PROTOCOL

Full/long title of the study

A randomised controlled pilot study to determine the effect of irrigation techniques used to enhance the release of endogenous signalling molecules from dentine matrix to treat apical periodontitis.

Short study title/acronym

Therapeutic Irrigation Procedures to Treat Apical Periodontitis (TIPTAP)

Protocol version number and date

- Project Version 2.0

Research reference numbers

IRAS Number	253012
Sponsor reference number	RG_19-062
ISRCTN number	
REC reference number	

This protocol has regard for the HRA guidance

Signature page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to adhere to the signed University of Birmingham's Sponsorship CI declaration.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:

Signature: ..... Date: ...../...../.....

Name:.....

Sponsor statement:

Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the IRAS form by the sponsor will serve as confirmation of approval of this protocol.

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Study summary

Title

A randomised controlled pilot study to determine the effect of irrigation techniques used to enhance the release of endogenous signalling molecules from dentine matrix to treat apical periodontitis.

Short Title

Therapeutic Irrigation Procedures to Treat Apical Periodontitis (TIPTAP)

Study Design

Parallel group randomised controlled pilot study

Objectives

1. Determine the clinical effect of using irrigation techniques to enhance the release of endogenous signalling molecules from dentine matrix in order to treat apical periodontitis.

2. Determine the change in profile of inflammatory markers as sampled from apical lesions during conventional non-surgical root canal treatment (NSRCT) and NSRCT using irrigation techniques to enhance the release of dentine Extracellular Matrix Components (dEMCs).

Population Studied

Inclusion Criteria:

- Patients diagnosed with apical periodontitis
- Single rooted permanent teeth
- Medically fit
- Adult patients (≥ 18)
- Voluntarily consent to partake in the study

Exclusion Criteria:

- Teeth in sextants with active periodontal disease (i.e. pocketing of ≤ 5 mm)
- Tooth unable to retain a rubber dam
- Teeth that have had previous endodontic treatment
- Root apex in close proximity to the maxillary sinus

- Patients who have had antimicrobial therapy within 3 months prior to the screening clinic
- Pregnant or breastfeeding women
- Do not have capacity to consent
- Patients that have systemic condition that would reduce immune function

Sample Size

Up to 40 patients with apical periodontitis will be enrolled in this study across 2 groups

Parallel Groups

Group 1: Two visit non-surgical root canal therapy (NSRCT) with conventional irrigation techniques (control arm)

Group 2: Two visit NSRCT with irrigation protocol to optimise release of soluble dentine extracellular matrix components (dEMCs) (intervention arm)

Outcome Measures

Primary Outcome Measure: Success Rate

- To analyse if the modified release of dECMs improve treatment success rates. Clinical/radiographic information (i.e. absence of pain and resolution of swelling, pain on percussion / palpation and reduction in size of periradicular lesion) will be collected at baseline and again at a 12 month follow-up. Treatment success will be determined based on criteria outlined by the European Society of Endodontology (ESE) Quality Guidelines for NSRCT (2006). In these criteria, outcomes are defined as being "favourable" (absence of pain, swelling and other symptoms, no sinus tract, no loss of function and radiological evidence of a complete healing), "uncertain" (absence of pain, swelling and other symptoms, no sinus tract, no loss of function and radiographic evidence of some healing) and finally "unfavourable" (tooth associated with clinical signs and symptoms of infection such as pain and swelling, sinus tract, loss of function and no radiographic evidence of healing. Comparisons in the success rate in the test and control groups will then be made to identify if there is any significant difference.

Secondary Outcome Measure: Profile of periradicular inflammatory mediators

- To analyse inflammatory mediator activity. Periradicular tissue fluid will be retrieved from the peri-radicular tissues through the root canal with a paper point and the concentration of various inflammatory mediators will be quantified via a multiplex bead-based assay technique.

Follow Up Period

12 months

Planned Study Period

24 - 30 months. 12 - 18 months for recruitment and then another 12 months for follow up and data analysis.

Funding and support in kind

FUNDER(S)	FINANCIAL AND NON FINANCIAL SUPPORT GIVEN
British Endodontic Society	£20 000

Role of study sponsor and funder

The University of Birmingham in the role of the sponsor will have oversight of the project. The funder, British Endodontic Society, will provide funding for the completion of the proposed study.

Roles and responsibilities of study management committees/groups and individuals

All members of the research team will work together to deliver the project. The individual roles/responsibilities of individual members are listed below:

Dr. Phillip Leo Tomson (PLT): As the Chief/Principal Investigator, PLT will take overall responsibility for the conduct of the project and provide effective supervision to SSV throughout the duration of the study. PLT will ensure the project is conducted in compliance with applicable laws and regulations and institutional policy governing the conduct of sponsored research. He will also be readily available to communicate with the Research Ethics Committee (REC) and other review bodies during the application process and where necessary during the conduct of the research. For the actual study, PLT will be involved in the recruitment of study participants, consenting eligible participants, collection of clinical/radiographic information at baseline / follow-up, collection of tissue fluid, performing treatment interventions, statistical analysis and dissemination of results..

Mr. Satnam Singh Virdee (SSV): As the PhD student, SSV will take responsibility for the day-to-day coordination of the project within appropriate safety standards and quality control parameters: he will be involved in recruitment of study participants, consenting eligible participants, collection of clinical/radiographic information at baseline / follow-up, performing treatment interventions, collection of PTF samples, conduction of assays in the laboratory, statistical analysis, data management, dissemination of results and meeting the reported requirements.

Dr. Josette Camilleri (JC): As a co-investigator, JC will be involved in the recruitment of study participants, consenting eligible participants, collection of clinical/radiographic information at baseline / follow-up, collection of tissue fluid, performing treatment interventions, statistical analysis and dissemination of results.

Professor Paul Cooper (PC): As a co-investigator, PC will be involved in overseeing the scientific laboratory analysis of samples, statistical analysis and dissemination of results.

Dr. Naomi Hubber (NH): As a co-investigator, NH will liaise with the team for ethical considerations throughout the course of the study.

Protocol contributors

The protocol has been created by the research team.

