SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

FORMAT BY SYRCLE (WWW.SYRCLE.NL)
VERSION 2.0 (DECEMBER 2014)

Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Extracorporeal perfusion of free tissue flaps and limbs – a systematic review	
2.	Authors (names, affiliations, contributions)	1. **Drs. A.S. Kruit** - Department of Plastic and Reconstructive Surgery – Article design, search strategy, screening, data extraction and -analysis, quality assessment, writing of the manuscript. 2. **H. Winters** – Department of Plastic and Reconstructive Surgery – screening, data extraction, quality assessment. 3. **J. van Luijk**, Department of SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE), Radboudumc, Nijmegen – Research protocol, meta analysis. 4. **Prof. dr. D.J.O. Ulrich** – Article design, supervision, cirtical revision of manuscript.	
3.	Other contributors (names, affiliations, contributions)	• **A. Tillema**, Medical library Radboud University, Nijmegen, Design search strategy	
4.	Contact person + e-mail address	A.S. Kruit, annesophie.kruit@radboudumc.nl	
5.	Funding sources/sponsors	None	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration	08 July 2016, Nijmegen	
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	Search and title and abstract screening completed, ongoing full-text screening.	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Extracorporeal perfusion with a modified heart lung machine has shown its efficacy in preventing tissue ischemia in solid organs, for example in kidney transplantation.[1] Following these successful results, the use of extracorporeal perfusion in free tissue flaps and extremity (auto)transplants is a relatively new topic of investigation. Whereas skin can be transplanted successfully after long periods of time in cold storage, fat and especially muscle tissue become ischemic and unusable within hours.[2] The ultimate goal is to lengthen the critical ischemic period with several extra hours to days, gaining operating time or time for transport and/or stabilisation of a patient in case of trauma with limb	

		extracorporeal perfusion.[3-10] In these studies, multiple perfusion fluids (blood products or preservation fluids) and experimental designs have been tested up to a period of 24 hours of extracorporeal perfusion. Results of these experiments have never been critically appraised and an overall conclusion remains undrawn. A systematic review of studies on extracorporeal perfusion of free tissue flaps and extremities provides a detailed overview of current knowledge on this topic and assesses the quality of research in this field. This information will be used to optimise future animal experiments as a step closer to implementing the extracorporeal perfusion of free flaps and extremities into human clinical trials. **References** 1. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplant outcomes. Br J Surg 2013;100:991–1001. 2. S. Gillani, J. Cao, T. Suzuki, DJ. Hak (2012). The effect of ischemia reperfusion injury on skeletal muscle. Injury, Int. J. Care Injured 43:670–675. 3. NJ Slater, H Zegers, B Kusters, T Beune, H van Swieten, D Ulrich (2015). Ex-vivo oxygenated perfusion of free flaps during ischemia time: a feasibility study in a porcine model and preliminary results. Article submitted to: Journal of surgical research. Under review. 4. CD Taeger, O Friedrich, A Dragu, et al (2015). Assessing viability of extracorporeal preserved muscle transplants using external field stimulation: a novel tool to improve methods prolonging bridge-to-transplantation time. Sci. Rep. 5, 11956. 5. CD Taeger, W Muller-Seubert, RE Horch, et al (2014). Ischaemia-related cell damage in extracorporeal preserved tissue – new findings with a novel perfusion model. J. Cell. Mol. Med. 18;5:885-894. 6. A Dragu, JA Kleinmann, CD Taeger, et al (2012). Immunohistochemical Evaluation after Ex Vivo Perfusion of Rectus Abdominis Muscle Flaps in a Porcine Model. Plast. Reconstr. Surg. 130: 265e. 7. K Ozer, A Rojas-Pena, CL Mendias, et al (2016). The Effect of Ex Situ Perfusion in a Swine Limb Vascularized Composite Tissue Allograft on Survival up to 24 Hours. J Hand Surg Am. 41(1):3e12. 8. K Ozer, A Rojas-Pena, CL Mendias, et al (2015). Ex Situ Limb Perfusion System to Extend vascularized Composite Tissue Allograft Survival in Swine. Transplantation 99: 2095–2101. 9. S Muller, MA Constantinescu, DM Kiermeir, et al (2013) Ischemia/reperfusion injury of porcine limbs after extracorporeal perfusion. J of surg Research. 181:170-182. 10. MA Constantinescu, E Knall, X Xu, et al (2011). Preservation of Amputated Extremities by Extracorporeal Blood Perfusion; a Feasibility Study in a Porcine Model. Journal of Surgical Research 171:291-299.	
	Research question		
11.	Specify the disease/health problem of interest	Hypoxic injury of free tissue flaps and extremities.	
12.	Specify the population/species studied	Humans and all animals	
13.	Specify the intervention/exposure	Extracorporeal perfusion	
14.	Specify the control population	Flush and cool storage ex-vivo, direct replantation, sham surgery or in-vivo perfusion (no dissection).	

