Microscopy Research and Technique

WILEY

MICROSCOPY RESEARCH & TECHNIQUE

RESEARCH ARTICLE OPEN ACCESS

In Vitro Evaluation of Novel Calcium Silicate-Based and Resin-Modified Calcium Silicate Materials: Cytocompatibility and Mineralization Potential on Human Dental Pulp Stem Cells for Pulp Repair

Francisco Javier Rodríguez-Lozano[1,2] | Nuria Pérez-Guzmán[1] | Paula García-Rios[1] | David García-Bernal[3] | Adrián Lozano[4] | Sergio López-García[1]

[1]Department of Dermatology, Stomatology, Radiology and Physical Medicine, Morales Meseguer Hospital, Faculty of Medicine, IMIB-Arrixaca, University of Murcia, Murcia, Spain | [2]Biomedical Research Institute of Murcia Pascual Parrilla–IMIB, Murcia, Spain | [3]Department of Biochemistry, Molecular Biology B and Immunology, Faculty of Medicine, University of Murcia, Biomedical Research Institute (IMIB), Murcia, Spain | [4]Departament D'estomatologia, Facultat De Medicina I Odontologia, Universitat De València, Valencia, Spain

Correspondence: Francisco Javier Rodríguez-Lozano (fcojavier@um.es)

Received: 21 July 2025 | **Revised:** 17 November 2025 | **Accepted:** 13 January 2026

Keywords: calcium silicate-based materials | dental pulp reparation | vital pulp materials

ABSTRACT

This study aimed to evaluate the biological effects of three calcium silicate-based materials—Biodentine XP (BD-XP), TheraCal PT (THPT), and TheraBase Ca (THB)—on human dental pulp stem cells (hDPSCs), focusing on cytocompatibility, immunomodulatory behavior (via IL-6 expression), and odontogenic/mineralization potential compared to control conditions. hDPSCs were cultured with eluates (25%, 50%, 100%) of BD-XP, THPT, and THB. Cytocompatibility was assessed via metabolic activity assay, cell cycle analysis, and cell migration. Morphology and adhesion were examined by SEM, while surface composition was analyzed by EDX. IL-6 secretion was quantified using ELISA. Gene expression of odontogenic/osteogenic markers (ALP, DSPP, RUNX2, COL-1) was analyzed via qRT-PCR at 14 and 21 days. Alizarin Red S staining was used to assess mineralization. Results were compared to unconditioned and osteogenic controls ($p < 0.05$). All materials exhibited acceptable cytocompatibility. BD-XP promoted the highest cell viability, migration, and adhesion. IL-6 secretion was significantly reduced in all treated groups, most notably with THB. SEM and EDX showed strong cell attachment and calcium-rich surfaces for BD-XP. BD-XP significantly upregulated DSPP and RUNX2 at both time points and COL-1 at day 21. ALP expression was mainly observed in the positive control. BD-XP also showed the greatest mineralized nodule formation. Biodentine XP demonstrated the most favorable biological behavior, showing high cytocompatibility, upregulation of odontogenic markers, and enhanced mineralization. These results highlight its potential for clinical use in vital pulp therapy and regenerative endodontics.

1 | Introduction

Despite the growing use of minimally invasive restorative techniques aimed at preserving healthy tooth structure in pediatric dentistry, the prevalence of caries in young permanent teeth—particularly proximal lesions in molars—remains high, highlighting the need for early diagnosis and preventive strategies. These cavities often progress rapidly, resulting in extensive lesions and, in severe cases, pulpitis or periapical periodontitis. (Bailleul et al. 2025; Elbahary et al. 2024).

Francisco Javier Rodríguez-Lozano and Nuria Pérez-Guzmán contributed equally to this study.

Highlights

- Calcium silicate-based and resin-modified materials were evaluated on human dental pulp stem cells (hDPSCs).
- Biodentine XP exhibited the highest cytocompatibility and mineralization potential among the tested materials.
- TheraBase Ca and TheraCal PT showed moderate cytotoxicity and limited regenerative performance.
- hDPSCs demonstrated enhanced odontogenic differentiation when exposed to calcium-releasing cements.
- For pulp repair applications, calcium silicate-based materials are more suitable than resin-modified formulations.