Key Words

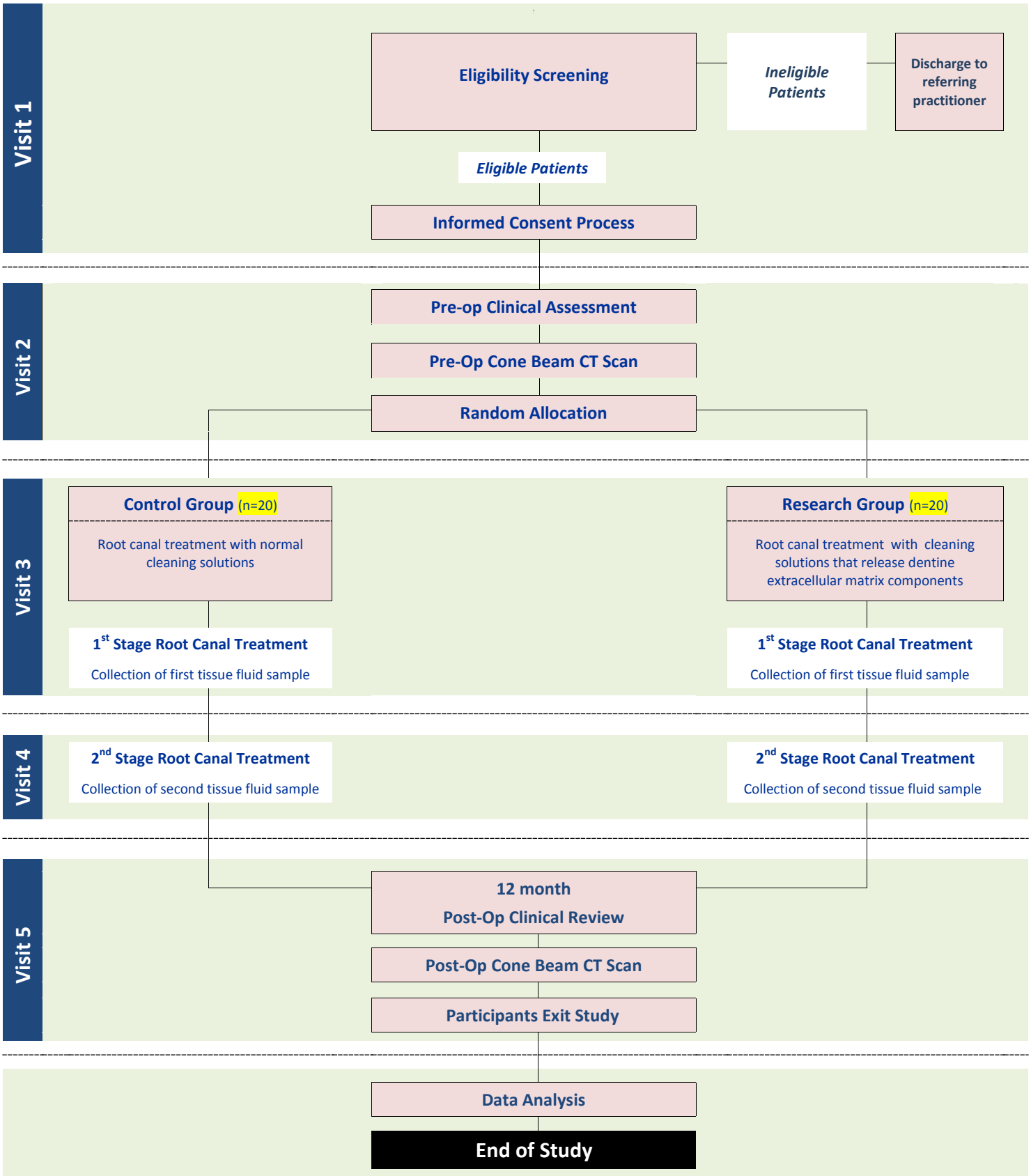
- Endodontics

- Dentine extracellular matrix components
- Growth factors
- Apical periodontitis
- Cytokines
- Periradicular tissue fluid
- Inflammation

Patient Pathway

Participants diagnosed with apical periodontitis will be initially identified and made aware of the study by a member of their direct care team on a routine consultation clinic held at the Birmingham Dental Hospital. Patients who express interest in the project will be given a patient information sheet (PIS) and referred (via letter or secure email of a signed reply slip containing the patients contact information) to a research screening clinic held at the Birmingham Dental Hospital. Here, a GCP trained clinical member of the research team will discuss the study with the potential participant, answer any questions and confirm that they are eligible against the predetermined inclusion/exclusion criteria. Those who are eligible for inclusion will then be consented by the GCP trained research member and subsequently booked onto 2 treatment sessions where they will be randomly allocated to receive either standard care (NSRCT with conventional irrigation techniques), or experimental care (NSRCT with irrigation protocol to optimise release of soluble dEMCs). The 2 appointments will be approximately 14 days apart and the patient will have to attend a 12 month review appointment for assessment and once completed they exit the study. These timelines comply with the routine care pathway of patients receiving treatment for apical periodontitis at the Birmingham Dental Hospital and are consistent with ESE 2006 quality guidelines so will place no extra burden on them or the treatment site.

Study flow chart


Visit 1
Eligibility Screening
Ineligible Patients
Discharge to referring practitioner
Eligible Patients
Informed Consent Process
Visit 2
Pre-op Clinical Assessment
Pre-Op Cone Beam CT Scan
Random Allocation
Visit 3
Control Group (n=20)
Root canal treatment with normal cleaning solutions
Research Group (n=20)
Root canal treatment with cleaning solutions that release dentine extracellular matrix components
1st Stage Root Canal Treatment
Collection of first tissue fluid sample
1st Stage Root Canal Treatment
Collection of first tissue fluid sample
Visit 4
2nd Stage Root Canal Treatment
Collection of second tissue fluid sample
2nd Stage Root Canal Treatment
Collection of second tissue fluid sample
Visit 5
12 month
Post-Op Clinical Review
Post-Op Cone Beam CT Scan
Participants Exit Study
Data Analysis
End of Study

Study Protocol

A randomised controlled pilot study to determine the effect of irrigation techniques used to enhance the release of endogenous signalling molecules from dentine matrix to treat apical periodontitis.

Background

Apical periodontitis is a diagnostic term used to describe a localised inflammatory reaction caused by interactions between the host's immune response, and pathogenic bacterial communities within the root canal of an infected tooth (Kakehashi et al., 1965; Nair 1997). Its prevalence within the UK is documented to be between 4.1% and 5.8% however, in poorly root treated teeth, its occurrence is much higher with instances ranging from 38.3% to 47.4% (Di Fillipo et al., 2014, Dutta et al., 2014). At a molecular level this inflammatory process, which clinically results in bone resorption with mild to severe pain, is regulated by a myriad of pro and anti-inflammatory signalling molecules (Metzger et al., 2003). Collectively these mediators result in regulatory immune responses that serve destructive and protective functions which respectively possess the ability to control the progression of the inflammatory lesion or if favourable conditions exist, repair and regenerate healthy tissue architecture (Marton & Kiss, 2014). These molecular changes orchestrate the inflammatory process and precede any clinical symptoms (Marton & Kiss, 2014).

Current strategies for treating apical periodontitis focus exclusively on eliminating microorganisms entrenched within the root canal space. This is typically achieved via endodontic treatment where a combination of bactericidal irrigating solutions and mechanical instrumentation to disinfect the canal and reduce the microbial load (ESE 2006). Conventionally, sodium hypochlorite (NaOCl) has been the irrigant of choice, largely due to its ability to dissolve organic tissue and its efficacy against a broad range of microbiota (Haapasalo et al., 2010). Ethylenediaminetetraacetic acid (EDTA) is also commonly used in conjunction with NaOCl due to its ability to break down the inorganic tissue and debris that is produced from instrumenting the root canal (Gulabivala et al., 2005). The resultant disinfection alters the dynamic equilibrium between bacteria and host's immune components to favour the latter, which permits healing. Although the equipment available to chemically and mechanically disinfect the canal has improved radically in the last 20-30 years (Tomson & Simon 2016), following such an ideology has only produced complete healing in 83% of cases when primary root canal treatment is undertaken and 80% of cases when teeth are retreated (Ng et al., 2011). This static nature of endodontic success rates over time has placed a significant burden on secondary care services as evidenced by rises in referrals (Al-Haboubi et al., 2014), extensive waiting lists and avoidable use of antimicrobials due to the difficulty of accessing such services (Segura-Egea et al., 2017).

For many years, it has been known that numerous bioactive molecules (dECMs), which are typically involved in wound healing processes such as proliferation and bone formation, are sequestered within the structure of the dentine (Smith et al., 2010). A commonly used chelating agent, EDTA irrigant solution, has been shown to release a complex cocktail of these soluble dEMCs (Graham et al., 2006, Tomson et al., 2007, Tomson et al., 2013). These liberated dECMs have gone onto show significant antibacterial properties in vitro (Smith et al., 2012) and the ability to induce the functional processes of dental tissue repair (Tomson et al 2017). Therefore, the release of these naturally occurring signalling molecules during NSRCT could modulate the localised inflammatory process and potentially improve success rates for the treatment of apical periodontitis, a concept which has not yet been investigated. *In vitro* experiments conducted by our group have optimised irrigation protocols aimed at releasing dECMs which is different to conventional treatment so they are made bioavailable and can interact with inflamed periradicular issues during NSRCT. We hypothesise this intervention could potentially promote a reduction in inflammation, improve healing and lead to more favourable outcomes for patients suffering from apical periodontitis.

The primary aim of this study is to identify if any difference in clinical/radiographic signs of periradicular healing exist when control and intervention arms are compared that may suggest or otherwise that irrigation procedures aimed at enhancing the release of dEMCs improve treatment outcomes. Secondly, we aim to determine if the

adjunctive release of dEMCs during NSRCT modifies periradicular inflammatory mediator activity in comparison to that of conventional treatment, for teeth diagnosed with apical periodontitis. This study will not only help us to understand the process of healing following treatment of apical periodontitis at a molecular level, but also help us explore if there is therapeutic potential in enhancing dEMC release during root canal therapy to develop more biologically based treatment approaches to manage endodontic disease.

Rationale

Our research group hypothesises that when compared to conventional NSRCT, the release of dEMCs will be associated with:

i) An increased rate of clinical/radiographic periradicular healing

ii) Modified inflammatory mediator activity

Theoretical framework

The general aim of the proposed study is to evaluate the impact of clinical intervention which aims at enhancing the release of dEMCs during root canal treatment which is proposed may modulate the inflammatory process to enhance healing. Understanding how the signalling molecules released in soluble dEMCs interact with the periradicular inflammatory lesion at a molecular level could lead to the development of more biologically driven treatment strategies which improve success rates in patients suffering from periradicular disease. By studying the clinically relevant wound healing properties of specific endogenous dECMs, novel intracanal medicaments, irrigants and sealers could also be developed and be used as adjuncts to conventional root canal treatment. Furthermore, studying specific inflammatory mediators within the apical lesion will improve understanding of their role in the development and repair mechanisms of periradicular lesions and could inform future research on which periradicular cytokines to target as well as how to retrieve and analyse them.

Research question/aims

1. Compared to routine care, does release of dEMCs from root dentine improve clinical outcomes of teeth with apical periodontitis?

2. Compared to routine care, does enhancing the release of dEMCs from root dentine modify the inflammatory process to promote healing of apical periodontitis?

Objectives

The key objectives of this research are:

- Determine if NSRCT that adopts irrigation protocols to optimise release of soluble dEMCs (Compared to conventional irrigation protocols) can improve clinical outcomes in teeth with apical periodontitis.

- Establish a profile of key inflammatory mediators during active disease (prior to any significant intervention) and be able to compare the effects following conventional NSRCT (control arm) and NSRCT adopting irrigation protocols that optimise release of soluble dEMCs (intervention arm).

Outcome

i) Primary Outcome:

- Success Rate

To analyse if the release of dECMs from the root canal improves the patient outcome following treatment the following clinical and radiographic parameters will be recorded prior to treatment, after one treatment intervention and after one year

- Clinical – presence of pain (recorded using a visual analogue scale), swelling, sinus tract, pain on palpation, pain on percussion or mastication,
- Radiographic – presence and size of lesion

Treatment success will then be determined based on criteria outlined by the European Society of Endodontology (ESE) Quality Guidelines for NSRCT (2006). In these criteria, outcomes are defined as being "favourable" (absence of pain, swelling and other symptoms, no sinus tract, no loss of function and radiological evidence of a complete healing), "uncertain" (absence of pain, swelling and other symptoms, no sinus tract, no loss of function and radiographic evidence of some healing) and finally "unfavourable" (tooth associated with clinical signs and symptoms of infection such as pain and swelling, sinus tract, loss of function and no radiographic evidence of healing). Comparisons in the success rate in the test and control groups will then be made to identify if there is any significant difference.

ii) Secondary Outcomes

- Determine profile of key inflammatory mediators in the presence of active disease and following treatment

To establish changes in the concentration of periradicular inflammatory mediators, periradicular tissue fluid (PTF) will be retrieved from the apical tissues associated with teeth at the start of and following NSRCT. This will be achieved by placing a paper point 2 mm beyond the end of the root canal for 60 seconds in a dry canal. The baseline sample will be taken on the first treatment visit prior to any intervention and then the second taken from the same tooth on the second treatment visit approximately 14 days later. The volume of extracted PTF will be determined using a wetted length to volume standard curve (Shimauchi et al., 1996). Once sampled, the PTF will be immersed in elution buffer and processed on clinic by vortexing for 60 seconds and then stored in a – 80^{o}C freezer at the Birmingham Dental School Research Lab. When ready to analyse, samples will be thawed at room temperature and the supernatant will undergo proteomic multiplex analysis to evaluate the concentration of a broad spectrum of inflammatory mediators, which will be represented in pg/ml. Through in vitro investigation our research group has optimised the sampling and elution procedure so that the maximum amount of PTF is absorbed onto the paper point, and the maximum amounts of analytes are recovered during elution.

Study design and methods of data collection and data analysis

5.1 Study Design

This pilot study has been designed as a parallel group randomised clinical trial design to compare the outcomes of standard care (NSRCT with a conventional irrigation technique) against the experimental intervention (NSRCT with an irrigation protocol to optimise release of soluble dEMCs). To generate preliminary data a total sample size of up to 40 participants, with up to 20 per group, will be required.

5.1.1 Number of Participant

As the proposed analysis has not been previously reported, a power calculation to determine a statistically appropriate sample size cannot be performed. The proposed sample size (up to 40) is therefore based on previous studies which have analysed the changes in concentrations of various periradicular inflammatory mediators during NSRCT.

5.1.2 Study Timeline

From the point of attaining ethical approval, the proposed research is expected to take 30-36 months to complete. The timeline (in months) of each individual stage is depicted in Figure 1.

	Time Scale - Months									
	3	6	9	12	15	18	21	24	27	30
Pilot Study										
Patient Recruitment										
Randomisation & Allocation										
Delivery of Treatment										
Molecular Analysis										
12 Month Review										
Data Analysis										
Dissemination										

Fig. 1 – Planned time scale for each stage of the proposed research project

5.1.3 Study Procedures

All suitable participants that have volunteered to take part in this study will undergo 2 visits for NSRCT, each appointment being approximately 14 days apart. The treatment of patients in both groups follows conventional treatment protocols apart from the irrigant regime used to optimise release of dEMCs. Briefly on the first visit, the tooth will be anaesthetised with local anaesthetic and isolated with a rubber dam. Using cooled diamond burs, the root canal will then be accessed and shaped using rotary nickel-titanium files after the root length has been determined using an electronic apex locator in conjunction with a radiograph. In the control group, a conventional irrigant regime which does not release dentine matrix growth factors will be used whereas in the test group, a novel regime which has been optimised by our group to release dEMCs will be employed. The irrigants used in both groups are widely used solutions in endodontic therapy (Haapasalo et al., 2010) however, it is the timings at which these irrigants are used during root canal therapy which will allow the release of dentine matrix growth factors. Once these stages have been completed, a temporary filling will be placed onto the tooth and the patient recalled for the second visit approximately 14 days later. On the second visit, the temporary filling will be removed and the after the root canal filled followed by a permanent restoration to prevent bacterial ingress.

5.1.4 Blinding

All irrigants used are clear fluids and are administered into the root canal using the same method. Therefore the operators (who will carry out the treatment) will be blinded to the irrigants being used during the experiment. The assessors (who will look at the results) will also be blinded once the data has been collected. In the case of a adverse event (i.e. inter-appointment flare up) the patient will be unblinded and the appropriate treatment provided.

5.1.5 Randomisation & Allocation

A third party provider "Sealed Envelope", which provides its services free to PhD students, will be used to provide a web based randomisation service with a telephone as back up. Once eligibility criteria have been confirmed, consent is obtained and stratification variables determined through clinical/radiographic examination, the

randomisation can be performed. The allocation of participants into either control arm or intervention arm will be concealed to the operator until all outcome measures have been collected.

To ensure the test and control groups are matched and chance imbalances are avoided in important stratification variables, a computer based "minimisation" algorithm will be used. The variables chosen are:

- Age (18 - 30 / 31 - 50 / > 51)
- Gender
- Ethnicity
- Size of apical lesion (1 - 5 mm / > 6 mm)

The procedures for randomisation will be fully documented and piloted prior to the start of the trial.

