PRILE 2021 guidelines for reporting laboratory studies in Endodontology: explanation and elaboration

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Abstract

Guidance to authors is needed to prevent their waste of talent, time and resources in writing manuscripts that will never be published in the highest-quality journals. Laboratory studies are probably the most common type of endodontics research projects because they are the majority of manuscripts submitted for publication. Unfortunately, most of these manuscripts fail the peer-review process, primarily due to critical flaws in the reporting of the methods and results. Here, in order to guide authors, the Preferred Reporting Items for study Designs in Endodontology (PRIDE) team developed new reporting guidelines for laboratory-based studies: the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines. The PRILE 2021 guidelines were developed exclusively for the area of Endodontology by integrating and adapting the modified CONSORT checklist of items for reporting *in vitro* studies of dental materials and the Clinical and Laboratory Images in Publications (CLIP) principles. The process of developing the PRILE 2021 guidelines followed the recommendations of the Guidance for Developers of Health Research Reporting Guidelines. The aim of the current document is to guide authors with an explanation for each of the items in the PRILE 2021 checklist and flowchart with examples from the literature , and to provide advice from peer-reviewers and editors about how to solve each problem in manuscripts prior to a peer-review. The Preferred Reporting Items for study Designs in Endodontology (PRIDE) website (http://pride-endodonticguidelines.org/prile/) provides a link to the PRILE 2021 explanation and elaboration document as well as to the checklist and flowchart.

Development of the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines.

The PRILE 2021 guidelines were developed to guide authors to avoid common peer-review problems that can prevent the publication of manuscripts describing laboratory studies in Endodontology by ensuring they are complete, accurate, and transparent (Nagendrababu *et al.* 2021). The PRILE 2021 development process adhered to the recommendations set out in the Guidance for Developers of Health Research Reporting Guidelines (Moher *et al*. 2010). The project leaders (VN, PD) identified the need for reporting guidelines for authors when writing-up laboratory studies in Endodontology and established a steering committee of ten members (PD, VN, PM, RZ, OP, IR, JS, EP, JJ, SP). The steering committee created the first draft of the guidelines based on the modified CONSORT checklist of items for reporting *in vitro* studies of dental materials (Faggion 2012) and the Clinical and Laboratory Images in Publications (CLIP) principles (Lang *et al*. 2012) and which included all the items necessary to report a laboratory study in Endodontology; they also create a flowchart.

Subsequently, the steering committee formed a PRILE Delphi Group (PDG) consisting of 30 members to achieve consensus on the items within the guidelines. The initial draft PRILE checklist and flowchart were revised based on the feedback obtained from the PDG members.

The steering committee then formed a PRILE Online Meeting Group (POMG) of 24 experts from around the world. The POMG held a Zoom call to discuss in detail the wording of each item in the checklist and the design of the flowchart. The POMG provided collective feedback to justify and support the inclusion or exclusion of each item and the steering committee revised the items of the checklist and flowchart based on the feedback obtained. The revised guidelines and flowchart were then piloted by several volunteer authors when drafting hypothetical manuscripts of laboratory studies in Endodontology to confirm they were fit for purpose, and a suitable and practical guide. The final PRILE 2021 guidelines consist of 11 sections and 40 individual items in a checklist along with a flowchart.

The PRILE 2021 explanation and elaboration document

The aim of the present document is to guide authors with an explanation for each of the items in the PRILE 2021 guidelines checklist and a flowchart, and to provide advice for peer-reviewers and editors about how to solve each problem in manuscripts prior to a peer-review. The document provides examples of good reporting practice from published laboratory studies to aid understanding. In several examples of the published studies, citations or web addresses have been removed, and abbreviations are stated in full. Hence, this explanation and elaboration document provides guidance for authors when using the PRILE 2021 guidelines whilst preparing or reviewing manuscripts of laboratory studies in Endodontology.

Item 1a: Title - The Title must identify the study as being laboratory-based, e.g. "laboratory investigation" or "*in vitro*," or "*ex vivo*" or another appropriate term

Explanation

Providing descriptive terms in the title will help readers quickly identify the type of study, such as: "laboratory investigation," or "*in vitro*" or "*ex vivo*," or "genetic phenotyping," or "micro-computed tomography (Micro- CT)" etc (*Examples 1a.1, 1a.2, 1a.3)*. The use of descriptive terms will also help database indexing as well as to refine the results within literature searches.

Example 1a.1

From Oliveira *et al.* (2020) – "A laboratory evaluation of cell viability, radiopacity and tooth discoloration induced by regenerative endodontic materials".

Example1a.2

From Persadmehr *et al*. (2014) – "Bioactive chitosan nanoparticles and photodynamic therapy inhibit collagen degradation *in vitro*".

Example 1a.3

From Naseri *et al.* (2019) – "The effect of calcium hydroxide and nano-calcium hydroxide on microhardness and superficial chemical structure of root canal dentin: An *ex vivo* study".

Item 1b: Title - The area / field of interest must be provided (briefly) in the Title

Explanation

Describing the area of interest (e.g. root canal preparation, canal disinfection) will help readers to clearly identify the focus of the laboratory study (*Examples 1b.1, 1b.2, 1b.3)*.

Example 1b.1

From Oliveira *et al.* (2020) – "A laboratory evaluation of cell viability, radiopacity and tooth discoloration induced by regenerative endodontic materials".

Example 1b.2

From Aksel *et al.* (2019) – "Micro-CT evaluation of the removal of root fillings using the ProTaper Universal Retreatment system supplemented by the XP-Endo Finisher file".

Example 1b.3

From Li *et al*. (2015) – "Occurrence of dentinal microcracks in severely curved root canals with ProTaper Universal, WaveOne, and ProTaper Next File Systems".

Item 2a: Keywords - At least two keywords related to the subject and content of the investigation must be provided

Explanation

The inclusion of relevant keywords is essential for readers to identify the focus of the laboratory investigation and will improve the precision of the search results, e.g. root canal, canal filling, canal disinfection, canal instrumentation, cytotoxicity. This is the reason why selecting terms from the MeSH (Medical Subject Headings) in the National Library of Medicine is strongly recommended (*Examples 2a.1, 2a.2)*.

Example 2a.1

From Oliveira *et al*. (2020) - "A laboratory evaluation of cell viability, radiopacity and tooth discoloration induced by regenerative endodontic materials". The keywords used were "Cytotoxicity; pulpotomy; radiopacity; staining potential; tooth discoloration".

Example 2b.2

From Aksel *et al*. (2019) - "Micro-CT evaluation of the removal of root fillings using the ProTaper Universal Retreatment system supplemented by the XP-Endo Finisher file". The keywords used were "XP-Endo Finisher; calcium silicate; endodontic retreatment; root canal filling".

Item 3a: Abstract - The rationale/justification of what the investigation contributes to the literature and/or addresses a gap in knowledge must be provided

Explanation

Peer-reviewers and editors prioritize the publication of research manuscripts according to its likely interest to the journal readers. The highest priority research for publication can identify a valid knowledge gap and yield information that can help solve a significant problem. For these reasons, a manuscript should provide a rationale/justification which is a brief background summary of the problem to be solved or the gap(s) in knowledge so as to justify the reason for performing the investigation (*Examples 3a.1, 3a.2)*. This item is subject to specific journal guidelines as some only require the Aim without the rationale/justification.

Example 3a.1

From Scattina *et al*. (2015) - "The finite element method (FEM) has been proposed as a method to analyze stress distribution in nickel-titanium (NiTi) rotary instruments but has not been assessed as a method of predicting the number of cycles to failure (NCF). The objective of this study was to predict NCF and failure location of NiTi rotary instruments by FEM virtual simulation of an experimental nonstatic fatigue test".

Example 3a.2

From Moore *et al.* (2016) - “Recently, we reported that in mandibular molars contracted endodontic cavities (CECs) improved fracture strength compared with traditional endodontic cavities (TECs) but compromised instrumentation efficacy in distal canals. This study assessed the impacts of CECs on instrumentation efficacy and axial strain responses in maxillary molars”.

Item 3b: Abstract - The aim / objectives of the investigation must be provided

Explanation

The aims / objectives must be described with precision and using professional terminologies to enable readers to quickly understand if the study is relevant to them (*Examples 3b.1, 3b. 2*).

Example 3b.1

From Aksel *et al.* (2019) - “To compare the removal of root fillings in extracted teeth using the ProTaper Universal Retreatment system (PTUR) followed by a supplementary preparation with the XP-Endo Finisher file”.

Example 3b.2

From Rathinam *et al.* (2020) - “The aim of this study was to compare the transcriptome-wide effects by next-generation RNA sequencing of custom-prepared human dental pulp cells (hDPCs) stimulated with tricalcium silicate (TCS)-based biomaterials: ProRoot

white Mineral Trioxide Aggregate (WMTA) (Dentsply, Tulsa; Tulsa, OK) and Biodentine (Septodont, Saint Maur des Fosses, France)".

Item 3c: Abstract - The body of the Abstract must describe the materials and methods used in the investigation and include information on data management and statistical analysis

Explanation

Due to word limits, the inclusion of a concise, unambiguous, and an accurate sequential description of the materials and methods used in the experiment and how data was managed/analysed is essential (*Examples 3c.1, 3c.2*).

Example 3c.1

From Aksel *et al.* (2019) - "The mesiobuccal root canals of 30 extracted mandibular first molars were instrumented with ProTaper Universal NiTi files up to F2 and filled with one of the following sealers using a single-cone technique (n = 10): AH Plus, NeoMTA Plus and EndoSequence BC. The root fillings were removed using the PTUR system with additional apical preparation using ProTaper F2 and F3 files. Then, an additional preparation with an XP-Endo Finisher file was performed. The samples were scanned using micro-CT before and after retreatment and again after the use of the XP-Endo Finisher to assess the volume of remaining filling material. Data were analysed by Kruskal-Wallis and Friedman's two-way analysis of variance tests with Bonferroni correction".