Vital pulp therapy (VPT) represents a clinically validated strategy to retain normal biological function of the dental pulp in primary teeth and incompletely developed permanent teeth. It is primarily indicated in cases involving compromise of the dentin-pulp complex due to extensive carious lesions, traumatic injury, or procedural mishaps during dental interventions (Koutrouli et al. 2024). This approach entails the utilization of pulp-derived materials designed to stimulate odontoblastic activity, stimulate the formation of reparative dentin at the lesion site and sustain pulp tissue viability. To achieve effective reparation of the dentin–pulp tissue, these materials must incorporate bioactive components capable of triggering cellular repair mechanisms, while also exhibiting biocompatibility and in vivo biodegradability (Rosa et al. 2025).

The effectiveness of vital pulp therapy is influenced by various elements, notably the regenerative potential of stem cells to multiply and develop into dental and osseous tissues. Among these, human dental pulp stem cells (hDPSCs)—isolated from the pulp of primary teeth, developing permanent teeth, and fully formed permanent teeth in young individuals—have been extensively investigated in experimental models for their capacity to contribute to tissue repair and regeneration (Osorio et al. 2024). Due to their accessibility and ability to differentiate into multiple lineages, hDPSCs have attracted significant interest in regenerative medicine. These cells exhibit multipotent potential, enabling differentiation into tissues such as bone, cartilage, fat, neural, and vascular structures, and are considered a promising source for neuroregeneration, bone repair, and related therapies (Spagnuolo et al. 2018). However, it is important to note that hDPSCs—particularly those derived from primary teeth—have not yet been applied in clinical regenerative procedures. In patients, actual tissue repair and regeneration are mediated by the activation of innate stem cells that reside within or around the affected tissue. Clinical materials and procedures are designed to stimulate these endogenous processes rather than to rely on exogenous cell transplantation (Spagnuolo et al. 2018).

Calcium silicate-based materials, also referred to as bioceramics or bioactive materials, promote bone and dental repair, as well as the osseointegration of dental implants (Spagnuolo 2022). These materials exhibit excellent biocompatibility, which enhances their potential as effective direct pulp capping (DPC) agents and demonstrate the ability to support repair and regeneration within the dentin–pulp complex.

Biodentine XP is a recently developed tricalcium silicate material that functions as a bioactive dentin substitute, thereby promoting tooth remineralisation while exhibiting excellent sealing properties (Mora et al. 2025). These characteristics ensure reduced sensitivity and minimal bacterial infiltration. As the successor to Biodentine, this material features an improved format that enhances ease of handling. TheraCal PT, a resin-modified calcium silicate cement with dual-curing properties, is intended for VPT procedures, including liner (Rodriguez-Lozano et al. 2021; Saber et al. 2023). TheraBase Ca (BISCO Inc., USA) is a novel dual-cure, calcium-releasing, self-adhesive base/liner designed for use in deep restorations and pulp-capping procedures. Its mechanism of action is based on a hydrophilic resin matrix that enables chemical bonding to dentin, allowing for the release and recharge of calcium ions over time (https://global.bisco.com/therabaseca/). This sustained calcium ion release creates an alkaline pH, which may support pulp vitality by neutralizing local acidity and favoring reparative dentin formation. The dual-curing capability of TheraBase Ca ensures complete polymerization even in areas with limited light penetration, such as deep cavities. However, despite these promising features, the biocompatibility, bioactivity, and regenerative potential of TheraBase Ca remain largely unassessed in comparison with established materials commonly used in vital pulp therapy.

This study aimed to evaluate, through in vitro experimentation, the biological behavior and mineralization capacity of Therabase Ca (THB), TheraCal PT (THPT), and the hydraulic calcium silicate-based cement Biodentine XP (BD-XP) on hDPSCs. The null hypothesis assumed the absence of statistically significant variation among the evaluated materials with respect to cytocompatibility, influence on cell plasticity, and mineralization potential in hDPSCs.

2 | Material and Methods

2.1 | Cell Culture and Characterizations of hDPSCs

Following approval of the research project by the Ethics Committee of the Institution (ID: 4930/2024), hDPSCs were harvested from the dental pulp of extracted impacted third molars from donors aged 16 to 22 years ($n = 10$). Cells were cultured under standard conditions in Dulbecco's Modified Eagle Medium (DMEM; LGC Biotecnologia, Cotia, São Paulo, Brazil) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (10,000 U/mL, Thermo Fisher Scientific, Waltham, MA, USA).

For phenotypic characterization, a total of 1×10^5 cells were resuspended in 100 μL of phosphate-buffered saline (PBS) supplemented with 1% fetal bovine serum (FBS). To verify their mesenchymal identity, the cells were stained with a panel of fluorescently labeled monoclonal antibodies targeting CD73,

TABLE 1 | Tested materials.

