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	Gene Therapy For Bone Defects In Oral And Maxillofacial Surgery: A Systematic Review And Meta-Analysis	
2.	Authors (names, affiliations, contributions)	**Riham Fliefel** • Experimental surgery and regenerative medicine, Ludwig-Maximilians-University, Munich, Germany, • Department of Oral and Maxillofacial Surgery, Ludwig-Maximilians-University, Munich, Germany, • Department of Oral and Maxillofacial Surgery, Alexandria-University, Alexandria, Egypt. **Jan Kühnisch** Department of Conservative Dentistry and Periodontology, Ludwig-Maximilians-University, Munich, Germany **Michael Ehrenfeld:** Department of Oral and Maxillofacial Surgery, Ludwig-Maximilians-University, Munich, Germany **Sven Otto** Department of Oral and Maxillofacial Surgery, Ludwig-Maximilians-University, Munich, Germany	
3.	Other contributors (names, affiliations, contributions)		
4.	Contact person + e-mail address	Riham Fliefel Riham.Fliefel@med.uni-muenchen.de, r_fliefel@yahoo.com	
5.	Funding sources/sponsors	No source of funding	
6.	Conflicts of interest	No conflict of interest	
7.	Date and location of protocol registration	04 May 2016, Experimed, LMU, Munich, Germany 01 August 2016, SYRCLE website	
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	Full-text analysis and data extraction	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Maxillofacial bone defects resulting from bone loss present a difficult and challenging problem for maxillofacial surgeons and scientists with the goal of restoring facial form, function and occlusion. There have been tremendous advances in gene therapy relevant to oral and maxillofacial complex.	
	Research question		
11.	Specify the disease/health problem of interest	Craniofacial anomalies and bone defects	
12.	Specify the population/species studied	Animal models with induced maxillofacial defects	

13.	Specify the intervention/exposure	Gene therapy	
14.	Specify the control population	Blank control or control gene or untransduced cells with scaffolds	
15.	Specify the outcome measures	Regeneration of bone in the maxillofacial defects histologically or radiographically	
16.	State your research question (based on items 11-15)	Was gene therapy successfully applied to regenerate bone or heal defects in the oral and maxillofacial region?	
	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: Cochrane Library □Specific journal(s), namely: International Journal of Oral and Maxillofacial Surgery, Journal of Cranio-maxillofacial Surgery, Gene therapy, Molecular therapy and Human gene therapy.	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	The data search included a combination of the following keywords: ‘‘Gene therapy’’ ‘‘AND’’ ‘‘Maxillofacial surgery’’ ‘‘OR’’ ‘‘Gene therapy’’ ‘‘AND’’ ‘‘Bone tissue engineering’’, ‘‘Genetic Engineering’’ ‘‘AND’’ ‘‘Maxillofacial bone’’, ‘‘Gene therapy’’ ‘‘AND’’ “Distraction Osteogenesis” ‘‘OR’’ ‘‘Gene therapy’’ ‘‘AND’’ “Alveolar bone” ‘‘OR’’ ‘‘Gene therapy’’ ‘‘AND’’ “Periodontal tissue” ‘‘OR’’ ‘‘Gene therapy’’ ‘‘AND’’ “Temporomandibular joint”. All the possible combinations of these words were explored. Medical subject headings (MeSH terms) without subheading restrictions was used and the heading sequence was ‘‘Gene therapy’’ ‘‘AND’’ ‘‘Dentistry’’. In addition, we performed hand-search to the references of the included articles, papers of interest and related systematic or non-systematic reviews. The International Journal of Oral and Maxillofacial Surgery, Journal of Craniomaxillofacial Surgery, Gene therapy, Molecular therapy and Human gene therapy journals were also screened to identify possible references not reported elsewhere.	
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: □Other, namely:	
20.	Define search strategy for these other sources	First, identification of the titles of related articles present on the reference lists of the included studies and relevant reviews. After wards, reading the full-text articles.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	After the identification of articles in the databases, the articles were imported into Endnote software to store search results and remove duplicates. Titles and abstracts identified were screened. The abstracts of the articles reviewed and the full-text was obtained for those articles with apparent relevance.	

22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Two reviewers screened the articles (RF and SO)with any differences resolved by discussion	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: In-vivo studies, combination of in-vitro/in-vivo studies. Exclusion criteria: In-vitro studies	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: Any animal model with induced maxillofacial defects Exclusion criteria: None	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Gene therapy in maxillofacial region Exclusion criteria: Gene therapy in bones other than maxillofacial, Calvarial bones defects, Oral cancer or soft tissue lesions, Studies based on the use of only growth factors or cell-based therapies.	
26.	Outcome measures	Inclusion criteria: Bone formation in the defect Exclusion criteria: None	
27.	Language restrictions	Inclusion criteria: Any Language Exclusion criteria: None	
28.	Publication date restrictions	Inclusion criteria: No restriction Exclusion criteria: After the 18th of December 2015	
29.	Other	Inclusion criteria: Exclusion criteria:	
30.	Sort and prioritize your exclusion criteria per selection phase	Screening phase: 1. Literature review 2. In vitro studies Selection phase: 1. letters to the editor 2. editorials, poster or oral presentations 3. articles with only abstract	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	First author, title, year, country, journal.	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of animals in the study, experimental groups,	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Alveolar bone defects with/ without dental implant, Periodontal disease with/ without alveolar bone, Distraction osteogenesis, Temporomandibular joint, Orthodontic tooth movement, Sinus floor elevation, Tooth restoration with bio-root regeneration, Central fissures Location: The defects were in the mandible and maxilla with the posterior mandible most frequently. Animal Model: Sprague-Dawley rats, Wistar rats, Lewis Fisher, ginue-pigs, mice, White New Zealand rabbits, dogs, pigs.	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Surgery and gene therapy	
35.	Outcome measures	The primary outcome measure for this meta-analysis was significant new bone formation by histology (% of area and % of volume) or radiograph (bone volume fraction) between the experimental and control group.	

36.	Other (*e.g.* drop-outs)		
	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 reviewers screened the articles (RF and SO)with any differences resolved by discussion	
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: □By use of CAMARADES' study quality checklist, e.g [22] ☑By use of CAMARADES' study quality checklist, adapted as follows: (1) published in a peer-reviewed journal; (2) random allocation to treatment or control; (3) treatment allocation concealment; (4) blinded assessment of outcome; (5) reporting of a sample size calculation; (6) statement of compliance with animal welfare regulations; (7) Statement of potential conflict of interest. □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)	Bone formation was assessed as continuous outcome variables by inverse variance (IV) method and recorded as the standardized mean difference (SMD) with 95% confidence interval (CI). A weighted fixed-effect model was used to estimate the overall effect size. Unit of measurement: Histology: 1. Percentage of area of bone formation. 2. Percentage of volume of bone formation. Radiograph: 1. Bone volume fraction.	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	Data was extracted from either text or tables in the results section of the included studies. Data that was presented as graphs was extracted electronically using WebPlotDigitizer software, version: 3.9 (WebPlotDigitizer, US, http://arohatgi.info/WebPlotDigitizer, 2015).	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	Two reviewers screened the articles (RF and SO)with any differences resolved by discussion	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Data were compared using both descriptive summary and meta-analysis.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Meta-analysis will be performed (using Review Manager (version 5.3) with subgroup analysis and sensitivity analysis for all outcome measures if possible. Otherwise descriptive summary.	
	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)	All the outcome measures are continuous variables. They will be expressed as standardized mean difference (SMD). Where outcomes are repeatedly measured at different points.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Fixed effects model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)		
48.	Any sensitivity analyses you propose to perform		
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)		
50.	The method for assessment of publication bias	Funnel plot will be visually inspected to determine the publication bias.	
Final approval by (names, affiliations): Riham Fliefel, ExperiMed, LMU, München, Germany		Date: 30.06.2016	