Example 3c.2

From Oliveira *et al.* (2020) - "Human dental pulp cells (hDPCs) stimulated with lipopolysaccharide (LPS) were placed in contact with several dilutions of culture media previously exposed to the experimental materials and tested for cell viability using MTT. Bovine teeth were prepared to simulate an open apex and to mimic extensive crown fracture. The roots were filled with a mixture of agar and blood, and the materials placed over this mixture. The control group consisted of teeth filled only with agar and blood. Colour assessment analyses were performed before and immediately after material insertion and repeated at 30, 45 and 60 days using a spectrophotometer. The total colour change (ΔE_{ab}, ΔE_{00} and whiteness index (WI)) was calculated based on the CIELAB colour space. Digital radiographs were acquired for radiopacity analysis. Cell viability was analysed by one-way anova, whilst differences in colour parameters (ΔE_{ab}, ΔE_{00} and WI) were assessed by two-way repeated measures ANOVA ($\alpha = 0.05$). Tukey's test was used to compare the experimental groups, and Dunnett's test was used to compare the experimental groups with the control group".

Item 3d: Abstract - The body of the Abstract must describe the most significant scientific results for all experimental and control groups

Explanation

Due to word limits, only the most important or surprising significant scientific results of likely interest to readers must be provided (*Examples 3d.1, 3d.2*).

Example 3d.1

From Persadmehr *et al.* (2014) – "As assessed by hydroxyproline release into the medium, collagen treated with bioactive chitosan nanoparticles (CSnp), photodynamic therapy (PDT), or a combination of CSnp and PDT exhibited less degradation than untreated controls (3.6-fold, 1.7-fold, and 7.9-fold reduction, respectively; $P < .05$). Compared with all other treatments, glutaraldehyde (GD) treated collagen was the most resistant to collagenolytic degradation (239.6-fold reduction, $P < .05$). The abundance of post-treatment residual collagen, as measured by picrosirius red staining, was inversely related to the extent of collagen degradation. Analysis of collagen cross-links with Fourier transform infrared spectroscopy showed that PDT or GD treatments enhanced collagen cross-linking. Immunoblotting of sedimented CSnp indicated that CSnp and collagenase bound with low affinity. However, CSnp-bound collagenase showed a significant reduction in collagenolytic activity compared with controls ($P < .05$)".

Example 3d.2

From Oliveira *et al.* (2020) – "MTA Flow (MTA), UltraCal XS (UC) and Bio-C Temp (BT) had similar cell viability to that of the control group (Dulbecco's Modified Eagles Medium) ($P > 0.05$), except for the BT group at the 1 : 1 and 1 : 2 dilutions, which had significantly lower viability ($P < 0.001$). All materials were associated with discoloration values greater than what is considered to be the acceptable threshold, and BT resulted in less or similar tooth colour change than MTA and UC, respectively. Decreasing radiopacity over

time was observed only in the MTA group (P = 0.007). Lower values of radiopacity were found in the BT group compared with the UC and MTA groups (P < 0.001)".

Item 3e: Abstract - The main conclusion(s) of the study must be provided

Explanation

Ideally, a conclusion explains how the scientific results solve a problem, or fills the gap in knowledge, and/or explains what remains unknown, without over-generalizing the concluding "take-away" lesson(s). Whereas, the conclusion cannot merely repeat the results, because that would be redundant. (*Examples 3e.1, 3e.2*).

Example 3e.1

From Rathinam *et al.* (2020) - "The results of the present study illustrate that several important signalling pathways are induced by human dental pulp cells (hDPCs) stimulated with Tricalcium silicate (TCS)-based biomaterials".

Example 3e.2

From Kishen *et al.* (2018) - "This study highlighted the maximum reduction of microbes after instrumentation-syringe irrigation. Although supplementary sonic agitation reduced the root canal biofilm further, it did not completely eliminate the biofilm from a single root canal model. The merits of combining microbiological and molecular

quantification methods with Confocal laser scanning microscopy for the comprehensive assessment of antibiofilm efficacy in root canals were emphasized".

Item 4a: Introduction - A background summary of the scientific investigation with relevant information must be provided

Explanation

The Introduction must provide relevant background information written in such a way as to justify the scientific approach. Scientific problems and relevant controversies must be described using accurately referenced background statements of fact, that are distinguished from opinions, written in a style that is interesting and original: Plagiarism is never acceptable. The manuscript aim's cannot be identical to a previous animal, clinical or laboratory study. This is because journals are seeking to publish novel research that uses the latest techniques, equipment, biomaterials, and therapeutics (*Examples 4a.1, 4a.2*).

Example 4a.1

From Moore *et al.* (2016) - "Contracted endodontic cavities (CECs), inspired by concepts of minimally invasive dentistry, emphasize tooth structure preservation including pericervical dentin. We previously reported that CECs, compared with traditional endodontic cavity (TECs), improved fracture strength under a continuous load in unrestored mandibular premolars and molars but not in maxillary incisors, and compromised instrumentation efficacy in distal canals of mandibular molars but not in

premolars and incisors. These results, suggesting that the impact of CECs varied in different tooth types when unrestored, might not be extrapolated to restored maxillary molars in which the morphology is distinctly different. Also, unlike available data on fracture strength of intact mandibular molars, respective data on maxillary molars are lacking. Therefore, this study assessed the impacts of CECs on canal instrumentation efficacy and biomechanical responses in maxillary molars restored with bonded composite resin".

Example 4a.2

From Scattina *et al*. (2015) - "Instruments that fail because of bending fatigue usually exhibit no specific macroscopic patterns, and failure may occur without any visible warning. Canal shape, instrument geometry, rotational speed, torque, instrument surface treatments, and the chemical composition of NiTi alloys are the main factors affecting the number of cycles to failure (NCF) of NiTi rotary instruments. Standardized experimental conditions are not possible for extracted teeth, but several self-designed devices and methods have been used to assess the NCF of NiTi rotary instruments in vitro. However, there is no international standard for testing the cyclic fatigue behavior of endodontic rotary instruments in vitro. A 3-dimensional computerized approach based on the finite element method (FEM) has been recently proposed to analyze stress distribution in bending fatigue. This approach takes cyclic loading conditions into consideration but makes no attempt to predict NCF".

Item 4b: Introduction - The aim(s), purpose(s) or hypothesis(es) of an investigation must be provided ensuring they align with the methods and results

Explanation

A well written introduction clearly describes the aim(s) or purpose(s) or hypothesis(es) for pursuing the research (*Examples 4b.1, 4b.2*). The Introduction must provide sufficient background information to enable readers to evaluate the approach taken and the research methodology, by explaining the knowledge gap, describing the research question(s) that need to be answered, and justifying the reasons for collecting the results. If hypothesis testing is required, the null hypothesis should predict a lack of differences in the results. Alternative hypothesis testing is encouraged to advance the frontiers of research. Reverse-engineering hypotheses to fit results is misleading and must be avoided.

Example 4b.1

From Moore *et al.* (2016) - "We tested the null hypotheses that CECs would not impact instrumentation efficacy, axial root strain, or fracture strength after cyclic fatigue".

Example 4b.2

From Scattina *et al*. (2015) - "The objective of this study was to assess the possibility of predicting the NCF and failure location of NiTi rotary instruments by the virtual simulation of an experimental fatigue test (nonstatic loading condition) using the FEM".

Item 5a: Materials and Methods - A clear ethics statement and the ethical approval granted by an ethics board, such as an Institutional Review Board or Institutional Animal Care and Use Committee, must be described

Explanation

Ethical oversight and ethical approval to use teeth, cells or tissues harvested from human subject donors and animals are required by laws, institutional policies, and author guidelines. If biological specimens such as, cells, tissues, teeth, bone, skin, fluids, DNA, antibodies, bacteria, biopsies, etc. were harvested from human subjects or animals, respective Institutional Review Board (IRB) or Institutional Animal Care And Use Committee (IACUC) approval information (name of the institution, reference number) must be described (*Examples 5a.1, 5a.2*).

Example 5a.1

From Martinho *et al*. (2018) - "The study was approved by the Ethics Committee for Clinical Research at São Paulo State University (São José dos Campos, São Paulo, Brazil) (083/2009 -PH/CEP)".

Example 5a.2

From Kato *et al*. (2016) - "The study protocol (no. 694.151) was approved by the Research Ethics Committee of the Sao Leopoldo Mandic Center for Dental Research, Campinas, Sao Paulo, Brazil".

Item 5b: Materials and Methods - When harvesting cells and tissues for research, all the legal, ethical, and welfare rights of human subjects and animal donors must be respected, and applicable procedures described

Explanation

Researchers must respect the legal, ethical and welfare rights of all human subjects/patients/guardians and obtain their informed consent to participate in research, and also obtain Institutional Review Board (IRB) prior approval to use human specimens in the laboratory. Human subjects should never be exposed to excessive treatment failure risks, unnecessary pain, suffering, discomfort, or disability, or care that falls below our professional standards of conduct. It is unethical to extract healthy permanent teeth or other essential healthy tissues from human subjects, or to deny or delay treatments, for purely research purposes. Authors must keep records to show that human subjects and or their guardians, have provided written permission to allow genetic testing, or for their cells and tissues (blood, saliva, biopsies, teeth etc.) to be used for research purposes. Researchers should respect the right to privacy of human subjects, and never publish their identifying information (names, addresses, or full-face photographs) (*Examples 5b.1, 5b.2*). Research that involves collecting tissues from donor animals, should avoid using non-human primates (monkeys) or pets (dogs, cats and rabbits etc) due to recent reader opposition to animal testing and any laws which prohibit

the use of such animals. It is never acceptable to cause severe animal suffering (failing to monitor pain and/or withholding analgesics and anaesthesia), cruelty and disability. Peer-reviewers and editors do not have to accept an IRB approval for human subjects, or an Institutional Animal Care and Use Committee (IACUC) for animals, as evidence that research has been performed legally or ethically, because they need to consider the worldwide legal and ethical standards.

Example 5b.1

From Martinho *et al*. (2018) - "The study was approved by the Ethics Committee for Clinical Research at São Paulo State University (São José dos Campos, São Paulo, Brazil) (083/2009 -PH/CEP). Primary cell culture of human pulp -derived cells. Impacted third molars, at the stage of starting root formation, were collected immediately following extraction from a patient (18 years old) at the Department of Oral Surgery and Diagnosis, University of Dentistry of São José dos Campos, UNESP. The use of human pulp cells was approved by the local research ethics committee".

Example 5b.2

From Rathinam *et al.* (2020) - "Informed consent was collected from all patients and ethical approval was obtained from the Ethical Committee of University Hospital, Ghent, Belgium, according to laws of ICH Good Clinical Practice (GE11-LM-go-2006/57)".

Item 5c: Materials and Methods - The use of reference samples must be included, as well as negative and positive control samples, and the adequacy of the sample size justified

Explanation

The use of standardized reference samples included in commercial assay kits or required by International Standards Organisation (ISO) standards etc., prepared identically to previously cited publications will improve the comparability of the research to the existing literature. The inclusion of negative and positive control samples will help troubleshoot problems to validate the accuracy of the results and enhance reader and reviewer confidence in the reliability of the results. Preparation of control samples/specimens must be described with the same level of detail as the experimental samples/specimens. Greater numbers of samples generally provide more reliable results, but due to time and financial constraints, most publications try to use the least number of samples. Reliable and reproducible research demands that sample sizes are adequate for sample group comparisons to detect true differences (of a clinically relevant magnitude) between the groups, if they exist. This would normally be done by performing *a priori* power analysis to avoid publishing underpowered research (power below 0.8); and also include a similar number of samples to those used in relevant cited published articles that have reported significant probability values (*Examples 5c.1, 5c.2, 5c.3, 5c.4*).

Example 5c.1

From Rover *et al*. (2017) - “The sample size was estimated based on studies comparing TECs and CECs, both with 10 teeth per group. Accordingly, for analysis with a = 0.05 and 80% power, at least 10 teeth were allocated for each of the following groups: CEC (experimental) and TEC (control)”.

Example 5c.2

From Moore *et al.* (2016) - “The sample size was estimated based on studies comparing fracture strength for TECs and CECs and the proportion of untouched canal wall, both with 10 teeth per group. Accordingly, for analysis with a = 0.05 and 80% power, at least 10 teeth were allocated for each of the following groups: CEC (experimental), TEC (control), and intact (negative control for fracture strength testing) for different aspects of the study”.

Example 5c.3

From Hussne *et al.* (2011) - “Prior to mechanical testing, 12 instruments of each of the eight types and sizes were randomly selected and photographed in a standardized manner using a high-resolution digital camera (Canon 20D, Tokyo, Japan) for the assessment of the tip angle and instrument diameter at 3 mm from the tip, based on ANSI/ADA specification number 101. One group comprising 12 of each of the eight instrument types (n = 12 each) was tested for bending resistance according to specification International Standards Organisation (ISO) 3630-1”.

Example 5c.4

From Martinho *et al*. (2018) - "The culture medium of unstimulated pulp fibroblast cells was used as negative control. The assay was carried out according to the manufacturer's instructions. Next, standard, control, or sample solution was added to the ELISA well plate, which had been pre-coated with specific monoclonal capture antibody".

Item 5d: Materials and Methods - Sufficient information about the methods/materials/supplies/samples/ specimens/ instruments used in the study must be provided to enable it to be replicated

Explanation

The methods used in the investigation must provide sufficient details for the study to be replicated by others. This requires accurate, detailed and precise information on the use of equipment and the settings, speeds, forces, and all parameters that were used. Each step used to create, prepare and/or standardise samples/specimens should be described in a clear sequence. Beyond the required manufacturer, city, country information, it is advisable to include the lot number and/or reference number for assay kits, antibodies, cell lines, strains, proteins, pharmaceutical compounds (purity/sterility), media, supplies, samples, instruments, solutions, and materials. Details of the weights, times, volumes and or dilutions of reagents and solutions must be described in their most commonly understood units, e.g. weights in grams [g], times in days [d]or seconds [s], volumes in centimetres cubed [cm^3], Solution reagent dilutions in percentages of weight/volume [%w/v]. If any materials or instruments were custom-made for the study, the deviations or changes from those instruments available commercially, must be explained (*Examples 5d.1, 5d.2, 5d.3, 5d.4*). Laboratory studies, with complicated designs (e.g. overall study design, sample preparation techniques) can be explained using a

simple line diagram or flowchart to complement the text and thus aid the understanding of readers. Whenever possible the methods should be referenced to professional standards and previous publications.

Example 5d.1

From Oliveira *et al*. (2020) - The pulp tissue was immersed for 1 h in the following solution: 3 mg mL^{-1} collagenase type I (Sigma- Aldrich, San Louis, MI, USA) and 4 mg mL^{-1} dispase (Sigma-Aldrich). The samples were centrifuged at 250 g (centrifuge 80-2B, Centribio, Curitiba, PR, Brazil) for 2 min and resuspended in basal medium. The cells obtained were plated in 25-cm^2 flasks and incubated for 4 days at 37 °C with 5% CO_2. The culture medium was first replaced after 3 days of incubation; thereafter, it was changed twice a week. The cells were expanded up to the 4th passage and frozen for later experimental use".

Example 5d.2

From Moore *et al.* (2016) – "Teeth were mounted up to 3 mm apical to the cementoenamel junction in customized cylinders fabricated with self-curing resin (SR Ivolen; Ivoclar Vivadent, Schaan, Lichtenstein), with a 0.2-mm-thick lining of polyvinyl siloxane (Aquasil Ultra Monophase Regular Set, Dentsply International) simulating the periodontal ligament. Foil strain gauges (N11-FA-2-120-11; Showa Measuring Instruments, Tokyo, Japan) were glued with rapid-setting cyanoacrylate adhesive (Instant Adhesive Aron Extra 4000; Toagosei, Tokyo, Japan) on the mesiobuccal and palatal cervical root surfaces and sealed with polyurethane varnish (PU140; Hottinger

Baldwin Messtechnik, Darmstadt, Germany). Teeth were mounted in the Instron Universal Testing machine (Instron, Canton, MA). Axial forces, directed at 3^0 angle from the tooth's long axis, were cycled between 50 N and 150 N and the voltage-change outputs from the strain gauges, connected by a half Wheatstone bridge, converted to strain measurements by a data acquisition module (DQ 430 EspressoDAQ; HBM Canada, Pickering, ON). Microstrain values were recorded using DAQ software (Catman Easy, EspressoDAQ version 1.02, HBM Canada) at each of the mesiobuccal and palatal surfaces".

Example 5d.3

From Mancini *et al*. (2013) - "Specimens were randomly divided into 2 control groups (n = 10) and 3 experimental groups (n = 15). Except for the negative control group, groups were shaped by means of ProTaper Ni-Ti rotary instruments (Dentsply Maillefer) according to the manufacturer's instructions until the ProTaper F4 file reached the working length (WL). Each instrument was used to shape only 4 specimens. After each instrumentation and before the next, canals were rinsed with 3 mL 5.25% sodium hypochlorite (NaOCl) at 37^0C (Chematek SpA, Rome, Italy). The apical patency was checked after each instrument with a #10 K-file. Each group was then irrigated with 17% EDTA (Chematek SpA) and left in the canal for 1 minute before being rinsed with 3 mL 5.25% NaOCl at Finally, 5.25% NaOCl at 37^oC was activated/delivered with different methods. Irrigating solutions were delivered by means of a 30-G syringe needle (NaviTip; Ultradent, South Jordan, UT) inserted deeply at 1 mm from the WL. All specimens were then irrigated with 5 mL distilled water and dried with sterile paper points".

Example 5d.4

From Martinho *et al*. (2018) - "For the cytotoxicity assay, the conditioned media were placed in contact with immortalized pulp-derived cells. The cells were maintained in DMEM medium (Gibco BRL, Karlsruhe, Germany) supplemented with 10% Fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37°C and 5% CO2. The amount of 8×103 cells was seeded in each well of the 96-well plates and then incubated at 37°C for 24 h. After this period, the old media was removed and the cell cultures were exposed to 200 µL of serial dilutions (1:2, 1:4, 1:8 and 1:16) of the original extracts (1:1) from the materials as well as to 200 µL of negative control medium (without cells) and 200 µL of positive control medium (with cells) before incubation in oven (5% CO_2 at 37°C) for 24 h. After this period, cell survival was determined for measurement of succinate dehydrogenase (SDH) activity, which indicates mitochondrial function, by using MTT (3-(4,5 dimetiltiazol-2il)-2,5-difenil-tetrazólio; Sigma, St Louis, MO, USA) assay. The SDH activity was quantified by dissolution of MTT with 0.1 N Sodium hydroxide (6.25 v/v%) in DMSO (dimethyl sulfoxide), whereas the resulting optical density reading was measured by using a spectrophotometer at 570 nm (Asdys Hitech). Cytotoxicity was expressed as a percentage of control group".