Materials	Manufacturer	Composition	Lot number
Biodentine XP	SEPTODONT. 58, rue du Pont de Créteil 94,107 Saint-Maur-des-Fossés Cedex, France	Powder: Tricalcium silicate, Zirconium oxide, calcium oxide, calcium carbonate and iron oxides. Liquid: Calcium chloride, polycarboxylate and purified water	**B33113AA**
Theracal PT	Bisco Inc. Shaumburg, IL, USA	SG-Mix cement, bisphenol A-glycidyl methacrylate (Bis-GMA), barium zirconate, ytterbium fluoride, initiator	**2,400,004,182**
TheraBASE Ca	Bisco Inc. Shaumburg, IL, USA	Ytterbium w/Barium Glass, Ethoxylated Bis A Dimethacrylate Portland Cement: tricalcium silicate, dicalcium silicate, alumina, tricalcium aluminate, and iron oxide. Ytterbium Fluoride, Brombenzenesulfinic Acid, Sodium Dihydrate, bisphenol A-glycidyl methacrylate (Bis-GMA), Titanium Dioxide, Acetyl-2-Thiourea.	**2,400,001,180**

CD90, CD105, CD14, CD20, CD34, and CD45 (Miltenyi Biotec, Bergisch Gladbach, Germany).

2.2 | Material Extracts and Preparation

The materials analyzed in this study are summarized in Table 1. All materials were prepared and placed into a cylindrical mold with a height of 5 mm and a radius of 2.5 mm, resulting in a surface area of 1.18 cm^2 and a volume of 0.1 cm^3, in accordance with the manufacturer's instructions. Following the manufacturers' specifications, the materials were mixed and allowed to cure fully. Each sample's extraction solution was passed through a 0.1 μm filter and stored at −20°C until needed. Dilutions at 25%, 50%, and 100% (v/v) were then prepared in line with the method reported by (Lozano-Guillen et al. 2022).

2.3 | Cell Viability and Cell Cycle Assays

Cell viability following exposure to different concentrations of extracts from VTP materials was studied using the MTT assay, in accordance with previously established protocols [10]. In summary, hDPSCs were cultured in 96-well plates at a seeding density of 2.5×10^4 cells per well using 100 μL of complete medium, followed by a 24-h incubation at 37°C with 5% CO2. After this incubation period, the cells were treated with material extracts at concentrations of 25%, 50%, and 100% (*v/v*) for 24, 48, and 72 h. This was conducted for 15 min at 37°C in a humidified environment with 5% CO_2. Untreated cells were used as the negative control. The formazan was dissolved with eluting agents, specifically dimethylsulfoxide and glycine buffer. The optical density (OD) of the solutions was measured with a microplate reader (ELx800; BIO-TEK, Winooski, Vermont, USA) at a wavelength of 570 nm. Additionally, to evaluate the impact of the VTP materials on cell proliferation, cell cycle profiling was conducted via flow cytometric analysis. hDPSCs were seeded in 25-cm^2 flasks at a density of 1×10^5 cells per flask and treated with the same extract concentrations (25%, 50%, and 100%) for 72 h. After treatment, cells were harvested by centrifugation (400 × g, 4 min), fixed in 70% ethanol overnight at 4°C, and stained with 40 μg/mL propidium iodide and 200 μg/mL RNase A for DNA content analysis. Flow cytometric analysis was performed using a FACSCanto II cytometer (Becton Dickinson, San Jose, CA) with excitation at 488 nm and emission at 617 nm. The proportions of cells in G0/G1, S, and G2/M phases were quantified using CellQuest and ModFit LT software (Becton Dickinson). Triplicate experiments were executed using three independent biological sources to validate the reproducibility of the results.

2.4 | Cell Migration

The migratory behavior of hDPSCs exposed to distinct vital pulp materials was examined by means of a standardized in vitro wound healing assay developed by our research group (Mora et al. 2025). Untreated cells were used as the negative control. Cells were seeded in either 12-well or 48-well culture plates at a density of 2×10^4 or 30,000 cells per well, respectively, and allowed to reach full confluence. After 24 h, a linear scratch was introduced using the sterile tip of a 100 μL or 1 mL micropipette to simulate a wound. The experimental media were then applied, and cells were incubated at 37°C in a humidified atmosphere containing 5% CO2. Wound closure was assessed at 0-, 24-, 48-, and 72-h post-scratch. For each sample, three regions along the wound were imaged using an EVOS inverted microscope (Advanced Microscopy Group) at 4× magnification. Wound closure was quantified by calculating the remaining wound area with ImageJ software (NIH, Bethesda, MD, USA). Each experimental condition was tested in triplicate or quadruplicate to ensure robustness and reproducibility of the results.