15.	Specify the outcome measures	Hypoxic injury and tissue function	
16.	State your research question (based on items 11-15)	What is the effect of extracorporeal perfusion of free tissue flaps or extremities on hypoxic injury and tissue function?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	☐**MEDLINE via PubMed** ☐Web of Science ☐SCOPUS ☐**EMBASE** ☐Other, namely: ☐Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	A search was performed on the 12th of June in PubMed and EMBASE, combining search strings for extracorporeal circulation (intervention) and free tissue flaps/extremities (domain). A supplementary file containing the search strategy was added: [Search Strategy AS Kruit 12-06-2016].	
19.	Identify other sources for study identification	☐**Reference lists of included studies** ☐Books ☐**Reference lists of relevant reviews** ☐Conference proceedings, namely: ☐**Contacting authors/ organisations, namely: in case of incomplete data regarding primary outcome. Unpublished data (eg conference proceedings) are not retrieved to prevent bias by possible selective response of authors.** ☐Other, namely:	
20.	Define search strategy for these other sources	- Screen reference lists of included studies for relevant additional articles that were not found in the PubMed/EMBASE search. - Identify relevant case reports and reviews in title/abstract screening and search reference list for additional relevant articles. - In case of incomplete data: email the corresponding author in order to retrieve original/raw data.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	Screening phases after removal of duplicates: 1. Pre-screening based on title/abstract 2. Full text screening	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Two independent reviewers (ASK and HW) will screen potentially relevant articles on title/abstract and full text. In case of discrepancies, final consensus will be reached after deliberation of both reviewers.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: • Original article Exclusion criteria: • No original article: review, case series <10 subjects	

		case report, editorial.	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: • All humans and animal species with or without comorbidities. Exclusion criteria: • In vitro studies.	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: • Extracorporeal circulation Exclusion criteria: • Single flush with preservation fluid or blood products. • Other organ preserving techniques, e.g. shockwave therapy. • Administration of chemotherapy or thrombolytic therapy via a (semi-)extracorporeal circuit.	
26.	Outcome measures	Inclusion criteria: • All outcomes related to tissue function and tissue vitality (e.g. histology, nerve stimulation). Exclusion criteria: • Outcomes not related to tissue function and tissue vitality.	
27.	Language restrictions	Inclusion criteria: None. Exclusion criteria: -	
28.	Publication date restrictions	Inclusion criteria: None. Exclusion criteria: -	
29.	Other	Inclusion criteria: • Domain: preservation of free tissue flaps. • Domain: preservation of extremities. Exclusion criteria: • Domain: preservation of solid organs (eg. liver, kidney). • Treatment of tumours or thrombosis in tissue flaps or extremities.	
30.	Sort and prioritize your exclusion criteria per selection phase	**Selection phase (title/abstract):** 1. Intervention (extracorporeal circulation) 2. Domain (free tissue flaps or extremities) 3. Article type (original article). **Selection phase (full text):** 1. Article type (original article) 2. Intervention (extracorporeal circulation) 2. Domain (free tissue flaps or extremities) 3. Outcome (tissue vitality and/or tissue function) 4. Unpublished and/or irretrievable data.	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	First author, year.	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	- Experimental groups. - Type of control group. - Number of animals per group.	