5.2 Data Collection

i) Profile of inflammatory mediators

A recent literature review conducted by our group has revealed the most common approach for sampling and analysing the concentrations of periradicular inflammatory mediators during NSRCT is using paper points and proteomic molecular analysis techniques respectively (Virdee *et al.* 2019). Through in vitro experimentation, our group has optimised the sampling and elution procedure so that the maximum amount of PTF is absorbed onto the paper point, and the maximum amount of cytokine is recovered during elution. Briefly, this will be passively achieved by placing an endodontic absorbent paper point (Panadent, Kent, UK) 2 mm past the apex for 60 seconds in a dry canal. The baseline sample will be taken on the first treatment visit prior to any intervention and then the second taken from the same tooth on the second treatment visit approximately 14 days later. The volume of extracted PTF will be determined using a wetted length to volume standard curve (Shimauchi et al., 1996). Once sampled, the PTF will be immersed in elution buffer and processed on clinic by vortexing for 60 seconds and then stored in a – 80^{o}C freezer at the Birmingham Dental School Research Laboratory (HTA licence – 12236). When ready to analyse, samples will be thawed at room temperature and the supernatant extracted and will undergo proteomic multiplex analysis to evaluate the concentration of a broad spectrum of inflammatory mediators.

ii) Clinical information

The following clinical information will be collected:

History	Presenting complaint, pain history, pain score	
Clinical findings	**Visual**	Condition of the hard tissue, presence of swelling and sinus
	Symptoms	Tenderness to percussion, palpation, mastication
	Periodontal	Periodontal pocket depth, mobility score
Special tests	Vitality of tooth	
Radiographic findings	Presence and size of radiographic lesion	

This data will be collected on a case report form (CRF) at the pre-operative appointment and the 12 month review by the chief or principle investigator.

5.3 Analysis of Outcome Measures

Appropriate statistical analysis will be conducted to identify if there is any significant difference in the concentration change of specific cytokines in both test and control group. Statistical analysis will also be conducted to identify correlations between clinical signs of apical periodontitis and individual mediator concentration.

Study setting

The whole study (i.e. recruitment, intervention, data collection and data analysis) will be conducted at the Birmingham Dental Hospital. This site receives referrals from general dental practitioners regarding patients requiring management of apical periodontitis and will therefore be ideal for recruiting patients into the study. Potential participants will be accessed through restorative consultation and acute dental clinics which are held on a daily basis. Birmingham Dental Hospital and School have its own HTA licence (No: 12313) for research and is experienced in handling human tissue samples (relevant material). Furthermore, there is an onsite laboratory which contains the necessary equipment to store, monitor and analyse the biological samples (periradicular tissue fluid) retrieved from patients.

Participant recruitment

Eligibility Criteria

5.3.1 Inclusion criteria

- Patients diagnosed with apical periodontitis
- Single rooted permanent teeth
- Medically fit
- Adult patients (≥ 18)
- Voluntarily consent to partake in the study

5.3.2 Exclusion criteria

- Teeth in sextants with active periodontal disease (i.e. pocketing of ≤ 5 mm)
- Tooth unable to retain a rubber dam
- Teeth that have had previous endodontic treatment
- Root apex in close proximity to the maxillary sinus
- Patients who have had antimicrobial therapy within 3 months prior to the screening clinic
- Pregnant or breastfeeding women
- Do not have capacity to consent
- Patients that have systemic condition that would reduce immune function

Recruitment target

6.1 Size of recruitment target

Up to 40 participants for this pilot experiment– explanation as per 5.1.1

6.2 Recruitment technique

A convenience sampling technique will be used to recruit the target population. This is a type of non-probability sampling method where the sample is being drawn from that part of the population that is close to hand. A similar technique has been used in previous studies which aim to study periradicular inflammatory mediator activity (Martinho et al., 2015).

Recruitment

7.1 Participant identification

Participants diagnosed with apical periodontitis will be initially identified and made aware of the study by a member of their direct care team (which includes clinical members of the research team) on routine consultation clinics held at the Birmingham Dental Hospital. Recruiting clinicians from the various departments within the

hospital will be invited to attend a formal minuted meeting at the Birmingham Dental Hospital. Here, the research team will provide clinicians with sufficient information and documentation (i.e patient information sheets) to allow them to discuss the study with potential participants and refer those interested in taking part to a weekly research screening clinic at the Birmingham Dental Hospital. The willingness of the clinicians to take part in this project will also be formally documented at this meeting, which will be kept in the investigator site file, and a follow up email alongside a digital version of the patient information sheet will be sent to each clinician.

Once a potential participant is identified, the clinician will make the patient aware of the trial through appropriate verbal and written means (i.e. patient information sheet). Those who express interest in taking part will be given a patient information sheet and asked to complete and sign a reply slip which will contain the participants name, DOB, address, telephone number and email. This reply slip will then be physically or electronically passed over securely to the research team, via a sealed envelope or encrypted NHS/University email respectively. This signed reply slip will act as a referral and contain enough personal information for the research team to identify and invite the potential participant to attend a screening clinic. The patient will be verbally informed of this passage of information by their clinical and the discussion, as well as their consent to transfer these details to the research team, will be documented in the participant's medical notes. Once the research team have received the referral/reply slip, they will contact the participant and arrange for them to attend a research screening clinic at the Birmingham Dental Hospital. Potential interested participants identified by clinical members of the research team on their routine treatment/consultation clinics will be booked directly onto the screening clinic and will not need to be referred or complete a reply slip. This is because the clinical researcher will already be part of the participants direct care team. At the screening clinic a research clinician will examine participants for eligibility, give appropriate information about the study (facilitated by the patient information sheet) and answer any questions throughout the discussion.

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Those who are eligible for inclusion and express interest in taking part will then be consented by the trained member of the research team (i.e. with GCP training, knowledge of the trial protocol and delegated authority from the principle investigator) and subsequently booked onto treatment clinics. Those who do not meet the eligibility criteria or are unwilling to take part will be discharged back to the referring clinician who will organise to have the treatment completed. For those wanting more time to think about participating, they will be contacted by a member of the research team 1 week following the discussion. At this point if the participant wants more time they will be discharged back to the referring clinician and have the option to contact the research team using details outlined on the PIS if and when they decide to participate. Withdrawing prior to enrolment or at any stage the trial will not result in any delay the patients' treatment a long a conventional pathway.

7.2 Consent

The consenting procedure will be held at a research screening clinic at the Birmingham Dental Hospital. It will be the responsibility of a member of the research team with the appropriate training (i.e. GCP) to obtain written informed consent for each participant before performing any trial related procedure. Prior to attending this clinic, potential participants will have been given a Patient Information Sheet (PIS), by a member of their direct care team, to facilitate this process. This will have allowed them sufficient time between being made aware of the study to attending the screening clinic to discuss their participation with others outside of the research team should they choose to do so.