2.5 | Cell Morphology

To evaluate potential cytoskeletal alterations in hDPSCs following exposure to undiluted (1:1) vital pulp materials, F-actin organization was visualized using a phalloidin-based fluorescent staining protocol, adapted from a previously established

method (Lozano-Guillen et al. 2022). After the incubation period, the cells were rinsed with PBS, and the samples were fixed with 4% paraformaldehyde for 15 min at room temperature. Cells were then labeled with 0.165 μM Alexa Fluor 594-phalloidin (Invitrogen) in PBS for 20 min at room temperature in the dark. The slides were then washed with PBS; nuclear labelling was performed with DAPI (ThermoFisher Scientific, Waltham, MA, USA) at 300 nM for 5 min. The slides were fixed, and images were taken using a high-resolution confocal microscope (Leica, Wetzlar, Germany) to assess cytoskeletal organization.

2.6 | Scanning Electron Microscopy

To assess cell attachment and material surface interaction, hDPSCs were seeded at a density of 5×10^3 cells per well onto material discs ($n = 9$) in 48-well plates and incubated for 72 h at 37°C with 5% CO2. After incubation, unattached cells were eliminated through three PBS washes. The adherent cells were then fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated through a graded ethanol series, and dried using hexamethyldisilazane (HMDS). After the drying process, the specimens were sputter-coated with a thin gold–palladium layer to enhance electrical conductivity for imaging. Surface characterization was performed using a scanning electron microscope (SEM) Hitachi TM3000 (Hitachi, Tokyo, Japan) equipped with an energy dispersive X-ray spectrometer (EDS; Oxford Instruments, London, United Kingdom) at magnifications of 100×, 300×, and 1500×, analyzing three distinct regions per sample.

2.7 | Analysis of Cell Apoptosis and Necrosis and Reactive Oxygen Species (ROS) Production

To assess cytotoxicity, hDPSCs were exposed to eluates (1:1, 1:2, and 1:4) of tested materials for 72 h at 37°C. Apoptosis was analyzed using Annexin-V and 7-AAD staining (BD Biosciences), followed by flow cytometry to identify viable, early apoptotic, and late apoptotic/necrotic cells. Additionally, intracellular reactive oxygen species (ROS) levels were assessed by incubating the cells with 10 μM CM-H_2DCFDA (Invitrogen) for 30 min before analysis. Untreated cells were used as the negative control. Untreated cells were used as the negative controlExperiments were conducted in triplicate using cells from three independent donors.

2.8 | ELISA: IL-6 Eexpression Analysis

The secretion of IL-6 by hDPSCs was evaluated using an ELISA assay, following protocols established in previous studies (López-García et al. 2024). After the adhesion period, the experimental groups were treated with material extracts from BD-XP, THPT, and THB and incubated for 72 h and 7 days.