33.	Animal model characteristics (*e.g.* species, gender, disease induction)	- Animal species, strain, age, weight, gender. - Comorbidities. - Flap/extremity harvest site. - Vessels (and nerves) in flap pedicle.	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	- Time interval between harvest of flap/extremity and start extracorporeal circulation. - Type of perfusion fluid(s) used. - Temperature of perfusion fluid(s) and flap. - Perfusion settings (e.g. pulsatile, flow rate, pressure). - Duration of perfusion. - Amount of perfusion fluid used and refills needed. - Addition of drugs into the extracorporeal circuit.	
35.	Outcome measures	- Timing of outcome collection. - Primary and secondary outcomes related to tissue vitality and function. - Edema formation. - Total follow-up.	
36.	Other (*e.g.* drop-outs)	- Drop-outs/failure of experiments. - Sample size calculation or posthoc power analysis. - Conflict of interest/acknowledgements.	
	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	Two independent reviewers (ASK and HW) will asses the study quality. In case of discrepancies, final consensus will be reached after deliberation of both reviewers.	
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] ☐**By use of SYRCLE's Risk of Bias tool, adapted as follows:** **Extra criteria: statement of compliance with animal welfare regulations, sample size calculation or posthoc power analysis performed, statement of potential conflicts of interest.** ☐By use of CAMARADES' study quality checklist, e.g [22] ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐**Other criteria, namely: Cochrane criteria will be applied to human studies.**	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	**Tissue vitality:** - Histology (preferably continuous, but also scales). - Immunological markers for hypoxia (preferably continuous, but also scales or dichotomous). - Serum markers (e.g. pO2 consumption, lactate), continuous. **Tissue function:** - Nerve or muscle stimulation (preferably continuous, but	

		also scales). - Muscle contractions (continuous or dichotomous). *If multiple outcome measurements for tissue vitality are performed on the same sample, the above order is applied as a priority for inclusion of outcomes in the meta analysis in order to prevent bias by 'multiple testing'.*	
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 text, tables and figures. 2. Extraction from graphs using a digital screen ruler. 3. Contact authors by e-mail for additional data in case of missing data or unclear outcomes. Authors will be contacted twice via email with an interval of two weeks. In case of no response within a month's time, the article will be excluded or data will be marked as missing.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	Two independent reviewers (ASK and HW) will extract data from the included articles. In case of discrepancies, final consensus will be reached after deliberation of both reviewers.	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Data are presented in tables (mean, CI/SD and number of animals per group) or in a descriptive summary, enabling comparison of results. If possible, data on tissue edema formation are presented in a separate table, as this is a secondary outcome of interest. This requires homogenous outcome presentation between part of the articles. A meta analysis is expected to be possible for at least 1 of the outcome measures. In case of clinically relevant differences in control groups between studies, a separate meta analysis will be performed according to type of control group. For subgroup analysis preferably >3 independent articles are used.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	A meta-analysis will be performed when >3 independent articles present data on comparable outcomes.	
	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)	In case of comparable outcome reporting (e.g. using similar scales or continuous outcomes), mean differences are used. When different units of measurements are used in outcome reporting, standardized mean differences are used.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	As a large heterogeneity in articles is expected in animal species, tissue harvest site and type of intervention, the random effects model will be used as the statistical model of analysis.	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	Review manager will be used for statistical analysis, with use of I^2 for heterogeneity testing.	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Type of perfusion fluid (blood vs. perfusion fluids), perfusate temperature (≤10°C vs. >10°C) and machine settings (pulsatile vs. continuous perfusion).	

48.	Any sensitivity analyses you propose to perform	Possible characteristics for the sensitivity analysis are: animal species, temperature cut off point, type of control group, type of perfusion fluid and vitality outcome measure.	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	If necessary, correction for multiple testing and multiple use of control group are applied.	
50.	The method for assessment of publication bias	If possible, using a funnel plot.	

Final approval by (names, affiliations): Date: