMW	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration	www.SYRCLE.nl, 27-07-2017	
8.	Registration number (if applicable)	ZonMW dossier:40-42600-98-412	
9.	Stage of review at time of registration	Preliminary searches performed	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	In retinal degenerative diseases, such as age related macular degeneration, the vision will be lost over time, as the retina will slowly degenerate. One cell layer of the retina is particularly important; the retinal pigment epithelium (RPE). This layer is essential for the normal function and health of the photoreceptor cells (PR). The PR are responsible for catching the light which shines into the eye. No effective therapy is currently available. For years, people have tried to exchange the diseased RPE in the eye of animal models with 'donor RPE'. However, a lot of problems have been faced. The type of animal model which should be used is not entirely clear and results are often inconclusive. Furthermore, the source of donor material which should be used is also not clear. Several options are possible; human donor RPE, human fetal RPE, cell lines, stem cell-RPE, neuroprogenitor cells etc. And then there is still the choice of the type of transplantation. This is either a suspension of cells or a sheet of cells either with or without a carrier membrane (scaffold). Up to now, it is not clear what is the best way to test and improve the intervention. We are hoping that, by means of this systematic review, we can gain more insight in all procedures concerning subretinal transplantations of RPE cells.	
	Research question		

11.	Specify the disease/health problem of interest	Age related Macular Degeneration (AMD) and other retinal degenerative diseases.	
12.	Specify the population/species studied	All animal models available, regardless of species, sex, age, genetic status or comorbidity.	
13.	Specify the intervention/exposure	Transplantation of cells to replace existing RPE. This will be either an injection of a cell suspension or a transplantation of a cell sheet possibly on a scaffold.	
14.	Specify the control population	No transplantation / PBS or vehicle treatment/ empty scaffold or healthy animals.	
15.	Specify the outcome measures	Morphological and functional outcome measures.	
16.	State your research question (based on items 11-15)	In animal models of retinal degenerative diseases, what is the effect of cell transplantation strategies to replace the RPE, compared to no treatment or placebo treatment, on morphology and function of the eye? Sub-questions: - what is the most suitable animal model? - what is the most suitable intervention to use for replacing existing retinal pigment epithelium?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	PubMed EMBASE Web of Science	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: [insert file name]	
19.	Identify other sources for study identification	X Reference lists of included studies ☐Books X Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources	Potentially eligible articles will be identified based on title, after which they will undergo the regular screening process as described below.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1) Screening on title and abstract 2) Screening for final inclusion based on full text assessment	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	A) Two reviewers per phase B) Discussion between the reviewers. A third reviewer will serve as arbiter if consensus cannot be reached.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: Controlled studies with a separate control group receiving no treatment or placebo treatment. Exclusion criteria: No suitable control group, cross-over	

		designs.	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: All animal models of retinal degenerative disease, regardless of species, sex, age, genetic status or comorbidity. Exclusion criteria: Studies in humans, in vitro or in silico, or no retinal degenerative disease model used.	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Transplantation of cells to replace existing RPE. This will be either an injection of a cell suspension or a transplantation of a cell sheet possibly on a scaffold. Exclusion criteria: Interventions not aiming to replace the RPE with cells	
26.	Outcome measures	Inclusion criteria: Outcomes related to morphology or function of the eye Exclusion criteria: All other outcome measures	
27.	Language restrictions	Inclusion criteria: All Exclusion criteria: None	
28.	Publication date restrictions	Inclusion criteria: All publication dates Exclusion criteria: None	
29.	Other	Inclusion criteria: - Publication type: original full paper presenting unique data. Exclusion criteria: - Reviews, abstracts, editorials, letters and data published in duplicate.	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: Title and abstract 1. Not and original full research article. 2. Not a study conducted in animals. 3. Not a study about retinal degenerative diseases. 4. No inclusion of a therapeutic intervention using cells to replace the RPE. Selection phase: Full text screening Same as above + 5. No outcomes related to morphology or function of the eye reported 6. No suitable control group. 7. Full text not retrievable	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year, journal	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of animals Which experimental groups	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	• Species • Strain • Sex • Age • Model: o Disease modelled (e.g. AMD, Stargardt disease, Retinitis Pigmentosa, etc.)	