At the research screening clinic, investigators will ensure that they adequately explain the aim, purpose of the project, anticipated benefits and potential hazards of taking part in the trial to the potential participant. They will also stress that participation is voluntary and that the participant is free to refuse to take part and may withdraw from the trial at any time. The participant will be given the opportunity to ask any questions throughout this discussion. It also gives the investigator the opportunity to assess that the participant understands the purpose

and nature of the research, what it involves, its benefits (or lack of benefits), risks / burdens, the alternatives to taking part, assess their ability to retain the information long enough to make an effective decision and ensure the decision is completely voluntary. Patients who meet these criteria and express an interest in participating in the trial will be asked to sign and date the latest version of the Informed Consent Form (ICF), which will contain details of the aforementioned discussion. The investigator will also sign and date the form. One copy of the ICF will be given to the participant, another copy will be filed in the medical notes, and the original placed in the Investigator Site File (ISF). Once the participant is entered into the trial, the participant's unique trial identification number will be entered on the ICF maintained in the ISF. Details of the aforementioned discussion will also be documented in the participant's medical notes. This will include date of discussion, the name of the trial, summary of discussion, version number of the PIS given to the participant, version number of ICF signed and the date / time the consent was obtained.

At each visit the participant's willingness to continue in the trial will be ascertained and documented in the medical notes. Throughout the trial the participant will have the opportunity to ask questions and they will be informed of how they can withdraw from the study if they no longer wish to continue. Any new information that may be relevant to the participant's continued participation will be provided. Where new information becomes available which may affect the participants' decision to continue, participants will be given time to consider and if happy to continue will be re-consented. In such cases, re-consent will be documented in the medical notes and the participant's right to withdraw from the trial will remain. The aforementioned consenting procedure will be the same and apply to all participants in both control and test groups.

This project does not involve obtaining consent from vulnerable groups.

7.3 Participant exit from study

A participant can withdraw consent and exit the study at any time. Participants that fail to attend appointments will be contacted by a member of the research team by text message and telephone call to remind them of appointments. Persistent non-attendance (e.g. 3 none attendances/late cancellations within 24 hours of appointment), will result in the participant being exited from the study.

Safety reporting

Adverse Events

All medical occurrences, including any unexpected out of range laboratory values, which meet the definition of an AE will be reported on an AE form.

Serious Adverse Events

Investigators will report AEs that meet the definition of an SAE.

Reporting period

Details of all AEs will be documented and reported from the date of consent to donate. All SAE will be reported within 24h of notification to any project team member listed on the delegation log.

Reporting Procedure

8.1 Adverse Events

AEs will be collected on an AE Form (and where applicable on an SAE Form). An AE Form will be completed and returned as soon as possible.

8.2 Serious Adverse Events

AEs defined as serious and which require reporting as an SAE will be reported on an SAE Form. When completing the form, the investigator will be asked to define the causality and the severity of the AE.

On becoming aware that a participant has experienced an SAE, the Chief Investigator will complete, date and sign an SAE Form. The form should arrive at the Trials Office *dentistryctt@contacts.bham.ac.uk* as soon as possible and no later than 24 hours after first becoming aware of the event.

On receipt the Trials Office will allocate each SAE a unique reference number which will be forwarded to the site as proof of receipt. If confirmation of receipt is not received within 1 working day the Chief Investigator will contact the Trials Office. The SAE reference number will be quoted on all correspondence and follow-up reports regarding the SAE and filed with the actual SAE in the Site File.

For SAE Forms completed by someone other than the Chief Investigator, the Chief Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form will then be returned to the Trials Office and a copy kept in the Site File.

Investigators will also report SAEs to their own Trust in accordance with local practice.

Any SAE related to this study (i.e. they resulted from administration of any of the research procedures) or unexpected (i.e. not listed in the protocol as an expected occurrence) will be submitted to the Sponsor and REC using the "non-CTIMP safety report to REC form". These will be sent within 15 days of the CI becoming aware of the event.

Ethical and regulatory considerations

Assessment and management of risk

Higher dose of ionising radiation than current recommended standards due to the use of pre and post-operative CBCT scans:

It is proposed in this study that participants will be required to have a pre- and post-operative CBCT scans to allow for accurate 3-Dimensional measurements of apical lesions to determine healing or progression of disease. Attempting to do this with conventional 2-Dimensional plain film radiography is crude and does not have the sensitivity / specificity to allow for accurate discrimination in outcomes between the control and intervention arms of the study. To limit the dose, a small field of view and the ALARA (As Low As Reasonably Acceptable) principles will be used and the scans will be taken by a qualified radiographer. These risks will be explained to the patient by a member of the research team on the screening clinic when obtaining informed consent

The collection and reporting of Adverse Events (AEs) will be in accordance with the Research Governance Framework for Health and Social Care and the requirements of the National Research Ethics Service (NRES), which are legislative frameworks adopted at the proposed site. A clinically trained investigator will then assess the seriousness and causality (relatedness) of all AEs reported by the trial participant with reference to the protocol.

Research Ethics Committee (REC) and other Regulatory review & reports.

Regulatory Review & Compliance

Monitoring will be carried out as required following a risk assessment and as documented in the monitoring plan. Any monitoring activities will be reported to the trials team and any issues noted will be followed up to resolution. Additional on-site monitoring visits may be triggered, for example by poor CRF return, poor data quality, SAE reports, excessive number of participant withdrawals or deviations. If a monitoring visit is required the Trials Office will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the project trial staff access to source documents as requested. The Principal Investigator will permit trial-related monitoring, quality checks, audits, ethical reviews, and regulatory inspection(s) at their site, providing direct access to source data/documents. The Principal Investigator will comply with these visits and any required follow up.

Amendments

All potential changes to the protocol will be submitted for consideration by the PI. Any changes to the protocol will be submitted by the clinical research co-ordinator after confirmation from the PI, who will process them via University of Birmingham research governance team and submit to HRA for approval. Substantial amendments as defined by the HRA, will be submitted administered the same way except they will be submitted for approval via IRAS. Approval for all amendments will be obtained before submission from the PI and the sponsor (where necessary). All stake holders (CI, PI, co-investigators, Trust R&D, Sponsor) will be notified of all amendments. Where approval is required before submission to HRA this will be co-ordinated by the research co-ordinator.

Peer review

For approval from the sponsor, this application successfully went through a peer review process at the University of Birmingham. Additionally to acquire funding, the application was assessed externally through an anonymised grant reviewing process by experts within this field who are independent from the institute.

Patient & Public Involvement

We will establish a patient advisory group who will be consulted on the design and feasibility of the project. They will be consulted during the recruitment and duration of the study for feedback and feedforward on study progress. We are planning dissemination events throughout the study period and at the end.

Protocol compliance

The project management group will be responsible for all aspects of running the project protocol compliance management, including adverse event reporting, data management, audit and steering. Members include:

- Chief Investigator: Dr. Phillip Tomson
- Project Manager: Mr. Satnam Singh Virdee
- Clinical research co-ordinator: Dr. Naomi Hubber
- All other members of the team – Dr. Josephine Camilleri, Prof. Paul Cooper

Source Data

In order to allow for the accurate reconstruction of the trial and clinical management of the subject, source data will be accessible and maintained. Source data will include any electronic or paper filled patient forms (which include sensitive data of age, ethnicity and clinical images). The key will be held by the lead clinician in a secure location Trial specific source data is kept, generated and maintained at site and is kept by the University of Birmingham, School of Dentistry. In addition, for this trial, all laboratory analyses performed will generate data that will be kept at the University of Birmingham, School of Dentistry. All other source data will be recorded in the patient record and will be transcribed to the CRF for research use and will be clearly Identifiable as transcribed data. The source data will be kept within the patient records at the hospital of origin Birmingham Dental Hospital) in line with local policy for a minimum of 10 years.

CRF Completion

Data reported on each Case Report Form will be consistent with the source data and any discrepancies will be explained. Staff delegated to complete CRFs will be trained on:

- *Date format and partial dates*
- *Time format and unknown times*
- *Rounding conventions*
- *Trial-specific interpretation of data fields*
- *Entry requirements for concomitant medications* (generic or brand names)
- *Which forms to complete and when*
- *What to do in certain scenarios, for example when a subject withdraws from the trial*
- *Missing/incomplete data*
- *Completing SAE forms and reporting SAEs*
- *Repeat laboratory tests*
- *Protocol and GCP non-compliances*

In all cases it remains the responsibility of the site's Principal Investigator to ensure that the CRF has been completed correctly and that the data are accurate. Where applicable for the trial this will be evidenced by the signature of the site's Principal Investigator on the CRF. The completed originals will be submitted to the Trials Office and a copy filed in the Investigator Site File.

Data protection and patient confidentiality

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the General Data Protection Regulation 2018 and Data Protection Act 2018. Participants will always be identified using only their unique trial identification number on the Case Report Form and correspondence between the University and participating site. Participants will give their explicit consent for the movement of their consent form, giving permission for the research team to be sent a copy. This will be used to perform in-house monitoring of the consent process. The research team will maintain the confidentiality of all participants' data and will not disclose information by which participants may be identified to any third party.

9.1 Data Management

9.1 Types of Data Created

Quantitative data: Pain score, concentration of periradicular inflammatory mediators, size of periradicular lesions and volume of periradicular tissue fluid collected.

Qualitative data: patient characteristics (age, gender & tooth to be treated), clinical data on the presence of absence of clinical symptoms related to periradicular disease including the participants presenting complaint, pain history, condition of the hard tissue, presence of swelling and sinus, symptoms of tenderness to percussion, palpation or mastication, periodontal pocket depths, mobility and the vitality of the tooth. Intra-oral images may also be taken.

Together, this data will inform an inflammatory profile of periradicular lesions.

9.2. Structuring, Documenting & Reporting Data

Source data will be split between CRF and patient record. Source data that is only relevant to the research will be kept only as part of the CRF, data that is clinically relevant will be kept in the patient record and simultaneously transcribed into the CRF for research purposes. All the research data will be analysed in graphical and statistical software such as excel, and will be exported or transcribed into a pseudo anonymised spread sheet numerical dataset. File formats will be Datasheets (.xls/.csv/.pzf), word documents (.doc/.docx/.txt) and Images (.bmp/.jpg).

Laboratory records will be maintained in a laboratory research file. These will be scanned to PDF for electronic storage. Source data stored in laboratory day/note books will be archived after the study with the project documents.

9.2.1 Maintaining Data Integrity

Integrity of clinical data will be maintained by a random spot check process by the PI. Patient submitted data cannot be verified without extra burden to the participant so will be assumed to be valid. Where data supplied by the participant does not make clinical or laboratory sense the participant will be contacted to verify the data at their next clinical contact point. Laboratory data integrity will be maintained by sample and experimental replicates, and electronic transfer of data using software export tools.

Data storage and archiving

Source data will be captured on NHS patient record systems and relevant data will be transcribed to usable research datasets for processing and project analysis.

All transcribed data retained on University servers will be pseudo anonymised by study ID, but will be accompanied by sensitive personal data such as demographical data required for publication (age, gender, ethnicity and pertinent disclosed medical history).

All data will be managed in accordance with the relevant Data Protection Laws and further details of the University of Birmingham data protection policies can be found at https://intranet.birmingham.ac.uk/legal-services/What-we-do/Data-Protection/index.aspx

All data shared with external collaborators will be pseudo anonymised by study ID and published personal data (age, gender, ethnicity) will be anonymised by group analysis.

Data will be archived at the end of the study for a minimum of 10 years in accordance with local and national policy. Data stored on University of Birmingham servers will be managed in accordance with the university of Birmingham guidance https://intranet.birmingham.ac.uk/as/libraryservices/library/research/rdm/Archiving-data/Archiving-and-sharing-data.aspx and local Data Management plan. NHS source data will kept indefinitely as part of the patient record.

9.2.2 Protecting Ethically/Commercially Sensitive Data

Where data is ethically sensitive these data will be pseudo anonymised using study ID and as such the participant will not be identifiable from the data set.