Item 5e: Materials and Methods - The use of categories must be defined, reliable and be described in detail

Explanation

The methods of many studies rely on categorization to describe their results, but the methods can lack a precise description of each category. One example is results that assign samples as a *success* or *failure*, without providing a definition for what success

actually means, when the difference between a failure can be arbitrary. Another example is a study that cites a categorization method from a previous publication, but then modified it, without explaining any of the modifications. Another example is a histologic study of pulpitis, which reports "none" or "no" inflammation based on the lack of inflammatory cells visible, yet inflammatory cells are always circulating. Therefore, the designations of "none" and "no" cannot be accepted as being accurate or reliable categories. As a general rule, always use professional standards and terminologies for categorizing results, and reference these to previously published categories for analysing results. It is almost never acceptable to invent new terminologies or new arbitrary evaluation criteria to replace existing professional terms or standards, because it creates reader confusion, indicates bias, and raises concerns about the validity of the new criteria. Whenever authors modify published categories, the reasons for the modifications must be justified (*Examples 5.e1, 5e.2*).

Examples 5e.1

From Kfir *et al*. (2000) – "The SEM images of each section, taken with × 1000 magnification, were examined and scored for the presence of erosion/etching using the three grade scoring system proposed by Kaya et al. score 1: no or minimal erosion (all tubules look normal in appearance and size), score 2: moderate etching/erosion (peritubular dentine is eroded), and score 3: severe etching/erosion".

Examples 5e.2

From Caballero *et al*. (2015)- "The criteria used for assessing the instruments included the observation of presence or absence of visible superficial defects, such as plastic deformation,

which was defined as the lost or regular geometry of the instrument; microscopic superficial defects, such as microcracks, which were defined as microfractures in the blades without a complete instrument separation; complete fractures, which was defined as the instrument separation during the tests; large crater, which was defined as the presence of large pits in the surface; disruption of the cutting edges, which was defined as the loss of the regular continuous shape of the blades; blunt edges, which was defined as the loss of the sharpness of the cutting edges; and the presence of dentine debris".

Item 5f: Materials and Methods - The numbers of replicated identical samples must be described within each test group. The number of times each test was repeated must be described

Explanation

Laboratory studies involving cell biology, microbiological techniques, or histological sample analysis usually involve the experiment being repeated multiple times. Details about the number of replications of the sample / experiment must be mentioned in the text or in Tables (*Examples 5f.1, 5f.2*).

Example 5f.1

From Kishen *et al*. (2018) - "All measurements were performed in duplicate for samples and triplicate for standards. In all experiments, triplicates of the appropriate negative controls containing no template DNA were subjected to the same procedures to exclude or detect any possible contamination or carryover".

Example 5f.2

From Widbiller *et al*. (2016) - "Enzyme activity on polystyrene served as a reference for each time point and was set to 1. Median values and 25–75 % percentiles were calculated from four independent experiments performed in triplicate. Amplification was performed in triplicate with the StepOnePlus™ Real-time Polymerase Chain Reaction (PCR) system (Applied Biosystems, Carlsbad, CA, USA) and two independent experiments were conducted".

Item 5g: Materials and Methods – The details of all the sterilization, disinfection, and handling conditions must be provided, if relevant

Explanation

Many biological and molecular studies require all the materials or samples to be sterilized or disinfected, and handled using aseptic techniques with sterile hand instruments inside a flow hood or aseptic cabinet. These conditions must be described (*Examples 5g.1, 5g.2*).

Example 5g.1

From Rathinam *et al*. (2020) - "The samples (n = 3) were allowed to set in 100% relative humidity at 37 °C for 3 h. The samples were removed from the mold and UV-sterilized for 30 min on each side. hDPCs (4th passage) were seeded in a 6-well plate at a density of 5 × 10^5 cells per well. ProRoot white MTA and Biodentine were then placed in transwell

inserts with a pore size of 0.4 µm and inserted in the well plate and maintained at 37°C and 5% CO_2 for 3 or 7 days. In total, n = 3 biological replicates were performed (four technical replicates per biological replicate)".

Example 5g.2

From Widbiller *et al.* (2016) - "After setting, pretreatment was equal for all samples. Specimens were covered with 200 µL aqua bidest. And exposed to UV-light in a laminar flow cabinet for 30 min to prevent bacterial contamination. After 24 h at 37 °C and 5 % CO_2, aqua bidest. was replaced with alpha minimum essential medium (αMEM) and incubated for another 24 h".

Item 5h: Materials and Methods – The process of randomization and allocation concealment, including who generated the random allocation sequence, who decided on which specimens to be included and who assigned specimens to the intervention must be provided (if applicable)

Explanation

The methods used to randomise samples must be provided. Allocation concealment prevents selection bias by concealing the allocation sequence from those assigning specimens to intervention groups until the moment of assignment. In laboratory studies, randomization usually takes place simultaneously for all the specimens. If the random sequence is generated just before allocation of the specimens to the different groups, then concealment is not really required. What is important is that this procedure

(randomisation and allocation to the different groups) takes place at the latest possible stage in the experiment so that all specimens are treated in exactly the same way until that stage (*Example 5h.1)*.

Example 5h.1

From Neelakantan *et al.* (2017) - "The specimens were coded and scanned in a blinded manner to determine the volume of the prepared root canal space. The specimens were randomly allocated to 12 experimental groups".

Example 5h.2

From Boutsioukis *et al*. (2014) – "The tooth–resin and resin–lid interfaces were further coated with cyanoacrylate (Pattex, Henkel, D€usseldorf, Germany) to ensure a fluid-tight seal. The apical 5 mm of the root, which was not embedded in acrylic resin, was also covered with cyanoacrylate, whilst a size 10 K-file (Dentsply Maillefer) protruded 2.5 mm from the surface to create a standardized artificial constriction of 0.15 mm. At this point, specimens were randomly divided into two groups, A and B (n = 16), by computer software (www.randomizer.org), to be used in the two separate phases of the experiments".

Item 5i: Materials and Methods – The process of blinding the operator who is conducting the experiment (if applicable) and the examiners when assessing the results must be provided

Explanation

The blinding of operators and examiners is essential to reduce the likelihood of performance, attrition, and detection bias (*Examples 5i.1, 5i.2*).

Example 5i.1

From Mancini *et al*. (2013) - "Two observers performed blind evaluation independently after examining 20 specimens for calibration purposes. Intra- and interexaminer reliability for field emission scanning electron microscopic assessment was verified by the kappa test".

Example 5i.2

From Kato *et al*. (2016) - "Two independent examiners, previously calibrated and blind to the study, scored the images according to the assessment criteria outlined previously".

Item 5j: Materials and Methods – Information on data management and analysis including the statistical tests and software used must be provided

Explanation

The statistical tests of significance and other information used for data analysis must be provided. For example: test of normality, type of tests (parametric/ non-parametric,

ANOVA, Student's t -test etc.), significance level, confidence intervals, software used (STATA, SPSS etc.) (*Examples 5j.1, 5j2*). Authors need to clearly specify if the assumptions for a particular test were met. For example: normality, independent observations, equal variance among others.

Example 5j.1

From Oliveira *et al.* (2020) - *"*Cell viability, colour assessment and radiopacity data were analysed for normality and homoscedasticity using the Shapiro–Wilk and Levene tests. One-way ANOVA followed by Tukey's test was used to compare data of the cell viability intragroup amongst dilutions and amongst the materials at each of the dilutions tested. Two-way repeated measures ANOVA and Tukey's tests were used to compare the radiopacity and colour parameters (*L,*a,*b, DEab, DE00 and WI), where 'time assessment' was used as a repetition factor. Dunnett's test was used to compare the colour in the experimental groups with the control group. A statistical analysis was performed using SigmaPlot 12.5 statistical software package (Systat Software Inc). The significance level was set at 95% for all data analyses".

Example 5j.2

From Kato *et al*. (2016) - "Kruskal-Wallis test was used to compare data on cleansing efficacy. Multiple comparisons were performed using the Dunn test when applicable. The Friedman test was applied to detect differences in cleansing promoted by each irrigation system at the different apical levels examined. All statistical calculations were performed

using the SPSS 20 (SPSS Inc, Chicago, IL) and BioEstat 5.0 (Fundacao Mamiraua, Belem, PA, Brazil) software programs. The level of significance adopted was 5%".

Item 6a: Results – The estimated effect size and its precision for all the objective (primary and secondary) for each group including controls must be provided

Explanation

The effect size, expressed as a data point estimate and confidence interval or standard deviation/error of the estimate, provides important information about the effectiveness of an intervention and the expected spread of this effectiveness within each group, which can affect the implications of the results precisely (*Examples 6a.1, 6a.2*).

Example 6a.1

From Moore *et al.* (2016) - "The mean load at failure values did not differ significantly between the Contracted endodontic cavity (1703 ±558 N; range, 1205–3021 N) and Traditional endodontic Cavity (1384 ± 377 N; range, 966–2381 N) groups but were significantly lower ($P < .05$) for both groups compared with the intact controls (2457 ± 941 N; range, 1252–3806 N)".

Example 6a.2

From Naseri *et al. (*2019) - "According to the results of this study, 1 week after intracanal medicament application, the mean microhardness values were 55.6 ±5.76 in the calcium

hydroxide (CH) group, 42.5 ±4.53 in the nano-calcium hydroxide (NCH) group, 40.6 ±7.96 in the sodium hypochlorite control (SHC) control group, and 47.4 ±5.48 in the intact control group. The mean microhardness values of dentin samples after 4 weeks were 24.0 ± 6.72, 38.2 ± 4.98, 36.7 ± 4.11, and 43.5 ± 6.13 in the CH, NCH, SHC control, and intact control groups, respectively. A significant reduction (from 55.6 to 24) was observed in the mean microhardness values of CH-treated samples (P< .05). The microhardness of the samples treated with NCH was reduced to a lesser extent (from 42.5 to 38.2), which was comparable with the reduction values of 2 control groups (from 40.6 to 36.7 in the SHC control group and from 47.4 to 43.5 in the intact control group). According to the results of the Tukey multiple comparison tests of different intracanal medicaments, there were no significant differences in the NCH group in comparison with the control groups after 1 and 4 weeks".

Item 6b: Results – Information on the loss of samples during experimentation and the reasons must be provided, if relevant

Explanation

It is never acceptable to discard samples or specimens or to exclude data. However, if a loss/contamination/lack of preservation of a sample occurs due to adverse events and/or technical problems, the reasons must be reported. It is good practice to replace lost specimens (when possible) or to repeat the experiment to compensate for the sample loss in order to maintain adequate sample size(s) (*Example 6b.1*).

Example 6b.1

From Meraji and Camilleri (2017) - "The composite resin with the self-etch primer was lost from all the Biodentine samples during thermocycling. The glass ionomer was dislodged from over the Biodentine and Theracal LC at the demolding stage, showing the weakness of both bonds.

Thermocycling also resulted in the loss of the composite using self-etch adhesive. Previous studies evaluating the bond strength of composite to Biodentine omitted the thermocycling; thus, the data obtained are questionable".

Item 6c: Results – All the statistical results, including all comparisons between groups must be provided

Explanation

The outcome of all statistical analyses, such as comparison between two experimental groups, should be reported precisely (e.g. P=0.032 rather than P<0.05) and be mentioned in either the text or tables. It is recommended that expert advice on data management and statistical analysis is obtained prior to the commencement of a study and during the development of the manuscript (*Examples 6c.1, 6c.2*).

Example 6c.1

From Scattina *et al.* (2015) - "The NCF of Protaper Next (PTN) X2 and X3 files was used for the tuning of material parameters involved in the C-S criterion. Their P values for the NCF were subsequently extremely large (P=.896 and .787, respectively). There was also

no significance in the NCF of the PTN X1 file (P= .098). Therefore, no significant statistical difference was found between the experimental NCF and the NCF predicted by finite elemental analysis (FEA)".

Example 6c.2

From Oliveira *et al*. (2020) - "The t-test did not indicate a difference between the initial (104.4 ± 8.1) and final (108.5 ± 8.6) radiopacities of the control teeth, which did not receive the material (P = 0.159). BT had a significantly lower initial radiopacity than UltraCal XS (UC) and MTA (P < 0.001), which had similar radiopacity (P = 0.97), at T1. After 30 days, all materials had different radiopacities, with the highest grey values in the UC group (vs. MTA: P = 0.045 and vs. BT: P < 0.001) and the lowest in the Bio – C Temp (BT) group (vs MTA: P < 0.002)".

Item 7a: Discussion – The relevant literature and status of the hypothesis must be described

Explanation

The discussion should focus on comparing the outcome of the investigation with a consideration of previous knowledge and the literature. Authors should try to describe both sides of arguments/hypotheses/biases by citing publications that confirm as well as contradict the results, and then try to justify which arguments/hypotheses/biases are stronger and succeed (*Examples 7a.1, 7a.2*). The repetition of information given in the

introduction should be avoided. The discussion must argue whether the results support or reject any hypotheses defined in the Introduction (*Example 7a.3*).

Example 7a.1

From Oliveira *et al*. (2020) - "The MTT results revealed that MTA and UC were not cytotoxic for human dental pulp stem cells (hDPCs) at all dilutions. MTA has been reported to induce proliferation of hDPCs by elution components such as calcium ions. The high proliferation of hDPCs at the 1: 4 dilution of extract corroborates previous studies using the MTT assay. Few researchers have evaluated the cytotoxicity of $Ca(OH)_2$ paste in the same formulation used in the present study). Previously, it was reported that UC extracts caused a significant increase in cell viability (Pires *et al*. 2016), which was not found in the present study. This could be because the referenced study used peripheral blood mononuclear cells, and $Ca(OH)_2$ has the capacity to induce an inflammatory response)".

Example 7a.2

From Mancini *et al*. (2013) - "Analyses of the 4 distances from the apex showed that the EA performed significantly better than the control groups at 5 and 8 mm from the apex and a significant increase of smear layer removal when compared with control groups and PUI at 3 mm from the apex. Similar results were described by Rodig *et al.*, who showed significantly greater smear layer removal when the EA was used rather than ultrasonic agitation and a canal brush. Conversely, these results are in contrast to those

from a recent study reporting no significant improvement of smear layer removal with the EA".

Example 7a.3

From Oliveira *et al.* (2020) - "The results support the rejection of the null hypothesis tested because significant differences were found between the materials regarding the viability of pulp cells, radiopacity and coronal discoloration in the presence of blood".

Item 7b: Discussion - The true significance of the investigation must be described

Explanation

The discussion must explain if the significance of the investigation is confirmatory of published facts, non-confirmatory of published facts, or provides novel facts that were not known previously. The scientific results must then be discussed according to their translational significance to a specific aspect of endodontic practice (*Examples 7b.1, 7b.2*); however, caution must be exercised when translating the results of laboratory-based studies to clinical practice as most do not have any direct clinical relevance. For example, it is inappropriate to suggest a root filling material would be ideal in clinical practice, when the study only evaluated the presence of voids or its radiopacity.

Example 7b.1

From Marciano *et al*. (2017) - "In the study, the spectrophotometer analysis indicated that all cements promoted color alteration for both models of study, which was more intense for MTA Angelus. Bovine teeth presented more intense color change, which can be attributed to the higher number of dentinal tubules in comparison with human teeth. The data of L indicated low values for MTA Angelus, suggesting darkening of teeth filled with MTA Angelus, which was not verified for the other groups. The discoloration of teeth was evident for MTA Angelus, with black areas close to the interface cement/dentin and marked dentin staining. The color alteration was also verified in buccal surface of the samples, suggesting that in clinical conditions MTA Angelus would promote darkening. On the contrary, the small amount of zinc oxide tested (5%) was sufficient to prevent tooth color alteration, indicating the efficacy of this substance to inhibit discoloration of MTA Angelus".

Example 7b.2

From Mullaguri *et al. (*2016) - "The results obtained from the study might have a strong clinical implication. Transforming Growth Factor- β1 (TGF- β1) has been proved to induce mineralization and osteodentin-like matrix and also plays a role in regulating the stem cells from the apical papilla. The increase in TGF- β 1 from Platelet Rich Fibrin membrane when layered with Biodentine observed in this study might improve the healing during pulp repair and regeneration of pulp".

Item 7c: Discussion - The strength(s) of the study must be described

Explanation

The Discussion should describe the unique benefits of the study: How it answered a problem, or filled a knowledge gap, e.g. this is the first evaluation of a new combination of biomaterials, devices or treatments, or this is the discovery of a new analgesic that can block receptors within the nerve signalling pathway etc. The significance of the study if it can be translated to benefit patients must be described. e.g. discuss how any ground breaking discovery can have a positive impact on endodontics and dentistry (*Examples 7c.1, 7c.2*).

Example 7c.1

From Pérez *et al.* (2020) – "One of the main purposes of the present study was to devise and propose a new correlative analytical approach that could make it possible to evaluate different effects of chemomechanical preparation in the same specimens. This study is innovative in the sense that the correlation between 2 analytical methods permits one to evaluate the main objectives of chemomechanical preparation in the same specimens (i.e., cleaning, shaping, and disinfection). Micro-CT imaging was used to assess the shaping ability of the instruments and identify areas that remained unprepared. Because micro-CT imaging cannot detect nuances of soft tissues, it was complemented with histobacteriology to evaluate the presence of pulp tissue remnants (cleaning) and residual bacterial cells or biofilms (disinfection) on the unprepared surface areas".

Example 7c.2

From Neelakantan *et al*. (2018) - “There are different paradigms within the realm of conservative access cavity (conservative endodontic access, ultraconservative ‘‘ninja’’ access, and orifice-directed ‘‘truss’’ access), and no ‘‘definitions’’ exist for each of these designs at this time. This study evaluated the orifice-directed approach. Our results indicated that the difference in debridement (or canal cleanliness) for the TEC design versus the DDC design was location dependent. Thus, the null hypothesis must be partially accepted. To the best of our knowledge, this is 1 of the first studies to address the relevance of modern endodontic access from the perspective of debridement of root canal systems.”

Item 7d: Discussion - The limitations of the study must be described

Explanation

Authors have a duty of care not to publish any errors and mistakes. On the other hand, every experiment has its inherent limitations which requires some discussion. A description of the limitations of a study identifies potential errors and allows the credibility of the conclusions to be judged (Ioannidis 2007). Authors can be hesitant to discuss the limitations of a study because they believe it might have a negative effect on the impact of their research. Clearly, laboratory investigations have environmental limitations, which prevent them from being directly extrapolated to the clinical care of patients. Therefore, it is essential to emphasise that any potential benefits that emerge from a laboratory study must be evaluated further in clinical trials, and not to over-emphasise and exaggerate the benefits to practitioners and/or patients (*Examples 7d.1, 7d.2*).

Example 7d.1

From Naseri *et al*. (2019) - "FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas. ATR can penetrate to a depth of 1–2 mm; thus, 1 of the limitations of evaluating the dentin chemical structure by FTIR is that the depth of penetration of infrared is limited. Therefore, the obtained data of the phosphate/amide I ratio are an indicator of changes in the chemical structure of superficial dentin subsequent to intracanal medicaments and do not provide information about the total thickness of the dentin".

Example 7d.2

From Rathinam *et al.* (2020) - "A limitation of the present study was the lack of a positive control group of hDPCs treated by osteogenic induction media. Future studies should include a positive control group that would enable the analysis of the differentiation data of hDPCs compared to the biomaterials".

Item 7e: Discussion - The implications for future research must be described

Explanation

The results of the study must be discussed in terms of how they can influence the future directions of similar research, and include suggestions for improving the research design to eradicate potential deficiencies (*Examples 7e.1, 7e.2*).

Example 7e.1

From Kato *et al.* (2016) - "Although this was a preliminary ex vivo study on the EC system, the results suggest that this novel final irrigation system with reciprocating activation promotes effective cleaning of the apical third. Future studies are warranted to confirm the effectiveness of the system in terms of the degree of cleansing and overall disinfection of the root canal system, including isthmus areas; to investigate possible adverse outcomes, such as the extrusion of debris or the formation of vapor lock; and to assess the possible correlation of these variables in vivo with measures of clinical success".

Example 7e.2

From Zuolo *et al.* (2017) - "It is important to stress that the crack propagation after instrumentation was not evaluated herein. However, we are aware that this is an important point, and future studies should focus on a three-dimensional longitudinal evaluation, thus allowing us to estimate the cracks propagation in a reliable way".

Item 8a: Conclusion – The rationale for the conclusion(s) must be provided

Explanation

The conclusions must accurately reflect the results of the statistical analysis (*Examples 8a.1, 8a.2*). There should never be a contradiction between the results and conclusions. e.g. if there were no significant differences amongst the groups in the results section, the

conclusion CANNOT claim that there were significant differences. The conclusions must not over-generalise laboratory results to clinical practice.

Example 8a.1

From Naseri *et al*. (2019) - "The use of CH as an intracanal medicament significantly decreases microhardness more than NCH and sodium hypochlorite after 4 weeks. The changes in microhardness were limited in the NCH group in comparison with the CH group, which suggests a possible replacement of NCH as an intracanal medicament for the preservation of intact dentin structure".

Example 8a.2

From Mancini *et al*. (2013) - "Based on the results of this study, the activation/delivering of 5.25% NaOCl at 37°C with different irrigating systems is not a currently viable technique for the consistent removal of the smear layer from endodontic walls. Nevertheless, the EV and EA showed statistically significant results at 1, 3, 5, and 8 mm and 3, 5, and 8 mm from the apex, respectively, thus showing how combinations of activation/delivery systems may help in straightforward clinical protocols".

Item 8b: Conclusion - Explicit conclusion(s) must be provided, i.e. the main "take-away" lessons

Explanation

Conclusion(s) and points of interest must be supported by the preponderance of the results combined with the outcomes of the discussion arguments/hypotheses/biases (*Examples 8b.1, 8b.2*).

Example 8b.1

From Kishen *et al*. (2018) - "In summary, this study emphasized the benefits of combining microbial quantification assays with confocal microscopy–based biofilm structural analysis for a comprehensive assessment of antibiofilm efficacies in root canals. The findings also highlighted the ability of instrumentation-syringe irrigation to achieve the maximum bacterial reduction quantitatively from the root canal lumen, whereas additional irrigation using sonic agitation of NaOCl further reduced surface adherent biofilm contents from the root canal wall".

Example 8b.2

From Rathinam *et al*. (2020) - "To the best of our knowledge, this is the first time where the RNA-Seq–based transcriptomic analysis was performed in hDPCs stimulated with TCS-based biomaterials. The results of the present study illustrate that several important signalling pathways are induced by hDPCs stimulated with TCS-based biomaterials. Despite the fact that the precise mechanisms that are involved remain largely unknown, this study has expanded our understanding systematically and provided a framework to further study the application of TCS-based therapy".

Item 9a: Funding and support - Sources of funding and other support (such as supply of drugs, equipment) as well as the role of funders must be acknowledged and described

Explanation

Details about the sponsorship, funding, or support of the research or its authors as consultants, and any potential conflicts of interest must be disclosed to the publisher, editors, and readers. (*Examples 9a.1*). Any conflicts of interest should be disclosed especially when there is sponsorship of research by a company for its own products. A conflict could be related to study design, conduct, analysis, and reporting (Lexchin *et al.* 2003, Moher *et al.* 2012). Authors should explicitly state whether the funder had any direct or indirect involvement in the conduct of study, supply of drugs and equipment, analysis of data or writing of manuscript (*Examples 9a.2, 9a.3*). The information on the person who supplied custom-made instruments, materials, chemicals, antibodies, or devices should be provided. Information on the individuals or organisations that translated or edited the manuscript should also be provided. It is important for authors to know that receiving supplies, funding, sponsorship, or consulting fees, or ownership of a dental business is not an automatic bar to publishing. But due to a severe conflict of interest, it is never acceptable to allow a sponsor who is not an author, to edit a manuscript prior to its submission for publication.

Example 9a.1

From Kishen *et al*. (2018) - "Supported by a grant from the University of Toronto (A.K.) (grant no. 928133). The authors thank Ms. Martha Cordova for technical support. The authors deny any conflicts of interest related to this study".

Example 9a.2

From Bayram *et al.* (2017) – "The authors thank FKG Dentaire for providing the XP-Endo Shaper instruments used in this study".

Example 9a.3

From Rathinam *et al.* (2020) – "The authors would like to thank the VIB Nucleomics Core (www.nucleomics.be) that performed the library preparation, sequencing, and statistical data analysis".

Item 10a: Conflict of interest - An explicit statement on conflicts of interest must be provided

Explanation

The presence of financial, commercial, legal or professional relationship of the researcher/clinician with external agencies that could influence the outcome of a study (bias) should be disclosed explicitly and reported as a conflict of interest. This is essential as any personal interests can create bias in a study (Bekelman *et al*. 2003, Romain 2015). The conflict-of-interest statement should also include relationships such as patent or

stock ownership, membership in a company, membership of an advisory board or committee within a company, and consultancy for or receipt of speaker's fees from a company. All authors of the manuscript should explicitly provide a conflict-of-interest statement, whether a conflict is present or not (*Examples 10a.1, 10a.2*). It is important for authors to know that having a conflict of interest is not an automatic bar to publishing, the most influential authors often have conflicts of interest to report.

Example 10a.1

From Rathinam *et al*. (2020) - "Dr. Govindarajan holds a post-doctoral fellowship from the Fund for Scientific Research – Flanders (FWO). The research was supported by grants from the Group-ID Multidisciplinary Platform (MRP) of Ghent University (Dr. Elewaut) and Fund for Scientific Research – Flanders (Dr. Elewaut). Dr. Rajasekahran holds a post-doctoral fellowship from the Fund for Scientific Research – Flanders (FWO). The authors would like to report research grants and non-financial support (i.e., cement materials free of charge) from Septodont, France. Outside of the submitted work, Dr. Martens and Dr. Rajasekharan report personal fees (honoraria/lecture fees/educational courses). In addition, Dr. Martens would like to report research grants from Septodont, France, negotiated with the Ghent University. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results".

Example 10a.2

From Widbiller *et al.* (2016) - "This article is based upon work partly funded by Septodont (Saint-Maur-des-Fossés, France). The authors declare that they have no conflict of interest".

Item 11a: Quality of Image - Details of the relevant equipment, software and settings used to acquire the image(s) must be described in the text or legend

Explanation

Information about the methods, equipment and software used to acquire and analyse image(s) must be provided. The information must include the make and model/version number of the equipment and its settings (e.g. Micro-CT, scanning electron micrograph etc.) (product; company, city, country), software developer and the version used for image processing (*Examples 11a.1, 11a.2*). There is no need to provide details of equipment that was not used in the methods to directly collect the results, e.g. a computer mouse.

Example 11a.1

From Oliveira *et al.* (2020) - "For radiopacity analysis of the materials, all teeth in the four groups were radiographed using the VistaScan Mini Plus_ photostimulable phosphor (PSP) system (Dürr Dental, Bietigheim-Bissingen, Germany). Each specimen was placed on the centre of a size 2 (3 X 4 cm) PSP plate along with a 10- mm aluminium step wedge. A Timex 70E X-ray unit (Gnatus, Ribeir~ao Preto, SP, Brazil) was used, operating at 70 kV, 7.0 mA, 0.14-s exposure time and 28 cm focus/film distance. After exposure, the plates

were scanned, and the 8-bit images were exported to ImageJ for Windows software (National Institutes of Health, Washington, WA, USA)".

Example 11a.2

From Moore *et al* (2016) - "A subset of 20 teeth assigned to the CEC and TEC groups was imaged with micro–computed tomography (micro-CT) (SkyScan 1172; Br€uker MicroCT, Kontich, Belgium) at 12-mm voxel size, 70- KVp beam energy, 10 frames/view, and 400-millisecond exposure, and the canals were captured (pre-treatment volumes). Mineral density was calibrated with mineral analogue rods and ReCon software (Br€uker MicroCT) used for 3-dimensional (3D) reconstruction".

Item 11b: Quality of Image – If an image(s) is included in the manuscript, the reason why the image(s) was acquired and why it is included must be provided in the text

Explanation

All images should be of the highest possible quality and be sufficient to provide the intended concepts or data. In addition, they must be accompanied by descriptive information in the text why they were included in the manuscript, that is, their purpose. Such descriptive information will improve the reader's understanding of the image and what it aims to illustrate, along with any possible limitations (*Examples 11b.1, 11b.2*).

