Study Protocol

I. Study Background

Root canal treatment is a key therapeutic approach for pulp diseases and periapical diseases. The sealing ability, antibacterial properties, and biocompatibility of the root canal sealer are critical factors determining the long-term hermetic seal of the root canal system, preventing reinfection, and promoting periapical tissue repair. Although traditional sealers such as zinc oxide eugenol and epoxy resin types are widely used, they still have issues like microleakage and insufficient biocompatibility. In recent years, bioceramic-based sealers have become a research focus due to their excellent physicochemical properties and bioactivity. The imported iRoot SP (injectable root canal sealer paste), as a representative of calcium silicate-based bioceramic materials, has been widely used in clinical practice because of its high pH, good radiopacity, hydrophilicity, and biocompatibility. In the past two years, the domestic new material nRoot SP (ENPUNUO root canal sealer paste), with its optimized physicochemical properties and potential cost advantages, has gradually entered clinical application. nRoot SP exhibits high heat resistance, high flowability, high sealing ability, and high radiopacity. Its core components react within the root canal to form hydroxyapatite, inducing periapical bone regeneration. Nevertheless, the comprehensive performance of nRoot SP has not been fully validated, and there is still a lack of long-term clinical outcome data for domestic materials and direct comparative studies with imported products. Existing clinical trials lack long-term follow-up data on the application of both materials in patients with chronic periapical periodontitis. Therefore, this study aims to conduct a clinical intervention trial to investigate the efficacy (including reduction range of periapical radiolucency on radiographs, postoperative pain, etc.) of root canal treatment using iRoot SP versus nRoot SP in patients with chronic periapical periodontitis, with a 3-24month follow-up. The goal is to provide evidence-based data for the optimization and clinical selection of domestic materials and to promote the precise application of bioceramic sealers.

II. Study Objectives

1. To compare the therapeutic effects of the two materials in patients with chronic periapical periodontitis through a randomized controlled trial.
2. To provide objective data support for the clinical selection of root canal sealers, promote the optimization and application of domestic materials, and offer direction for the research and development and improvement of root canal sealers.

III. Study Design and Methods

(I) **Type of Study**: Interventional, single-center study.
(II) **Overall Project Duration**: 3 years. Start date: Upon ethics committee approval. End date: June 30, 2028.
(III) **Patient Inclusion, Exclusion, and Withdrawal Criteria**
Inclusion Criteria:

* Age ⩾ 18 years.
* Meeting the diagnostic criteria for periapical periodontitis according to "Endodontics".
* The affected tooth is deemed restorable after evaluation, with non-vital pulp requiring extirpation.
* The affected tooth is a single tooth with a fully developed apical foramen.
* No root resorption and no significant variation in root canal morphology.
* The affected tooth is undergoing initial root canal treatment.
* Radiographic examination reveals periapical bone destruction with a diameter of 1-5 mm and no surrounding sclerotic line.
* The patient is in normal mental state, conscious, and capable of normal communication.
* The patient provides informed consent.

Exclusion Criteria:

* Alveolar bone destruction exceeds one-third of the root length.
* The affected tooth has root fracture, root canal obstruction, calcification, or root surface defects.
* Presence of severe systemic diseases (e.g., osteoporosis, psychiatric disorders, hepatic or renal insufficiency).
* Use of analgesics, immunosuppressants, or antibiotics within one week prior to enrollment.
* Concurrent oral diseases such as periapical cysts or oral tumors.
* Pregnancy or lactation.
* Dental anxiety/phobia, severe gag reflex, limited mouth opening, or poor compliance.
* Allergy to the root canal sealer materials.

Withdrawal Criteria:

* Non-compliance with the study physician's instructions or failure to attend follow-up visits on time over an extended period.
* If, during the trial, the participant's condition no longer meets the inclusion criteria.
* Occurrence of serious adverse events or reactions requiring treatment, and the investigator deems it necessary to terminate participation.
* The participant has the right to voluntarily withdraw at any time without providing a reason.

(IV) **Detailed Study Plan**

1. General Information

Eighty patients with chronic periapical periodontitis admitted to the Affiliated Stomatology Hospital, School of Medicine, Zhejiang University from the date of ethics approval until June 30, 2028, will be selected (see above for inclusion/exclusion/withdrawal criteria; sample size calculation below). Patients will be divided into the iRoot SP group and the nRoot SP group (40 patients each) using stratified block randomization.

2. Randomization Method: Stratified Block Randomization

2.1 Determination of Stratification Factors and Sample Size: Stratification by tooth position into 4 strata: maxillary anterior teeth, maxillary posterior teeth, mandibular anterior teeth, mandibular posterior teeth. Each stratum will have 5 blocks with a block size of 4. A 1:1 allocation ratio between iRoot SP and nRoot SP will be maintained within each block.

