Supplementary Table S4: Experimental Protocol for Establishing and Maintaining Ex Vivo Whole-Tooth Cultures

1. Sample Selection	Recommended tooth type: immature human third molars extracted for clinical reasons. Typical age range: 15–20 years. Teeth should present open apices and vital pulp tissue to ensure preservation of pulp architecture and cellular viability.
2. Transport and Initial Storage	Immediately after extraction, teeth should be placed in sterile tubes containing 10 mL of DMEM supplemented with 10% fetal bovine serum (FBS), antibiotics (e.g., penicillin–streptomycin), and an antifungal agent (e.g., amphotericin B). Transport time to the laboratory should ideally not exceed 4 hours.
3. Surface Decontamination and Preparation	Under sterile conditions, teeth should be briefly rinsed in 70% ethanol (approximately 1 minute), followed by sterile PBS, or alternatively disinfected by immersion in 0.2% chlorhexidine solution and PBS for 10 and 30 seconds, respectively. Residual periodontal ligament tissue should be removed using a sterile scalpel. Teeth should be handled with sterile gauze moistened with culture medium to avoid dehydration.
4. Cavity Preparation and Pulp Exposure	A standardized Class I cavity should be prepared using a sterile high-speed bur under abundant sterile saline irrigation. Pulp exposure should be achieved with a low-speed round bur under copious irrigation, creating a controlled exposure area of approximately 1 mm^2. The cavity should be rinsed with sterile saline and gently dried with sterile cotton pellets.
5. Biomaterial Application and Coronal Restoration	The tested biomaterial should be placed in direct contact with the exposed pulp, typically in a 2–3 mm layer, and gently compacted using sterile cotton pellets. Coronal sealing may be achieved by: Filling the entire cavity with biomaterial, or Covering the biomaterial with glass ionomer cement. These variations do not appear to be the main determinant of biological outcomes, provided that an adequate seal and proper tooth suspension are ensured.

6. Tooth Suspension and Culture Conditions	Teeth should be fixed to an orthodontic wire and suspended in culture plates (commonly 24-well plates) so that the apex does not contact the bottom of the well. Each well should contain approximately 1.5 mL of DMEM supplemented with 10% FBS, antibiotics, and antifungal agents. Incubation conditions: 37°C, 5% CO_2. The culture medium should be replaced daily.
7. Culture Duration	A culture period of 3–4 weeks (21–28 days) is recommended to allow observation of early reparative dentinogenic events. Available evidence supports cell survival and maintenance of mesenchymal stem cell characteristics for up to 4 weeks in this model.
8. Histological Processing	Following the culture period, specimens should undergo standard histological processing prior to analysis.
9. Recommended Outcome Assessment	Core histological staining: Hematoxylin and Eosin (H&E) Optional complementary analyses: Immunohistochemistry markers: Nestin, Dentin sialoprotein (DSP), Collagen I, Osteonectin