Example 11b.1

From Tokita *et al*. (2017) - "An original cyclic fatigue test device was used comprising 3 stainless steel pins. The pins, possessing a 2-mm internal diameter, were adjusted horizontally and fixed to bend 2 mm from the tip of the instrument. The instrument curvature was set at 38º with a curvature radius of 5 mm. Silicone oil (KF-96-100CS; Shin-Etsu Chemical Co, Ltd, Tokyo, Japan) was used to reduce friction and heat generation. A load cell (LUR-A-50NSA1; Kyowa Electronic Instruments Co, Ltd, Tokyo, Japan) was fixed to 1 pin to measure the deflection load during rotation where the output of the load cell was connected to an analog-to-digital converter with a bridge box (TUSB-S01LC; Turtle Industry Co Ltd, Tsuchiura, Japan), and the output of the converter was connected to a personal computer (Vaio VGF-FE; Sony, Tokyo, Japan)".

Example 11b.2

From Pérez *et al.* (2020) – "Histobacteriologic evaluation of the 3 selected regions with unprepared walls (as identified by micro-CT imaging) showed that 30% of the main mesial canals from mandibular molars prepared with Reciproc Blue instruments were free of residual bacterial infection, and 20% had no detectable pulp tissue remnants. The apical part of the mandibular molar mesial canals were free of bacteria in 60% and pulp remnants in 50% of the specimens. Intratubular bacteria were detected in 30% of the cases. Whenever present, the isthmus between the mandibular molar mesial canals contained residual bacteria and pulp tissue remnants".

Item 11c: Quality of Image – The circumstances (conditions) under which the image(s) were viewed and evaluated by the authors must be provided in the text

Explanation

Information about the process of analysing and interpreting the images should be provided. Details about the image equipment (image resolution, size or pixel ratio) and the settings should be reported. The experience and eligibility of the examiners and their calibration training should be provided. The level of agreement can be given as inter and / or intra-rater agreement (Kappa statistic), and the method used to resolve any rater disagreements about the interpretation of images should be explained (*Examples 11c.1, 11c.2*).

Example 11c.1

From Mancini *et al.* (2013) - "Cleanliness was evaluated by micrographs taken at 1, 3, 5, and 8 mm from the apex at a 1,000X magnification. Two observers performed blind evaluation independently after examining 20 specimens for calibration purposes. Intra- and inter-examiner reliability for field emission scanning electron microscopic assessment was verified by the kappa test".

Example 11c.2

From Kato *et al.* (2016) – "Each image obtained was coded according to the group (negative control, blank control, PUI group, or EC group), the tooth (from 1 to 10), and the level at which the reading was taken (L1, L2, L3, L4, L5, or L6). All of the images from the control and experimental groups for the same level were loaded into the Microsoft Office PowerPoint application (Microsoft Corporation, Redmond, WA) and displayed in slide format on an LCD monitor. Two independent examiners, previously calibrated and

blind to the study, scored the images according to the assessment criteria outlined previously. The level of inter-examiner agreement was determined using the kappa test".

Item 11d: Quality of Image – The resolution and any magnification of the image(s) or any modifications/ enhancements (e.g. brightness, image smoothing, staining etc.) that were carried out must be described in the text or legend

Explanation

Details about the editing of images, including resolution enhancement and magnification should be provided in the image legend or text (*Examples 11d.1, 11d.2*). A scale bar should be provided inside the images if relevant. The entire image can be modified or enhanced by editing, but should not distort or enhance a particular part of the image leading to misinterpretation by readers (Lang *et al.* 2012). Authors must disclose the source of image(s) and/or data if it was prepared by someone other than the authors. Authors must obtain copyright permission to reproduce any data or images that were published in the past.

Example 11d.1

From Kishen *et al*. (2018) - "The specimens were serially dehydrated using ethanol; critical point dried in a critical point drier (CPD030; BalTec, Balzers, Liechtenstein); sputter coated with palladium (SCD005, BalTec); and examined using scanning electron microscope (SEM) at magnifications of 1000X, 3000X, and 5000X. Each sample was imaged at 9 random locations along the root canal lumen".

Example 11d.2

From Mavridou *et al.* (2016) - "The teeth were dehydrated in a graded series of ethanol concentrations (70%, 80%, 90%, 97%, and 100%) and then embedded in methylmethacrylate. The histologic sections were prepared in a transaxial, sagittal, or coronal direction according to the plane of interest (as defined previously by nano-CT imaging) using a rotary microtome and a diamond saw. The sections were evaluated using a DM2500 M Leica microscope with a digital high-definition camera (Leica DFC295) by means of the Leica Application Suite color image software. Overview and detailed images were obtained using a magnification up to 63. By using Application Suite LAS software, multiple histologic images were stitched Leica Suite together to reconstruct the whole histologic section".

Item 11e: Quality of Image – An interpretation of the findings (meaning and implications) from the image (s) must be provided in the text

Explanation

Comprehensive information about the results obtained from an image, through its assessment, interpretation, relevance and meaning, must be provided (*Examples 11e.1, 11e.2*).

Example 11e.1

From Widbiller *et al.* (2015) – "SEM imaging showed the typical morphology of tricalcium silicate cement. As a product of gelation, calcium hydroxide particles were present on the surface, which reportedly play a key role in dentinogenesis in vivo. Dental pulp stem cells adhered directly to the surface and extended processes, affirming the cytocompatibility of tricalcium silicate cement".

Example 11e.2

From Kishen *et al.* (2018) – "In our study, CLSM revealed a higher bacterial biomass and viable bacteria in controls, which decreased markedly in the instrumentation-syringe irrigation group. Additional sonic agitation of NaOCl resulted in an additional reduction in the residual volume and number of live bacteria from the root canal walls".

Item 11f: Quality of Image – The legend associated with each image must describe clearly what the subject is and what specific feature(s) it illustrates

Explanation

Legends for images should describe what anatomical and pathological features are shown within the image, as well as measurements, magnifications, and radiographic view. e.g. subject age, sex, history, tooth number, type of radiographic view, resorption, wear, periapical lesion, plaque, treatment type, working length, and distance from apex etc. The use of these terms should be self-explanatory and comprehensive (*Examples 11f.1, 11f.2*).

Example 11f.1

From Chan *et al.* (2019) - “Validation of the method used in this study shown by the correlation between micro-CT and scanning electron microscopic images regarding the presence of hard tissue debris within the mesial root canal system. (A) A representative 3-dimensional model of a mesial root canal system after experimental procedures depicting the presence of AHTD (in black), (B) the axial cross-sectional slice at 9.9 mm from the apex of the representative specimen, and (C–E) corresponding scanning electron microscopic images of the same slice in B at different magnifications (35X ,150X and 250X, respectively)”.

Example 11f.2

From Mavridou *et al.* (2016) - “External Cervical Resorption of tooth #6. (A) Radiographic view. (B) Clinical view showing signs of pathological tooth wear and plaque accumulation. (C) Macroscopic view after extraction. (D) Enlargement at the portal of entry. (E) coronal, sagittal, and transaxial nano-CT images of tooth #6 showing the ingrowth and apposition of reparative bonelike tissue through the portal of entry, resorption channels, and the pericanalar resorption resistant sheath. The interface between resorption and repair is clearly visible as a radiopacity shift (white arrows)”.

Item 11g: Quality of Image – Markers/labels must be used to identify the key information in the image(s) and defined in the legend

Explanation

Marker labels, or index keys (a, b, c etc) with arrows should be used to identify important information within the images and explained in the legend (*Example 11g.1*). The marker labels and arrows should not mask or obscure important features of the image (e.g. apex or coronal access), rendering it difficult to understand. The legend and image should be self-explanatory without the aid of the corresponding text in the manuscript (Lang *et al.* 2012).

Example 11g.1

From Kishen *et al*. (2018) – Figure 1: Arrows have been used to identify key information in the image and details are mentioned in the legend.

Item 11h: Quality of Image - If relevant, the legend of each image must include an explanation whether it is pre-experiment, intra-experiment or post-experiment and, if relevant, how images over time were standardised

Explanation

The information about the experimental stage of the image (i.e. pre-experiment, intra-experiment or post-experiment) as well as the time, measurements, treatment, instruments or materials seen within each of the sequential images to allow comparisons must be described (*Examples 11h.1, 11h.2*).

Example 11h.1

From Kishen *et al.* (2018) – "Confocal laser scanning microscopic representative images of the root canal lumen before and after different irrigation protocols. Bacterial biomass and viable cells were higher in the (A) no treatment group and (B) group 3 (distilled water). Group 1 (syringe disinfection) and group 2 (with added sonic agitation) showed (C)markedly decreased biofilm volume and (D) viable bacteria. The biofilm thickness of the no treatment control group and group3were in (E) a range of 56–90 mm, which significantly decreased after the NaOCl disinfection protocols to (F) 10–30 um ($P < .05$)".

Example 11h.2

From Zuolo *et al.* (2017) – "Representative cross-section images of 4 mandibular incisors showing the presence of dentinal micro-cracks (yellow arrows) before and after root canal preparation with TRUShape, BioRace, self-adjusting file, and Reciproc systems".

PRILE 2021 flowchart

Explanation

The PRILE 2021 flowchart summarises the steps to be followed by researchers during the performance of laboratory studies. The flowchart can be customized and populated with the information that needs to be included in the submission of the manuscript. The PRILE 2021 flowchart is freely accessible and downloadable from the 'Preferred Reporting Items for study Designs in Endodontology (PRIDE) website':

http://pride-endodonticguidelines.org/prile/.

Example

Figure 2: PRILE 2021 flowchart explains the steps involved in conducting laboratory studies.

Discussion

Manuscript peer-review rejection is an occupational hazard for all authors. Guidance to authors is needed to prevent their waste of talent, time and resources in writing manuscripts that will never be published in the highest-quality journals. Here, we strongly recommend that authors apply the PRILE 2021 flowchart and guidelines during the preparation of manuscripts for publication.