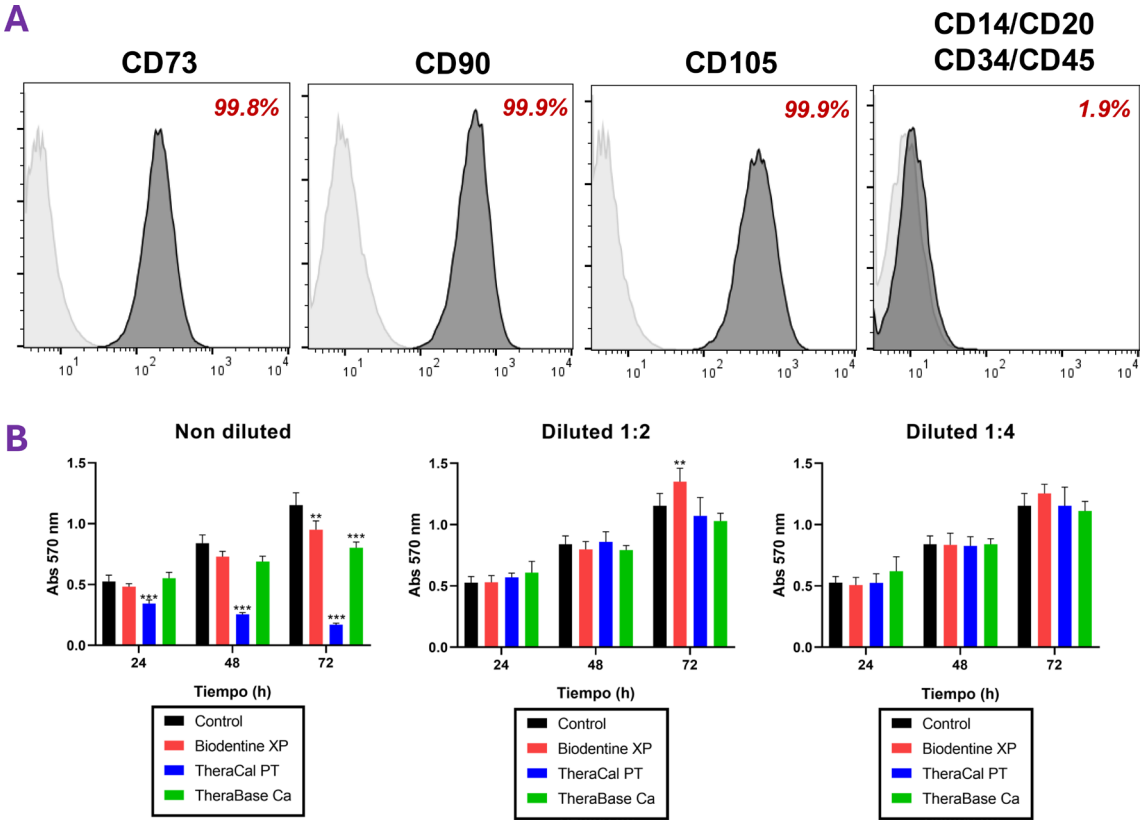

FIGURE 1 | (A) Flow cytometric analysis was used to assess the immunophenotype of hDPSCs. Cells were labeled with antibodies against MSC surface markers (CD73, CD90, CD105) and hematopoietic lineage markers (CD14, CD20, CD34, CD45). Representative histograms show expression profiles from three independent donors. (B) Cell metabolic activity measured by MTT assay after exposure to eluates (1:1, 1:2, 1:4) of BD-XP, THPT, and THB for 24, 48, and 72 h. BD-XP preserved higher viability compared to the other groups, especially at diluted concentrations. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (ANOVA).

Following incubation, supernatants were collected and centrifuged at 2500 rpm for 5 min at 2°C–8°C to remove any remaining cells. The ELISA was carried out according to the manufacturer's instructions (FineTest ELISA kit, FineTest Biotech Inc., Boulder, CO, USA). Absorbance was measured at 450 nm using a microplate reader. This analysis allowed quantification of IL-6 secretion, providing insight into the immunomodulatory effects of the tested pulp capping materials on hDPSCs.

2.9 | Gene Expression Profiling

The expression of cemento/osteogenesis-related mRNAs—including alkaline phosphatase (ALPL), dentin sialophosphoprotein (DSPP), runt-related transcription factor 2 (RUNX2), and collagen type I alpha 1 chain (COL1A1)—was detected using RT-qPCR assay. Cells were treated with eluates during 14 and 21 days. Total RNA was extracted from hDPSCs using the TRIzol Plus (Thermo Fisher, USA). Reverse transcription and PCR amplification were performed using the OneStep RT PCR Kit (QIAGEN, Germany) and the miRNA qRT-PCR Kits (RiBoBio, China). The 2-ΔΔCT method was employed to determine relative gene expression, using GAPDH as the reference gene. Untreated cells and those cultured in osteogenic differentiation medium (OsteoDiff, Miltenyi Biotec) served as negative and positive controls, respectively. All assays were run in triplicate.

2.10 | Evaluation of Calcified Nodule Formation

Alizarin Red S (ARS) staining was employed to assess the mineralization-inducing potential of VPT materials in hDPSCs. The assay was conducted under conditions consistent with those used for gene expression analysis. Two controls were included: a negative control (untreated cells) and a positive control (Osteodiff = osteogenic induction medium). Following a 21-day incubation period, cells were subjected to two PBS washes, fixed with 4% paraformaldehyde, and stained with ARS (Sigma). After a final rinse, images were taken under natural light conditions. All conditions were tested in triplicate to ensure experimental reliability.