9.2.3 Long Term Archiving of Data

At the publication of the PhD thesis, a subset of the data that underpins the thesis will be archived in the University of Birmingham UBIRA eData repository. The repository uses the University Research Data Archive as storage solution which has been created to be highly resilient and is located at two data centres in two different sites, with a backup placed in a third site. Data will be stored for 10 years, should access to the data be requested within a 10 year period, the 10 year clock is then reset from the point of last access. After the 10 year period the data will be deleted.

All paper documentation will be archived initially within the Dental Hospital. As resources decrease data may be moved to a GCP accredited offsite facility with a high reputation for data management and who can adhere to all the principles set out in this DMP.

9.2.4 Data sharing

For the sharing anonymised data between stake holders (Investigators, researchers, collaborators, sponsor, HRA, REC, university governance and associated research and development teams), the University of Birmingham has a service similar to Dropbox called BEAR DataShare.

This is a file synchronisation and sharing service provided by the University, which allows University of Birmingham research staff and postgraduate research students to share files securely with others (Non-University) and across different desktop computer systems and mobile devices. This facility will be used for the benefit of all partners within the project. The service is free for University members to use as it is provided centrally, and there is no additional charge. Only anonymized data will be shared via this method.

For documents associated with the ethical approval of the project the integrated research application system and encrypted email systems of the university will be used.

We will use the University of Birmingham UBIRA eData repository (https://edata.bham.ac.uk/) which will also archive the data for at least 10 years. The eData repository uses Dublin Core as a metadata standard and the minimum metadata provided for published datasets will cover amongst others title, type of data, creators, publication date and related publications. The eData repository is indexed by Google and other search engines and thus datasets shared there are widely discoverable.

In addition, a metadata record will be created in the University's research information system PURE and the dataset is thus also discoverable through the University of Birmingham Research Portal (https://research.birmingham.ac.uk/portal/en/).

Proprietary data

It is envisaged that the parties involved will mainly share and use publicly available information. Whenever pub information (i.e. patient information) will need to be shared, standard confidentiality agreements will be put in place. In particular, the parties involved will use reasonable endeavours to ensure that any confidential information disclosed or submitted in writing or any other tangible form to one party ("Receiving Party") by the other ("Disclosing Party") shall be treated with the same standard of care and discretion to avoid disclosure as the Receiving Party uses with its own similar information which it does not wish to disclose. Any information disclosed orally or visually that is identified (orally or in writing) by the Disclosing Party as confidential information shall be treated the same as if it had been reduced to writing at the time of disclosure to the Receiving Party. Each party,

through their respective Technology Transfer Offices, have already in place standard operating procedures to record and protect any Background IPR belonging to the Parties. At the same time, ownership of any Foreground IPR generated during the project will be apportioned to the Parties on the basis of their contribution to the arising IPR.

In accordance with normal academic practice, all employees, students, agents or appointees of the University (including any others who may work on the project) shall be permitted, to discuss resulting IP in internal seminars, and to give instructions within the University on questions related to such work. All of the parties involved shall be permitted to publish the results of the project in accordance with normal academic practice and each party shall send the other parties a draft of all intended publications in advance of publication, for the other parties to review them for the possible inclusion of any of its confidential information. The other parties shall have 28 days, after the receipt of the draft to request in writing the delay or amendment of such proposed publication on the grounds that there is subject matter which needs patent protection or similar protection or to prevent publication of any confidential information of the other parties. Where in the reasonable opinion of the other parties a proposed publication contains patentable or commercially sensitive subject matter which needs protection then a request to refrain from doing so for a maximum of six months in order to allow for application for patent protection in the name and at the cost of the owner of the resulting IP can be made.

Timeframes for data release

Where appropriate and possible studies undertaken at the dental hospital will aim to release all experimental data that underpins published work. Experimental data will be released after the point of publication. This will either be at the time a journal publishes a pre-print of a paper online or at which we publish work ourselves. Where work does not get accepted for publication or is not intended for publication (such as methodology work up), this data will be released after appropriate quality standards have been met.

We will ensure that the experimental data is in a non-proprietary, open format at the point of release. However, if intellectual property is generated during the course of the project, this may be subject to the standard procedures for the recording and protection of IPR, in which case an embargo period will apply prior to the release of this information to third parties and the general public as described above.

Indemnity

The University of Birmingham has in place Clinical Trials indemnity coverage for this trial which provides cover to the University for harm which comes about through the University's, or its staff's, negligence in relation to the design or management of the trial and may alternatively, and at the University's discretion provide cover for non-negligent harm to participants.

With respect to the conduct of the trial at Site and other clinical care of the patient, responsibility for the care of the patients remains with the NHS organisation responsible for the Clinical Site and is therefore indemnified through the NHS Litigation Authority.

The University of Birmingham is independent of any pharmaceutical company, and as such it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for participant compensation.

End of study and archiving

The end of study will be 6 months after the last data capture. The Trials Office will notify the REC that the trial has ended and a summary of the clinical trial report will be provided within 12 months of the end of trial.

A copy of the end of trial notification as well as the summary report is also sent to the University of Birmingham Research Governance Team at the time of sending these to the REC.

It is the responsibility of the Principal Investigator to ensure all essential trial documentation and source documents (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, participants' hospital notes, copies of CRFs etc.) at their site are securely retained for at least 10 years.

Access to the final study dataset

The CI will have access to the full dataset. They will disseminate data to the appropriate research team member to ensure complete analysis can be achieved. Secondary data analysis may wish to be undertaken and has been outlined in the ICF and PIS appropriately. Study data will be stored and transferred as outlined in the Data management plan.

Dissemination policy

Results of this trial will be owned by the sponsoring organisation and submitted for publication in a PhD thesis and peer reviewed journal. The manuscript will be prepared by the team and authorship will be determined by mutual agreement.

Additionally the team will make available the results of the trial in lay terms and will hold at least one participant event to discuss the results with patients. This will be organised by the principle investigator.

Authorship eligibility guidelines and any intended use of professional writers

All members of the research team will be granted authorship on the final study report. Authors must acknowledge that the trial was performed with the support of the British Endodontic Society.

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