Laboratory research present its own challenges compared to clinical research. The preparation of specimens and calibration of methods to be used in laboratory research can take a significant amount of time and resources to create. It is also a mistake to believe that laboratory research is not expensive specially when newer materials are developed or cutting-edge equipment are used. Some samples and equipment require labour-intensive monitoring over long hours (cells, biofilms, molecular techniques, etc) and specialized staff. Thus, proper planning and a clear rationale/justification and testing an hypothesis are important to achieve a successful publication.

This PRILE Explanation and Elaboration document supports the PRILE 2021 guidelines by enhancing their comprehension, adoption, and dissemination. The examples provided in this document are derived from actual studies. The PRILE

guidelines provide a structure for authors to follow when preparing manuscripts reporting laboratory studies in Endodontology and are similar to other published guidelines, e.g. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Liberati *et al*. 2009) and Preferred Reporting Items for RAndomized Trials in Endodontics (PRIRATE) 2020 (Nagendrababu *et al.* 2020).

Fifty percent of the content of articles in the field of Biology have been reported to be related to images/figures (Futrelle *et al.* 2004). Indeed, authors invariably use images to summarise and describe their findings visually. Thus, the images included in research articles form an important source of information and evidence for the reader (Kotz *et al.* 2013, Polepalli Ramesh *et al*. 2015). As a consequence, several items in the PRILE 2021 checklist are related to images in the expectation the advice will result in an improvement in the use of images, their quality and their description within reports.

It has been reported that quality of reporting of randomized controlled trials and systematic reviews (Egger *et al*. 2001, Vu-Ngoc *et al.* 2018) has been enhanced by incorporating flowcharts within manuscripts. The completeness of a flowchart is important to assist readers to critically appraise the external and internal validity of trials (Egger *et al.* 2001). Similarly, in a systematic review, a well-constructed flowchart helps readers understand the sequence and flow of the review process and allows them to assess sources of bias (Vu-Ngoc *et al.* 2018). Those reviews that included a flowchart have been reported to be of higher quality than those that did not (Vu-Ngoc *et al.* 2018). The various stages in the preparation of reports describing laboratory studies in Endodontology have been captured in a flowchart within the PRILE 2021 guidelines and are expected to bring similar benefits to readers.

When authors submit manuscripts that have been developed using the PRILE 2021 guidelines, they should include the phrase - ‘‘This laboratory study was prepared according to the PRILE 2021 Guidelines’’ and include a reference to the PRILE 2021 consensus publication (Nagendrababu *et al.* 2021). This ensures that editors and readers of the laboratory-based research report are aware that the guidelines have been followed. The potential increase in word counts in manuscripts that follow the PRILE 2021 guidelines is offset by the advantages they confer. The adoption of the PRILE 2021 guidelines by journals in their ‘‘Instructions to Authors’’ will ensure that the maximum benefit is achieved. This will inevitably mean that journal editors will need to revise their submission criteria to accommodate larger word counts in structured (with headings) abstracts and the main text.

The PRILE 2021 checklist has a logical sequential structure from 1 to 11 of the key domains. The sequence can be followed as described or altered to accommodate the structure and context of any particular study. The key issue is the inclusion of all the required information rather than the numerical sequence of the items within each section/topic.

Conclusion

The PRILE 2021 guidelines checklist and flowchart, provides advice from peer-reviewers and editors about how to solve specific problems that can arise in manuscripts submitted for publication. In addition, the present document provides examples of good reporting

practice from published laboratory studies to explain to authors how they can be more successful at writing and publishing higher-quality research.

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Legends

Figure 1: Arrows (Open/closed arrow) have been used to identify the key information in the image. Legends from Kishen *et al.* (2018) - "Surface topography of the bacterial biofilm on the root dentin surface was evaluated using SEM. (A–C) Scanning electron microscopic images of 3-weekold E. faecalis biofilms on the root canal dentin surface. The biofilms presented as a uniformly thick mat like structure covering the entire dentin surface. The biofilm showed an abundant polymeric matrix (area shown by the open arrow). (D–F) Conventional cleaning and shaping and syringe irrigation resulted in the disruption of the biofilm. Areas of (E) clean and open dentinal tubules as well as with the (F) remaining biofilm were observed (closed arrow). (G–I) Sonic agitation in addition to conventional disinfection resulted in a cleaner dentin surface. Aggregates of disrupted bacteria and EPSs were found on the dentin surface". Reprinted from *Journal of Endodontics*, Vol 44, Kishen A, Shrestha A, Del Carpio-Perochena A. Validation of Biofilm Assays to Assess Antibiofilm Efficacy in Instrumented Root Canals after Syringe Irrigation and Sonic Agitation. Pages No. 292-80, Copyright (2018) with permission from Elsevier.

Figure 2: PRILE 2021 flowchart [Adapted from: Boutsioukis C, Psimma Z, Kastrinakis E (2014) The effect of flow rate and agitation technique on irrigant extrusion *ex vivo*. *International Endodontic Journal* **47**, 487-96.

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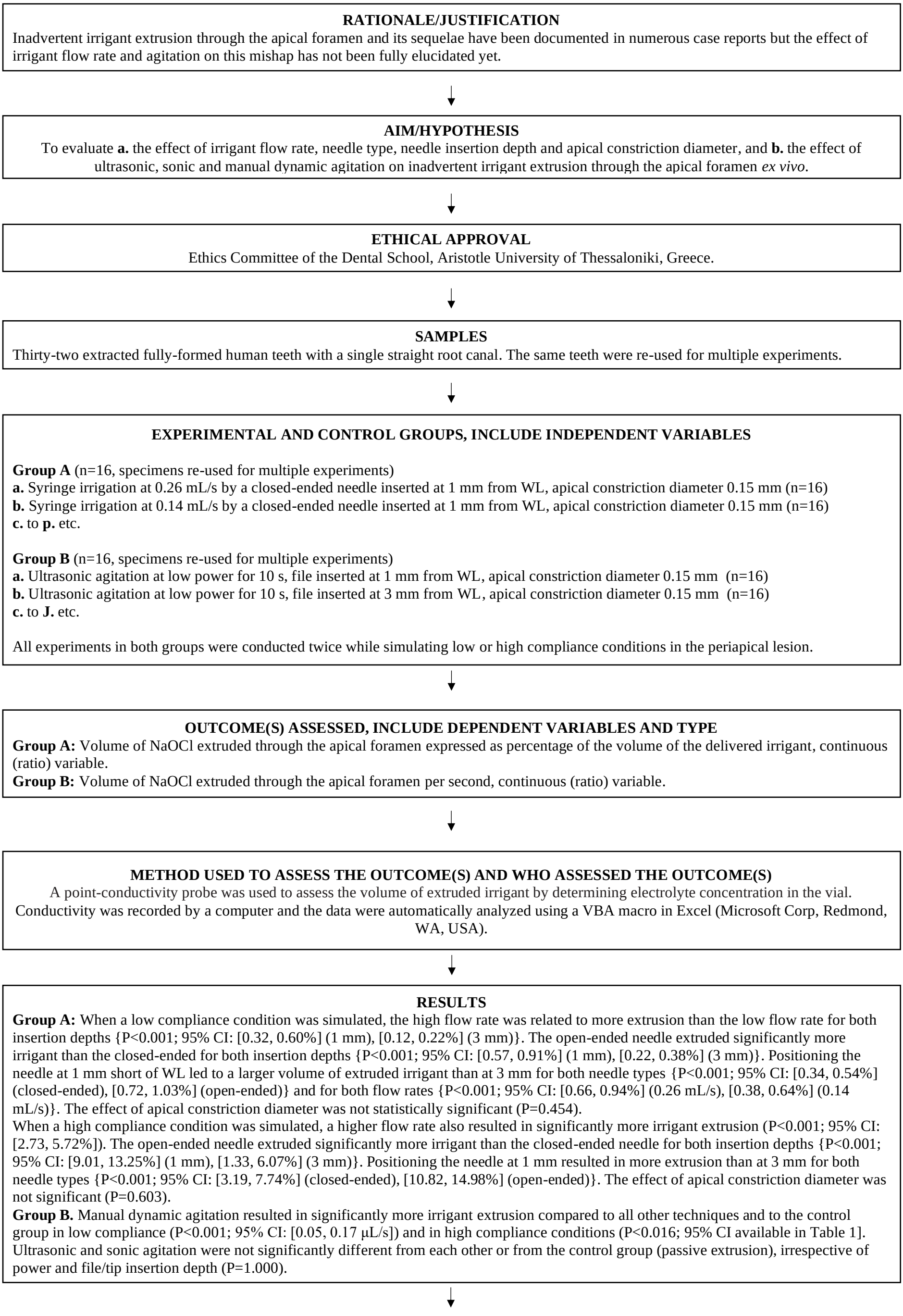
Figure 2: PRILE 2021 flowchart [**Adapted from:** Boutsioukis C, Psimma Z, Kastrinakis E (2014) The effect of flow rate and agitation technique on irrigant extrusion *ex vivo*. *International Endodontic Journal* **47**, 487-96.]



CONCLUSIONS

An increase in the irrigant flow rate resulted in increased irrigant extrusion. The open-ended needle extruded more irrigant than the closed-ended needle. Irrigant extrusion decreased as needles moved away from WL. The effect of apical constriction diameter was not significant. Manual dynamic agitation extruded significantly more irrigant than ultrasonic or sonic agitation. No significant difference was found between the various ultrasonic or sonic agitation protocols tested.

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CONFLICT OF INTEREST

The authors deny any conflicts of interest related to this study.