2.2 Generation of Stratified Block Random Sequence: Using Python. Code example:

```python
import random
# Stratification factors (4 strata)
strata = ["Maxillary Anterior", "Maxillary Posterior", "Mandibular Anterior", "Mandibular Posterior"]
total_samples = 80
samples_per_stratum = 20 # 20 cases per stratum
block_size = 4
groups = ["iRoot SP", "nRoot SP"]
# Generate sequence
random_sequence = {}
for stratum in strata:
    blocks = samples_per_stratum // block_size
    sequence = []
    for _ in range(blocks):
        block = random.sample(groups * (block_size // 2), block_size)
        sequence.extend(block)
    random_sequence[stratum] = sequence
# Output result
for i, stratum in enumerate(strata):
    print(f"{stratum} Stratum")
    for j, group in enumerate(random_sequence[stratum]):
        print(f"Patient {i*20 + j + 1} assigned to {group} group")
```

2.3 Allocation Concealment: The random sequence will be sealed in envelopes. A third party (e.g., a nurse) will open the envelopes in the order of patient enrollment and assign the group, keeping both the investigator and the patient blinded to the allocation to avoid selection bias.
2.4 Implementation Process: Patient enrollment → Baseline assessment → Stratum determination → Open corresponding stratum envelope → Execute assigned treatment.
2.5 Handling of Special Situations: For patients with multiple eligible teeth, only one tooth per patient will be included, prioritizing the tooth position needed for sample size. If a stratum is filled early, remaining patients in that stratum will continue allocation by block. If a patient withdraws, the original group assignment is retained, but their data is excluded from analysis.
3. Treatment Methods
iRoot SP Group: iRoot SP root canal sealer (Manufacturer: Innovative Bioceramix Inc.) will be used with the warm vertical compaction technique for root canal obturation.
nRoot SP Group: nRoot SP root canal sealer (Manufacturer: Changsha Enpunuo Biotechnology Co., Ltd.) will be used with the warm vertical compaction technique for root canal obturation.
4. Observation Indicators
4.1 Treatment Efficacy Comparison Post-treatment:** Efficacy will be compared between groups based on the following criteria:
Markedly Effective: Post-treatment radiographic examination shows disappearance of periapical radiolucency. Chewing function is largely restored. Symptoms such as gingival

swelling/pain, tooth discoloration, and occlusal discomfort disappear.
Effective: Post-treatment radiographic examination shows significant reduction of radiolucency. Patient reports mild percussion pain or discomfort. Chewing function is largely normal. Symptoms show improvement.
Ineffective: Post-treatment radiographic examination shows no reduction or even enlargement of radiolucency. Patient reports significant percussion pain. Symptoms show no improvement.
4.2 Comparison at baseline, 3, 6, 12, and 24 months post-treatment: Changes in tooth mobility, Periodontal Pocket Depth (PD), degree of percussion pain, and the range of periapical radiolucency on radiographs.
5. Statistical Methods
Data will be analyzed using SPSS 24.0. Continuous data like PD will be presented as mean ± standard deviation. Inter-group differences between iRoot SP and nRoot SP groups will be compared using the independent samples t-test. Intra-group comparisons will use the paired samples t-test. Categorical data like treatment efficacy rates will be presented as n (%) and compared using the Chi-square test. A P-value < 0.05 will be considered statistically significant.

IV. Sample Size Calculation

For this randomized controlled trial comparing the two sealers, the primary evaluation indicators involve comparison of means (e.g., reduction range of periapical radiolucency) and comparison of rates (e.g., success rate of root canal treatment). According to the formulas:

* Comparison of means: $n = [2(z_\alpha + z_\beta)^2 * \sigma^2] / \delta^2$

* Comparison of rates: $n = [2\bar{p}(1 - \bar{p})(z_\alpha + z_\beta)^2] / (p_1 - p_2)^2$

Where $\alpha = 0.05$ (two-tailed), $z_\alpha = 1.96$; $\beta = 0.2$, $z_\beta = 0.84$. Based on estimates of p_1, p_2, σ, δ from existing literature, and considering a 10% loss-to-follow-up rate, the required sample size was calculated using G*Power software. The final estimated sample size per group is $n = 40$, resulting in a total sample size of 80.

V. Data Management and Confidentiality

1. All records identifying participants will be kept confidential. All data will be managed by designated personnel for internal use only, protected by dedicated passwords inaccessible to others.
2. Missing data, unused data, and illogical data will be excluded from analysis.
3. All records identifying participants will be kept confidential and will not be publicly disclosed beyond the scope permitted by relevant laws and/or regulations.

VI. Informed Consent

The investigator, Tu Yan, will be responsible for obtaining written informed consent from participants prior to root canal treatment. The informed consent process will adhere to the

principles of full disclosure, adequate comprehension, and voluntary choice. The language used in the consent form will be clear and comprehensible to the participant population. This study does not involve vulnerable groups. All participants will sign the informed consent form personally.

VII. Adverse Event Reporting

1. Potential Risks for Participants: In case of allergic reaction, the use of the sealer will be terminated immediately, and residual material within the root canal will be cleaned. The affected patient will undergo re-treatment and anti-allergy therapy, and the cause will be documented.

2. Adverse Event Reporting Procedure:

All Adverse Events:** Managed promptly, recorded in the Case Report Form (CRF).

Serious Adverse Events (SAE): Managed promptly, recorded in the CRF. The investigator will decide to discontinue or suspend the use of the sealer. The SAE must be reported immediately to the Ethics Committee, the institutional drug clinical trial authority, and the sponsor, and within 24 hours to the National and Provincial Medical Products Administrations.