		○ genetic *versus* induced ▪ if genetic: genotype ▪ if induced: method of induction (e.g. chemical, laser, etc.)	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	• Donor species of transplanted cells • Cell type of transplanted cells • Number of transplanted cells • Route of administration • Medium used for delivery (e.g. suspension or sheet) ○ If sheet: type of scaffold (or no scaffold) • Volume of transplant medium • Timing of administration • Frequency of administration	
35.	Outcome measures	List all reported outcomes related to morphology or function of the eye. Data extraction and synthesis only for the outcomes defined below.	
36.	Other (*e.g.* drop-outs)	Adverse events, auto fluorescence (yes/no), blood leakage in the retina (angiography) (yes/no).	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	A. Two B. Discussion between the reviewers. A third reviewer will serve as arbiter if consensus cannot be reached.	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	☐By use of SYRCLE's Risk of Bias tool[4] **X** By use of SYRCLE's Risk of Bias tool, adapted as follows: addition of an assessment of reporting of: any randomisation, any blinding, a sample size calculation, a conflict of interest statement ☐By use of CAMARADES' study quality checklist, e.g [22] ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Primary outcome: ERG measurements (functional outcome) • a-wave amplitudes (continuous; Volts) • b-wave amplitudes (continuous; Volts) • c-wave amplitudes (continuous; Volts) Secondary outcomes: OCT (morphological outcome): • Thickness of the retina and the specific cell layers (continuous; µm). Behavioural experiments (functional outcome): • Improvement of vision-based behaviour Transplant survival (morphological outcome) • Presence of transplant at follow-up yes/no	

		(dichotomous; incidence) (SLO imaging or immunohistochemistry) • Number of cells present at follow-up (continuous; total number of cells, or cells per mm^2) (Immunohistochemistry)	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1. Direct extraction of data from tables of text. 2. Extraction from graphs using a digital screen ruler (e.g. ImageJ). 3. Contacting the authors. A maximum of two attempts (emails) will be made. After the second attempt, we will attempt to reach authors by phone. If no response, we will wait another two weeks for an answer.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	a) One, a second reviewer will randomly check 25% of the extracted data for errors. b) Discussion between the reviewers. A third reviewer will serve as arbiter if consensus cannot be reached.	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	For all outcomes listed under 39. we plan to perform meta-analysis if sufficient data are available (see 43). If this is not the case, a descriptive synthesis will be performed.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Meta-analysis will be performed if there are at least 4 studies reporting on a specific outcome measure. Subgroup analyses will be performed when there are comparisons from at least 4 studies included in at least two of the subgroups.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Primary outcome: ERG measurements • a-wave, b-wave and c-wave amplitudes (continuous; Volts; mean difference (MD) if one species, standardized MD (SMD) if multiple species), normalized MD (NMD) wherever possible. Transplant survival (morphological outcome) • Presence of transplant at follow-up yes/no (SLO imaging or immunohistochemistry) (dichotomous; incidence; Risk Ratio) • Number of cells present at follow-up (continuous; total number of cells, or cells per mm^2; SMD) (Immunohistochemistry) OCT: • Thickness of the retina and the specific cell layers (continuous; µm; MD). Behavioural experiments: • Improvement of vision-based behaviour (continuous; any UoM reported; SMD)	

45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model for all outcome measures	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2 an residual R^2 for any subgroup analyses performed	

47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Potential sources of heterogeneity:
		• Species (stratified meta-regression)
		• Sex (stratified meta-regression)
		• Therapeutic intervention type (suspension/sheet; (stratified meta-regression)
		• Source/Cell type
		• Type of animal model (stratified meta-regression)
		  ○ Disease modelled (e.g. AMD, Stargardt disease, Retinitis Pigmentosa, etc.)
		  ○ genetic *versus* induced
		  ▪ if genetic: genotype
		  ▪ if induced: method of induction (e.g. chemical, laser, etc.)
		• Age (stratified meta-regression)	

48.	Any sensitivity analyses you propose to perform	For meta-analysis of dichotomous outcomes: odds ratio instead of risk ratio For meta analyses about the presence of the transplant at follow-up: pooling SLO results with immunohistochemistry results versus not-pooling. Other sensitivity analyses may be performed depending on decisions we have to make during the review process regarding the (data from the) included studies	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	For primary studies, whenever more than one treatment group is compared to the same control group, we will extract data for both comparisons and correct the number of control animals by dividing the number of animals in the control group by the number of comparisons. Where applicable (testing the same comparisons in multiple subgroup analyses), we will correct the p-value for testing differences between subgroups using the method of Holm-Bonferroni.	
50.	The method for assessment of publication bias	We will produce funnel plots and perform visual analysis of these plots for outcome measures containing 20+ studies. For SMDs, we will use an n-based precision estimate to avoid distortion of the funnel plots. In addition, we aim to perform Egger's test for small study effects for outcome measures containing 20+ studies.	
Final approval by (names, affiliations): Céline Koster (AMC) and Kim Wever (Radboudumc)		Date: August 10th 2017	