2.11 | Statistical Methodology

The results were tabulated and analyzed using GraphPad Prism version 8.1.0 (GraphPad Software Inc., San Diego, CA, USA). Data are reported as mean ± standard deviation (SD) from

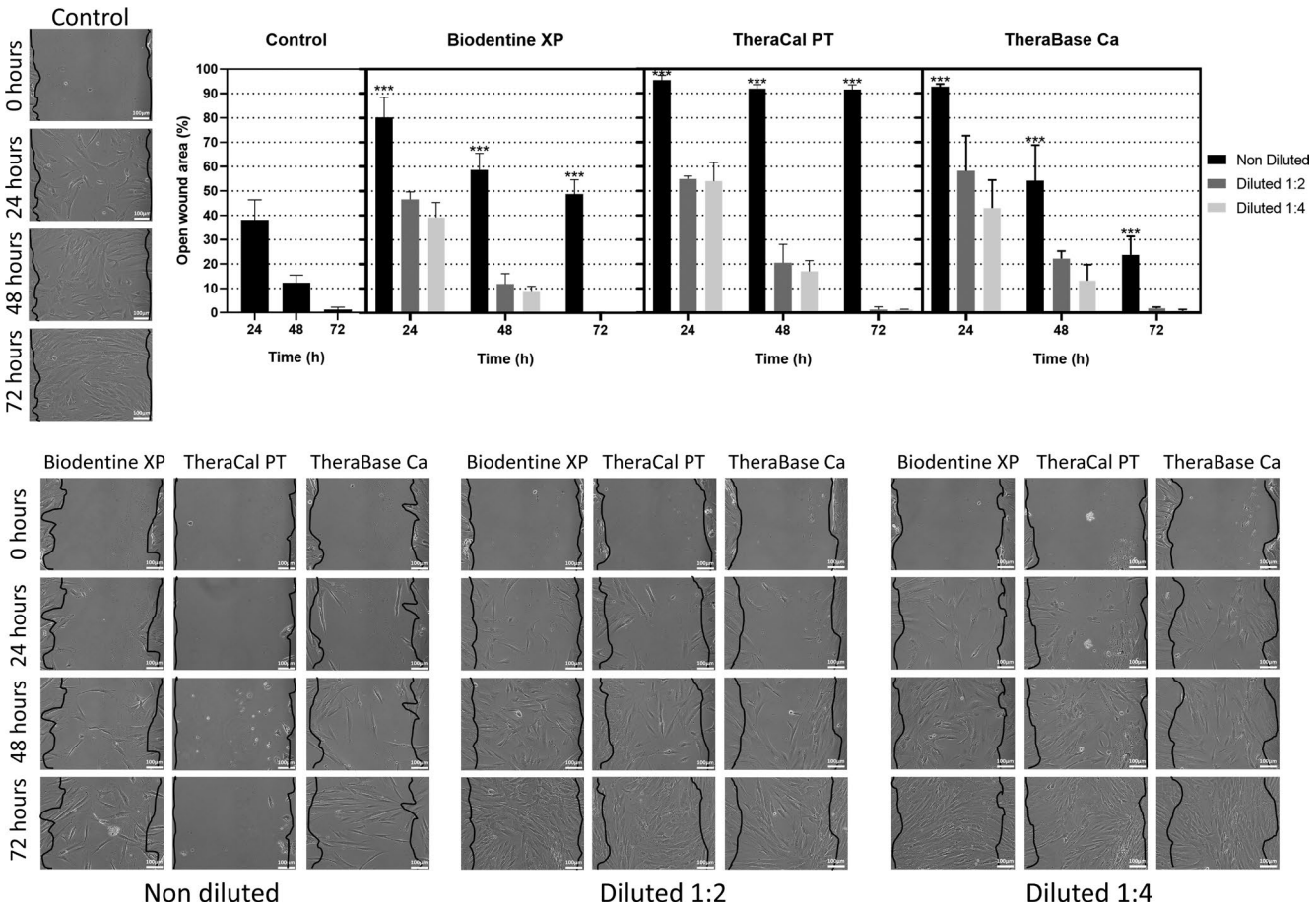

FIGURE 2 | Assessment of hDPSC migration using the wound healing assay after exposure to material eluates (1:1, 1:2, 1:4) of the VTP materials (BD-XP, THPT, and THB) at 24, 48, and 72 h. Wound area measurements are expressed as a percentage relative to the negative control. Cells treated with BD-XP demonstrated significantly enhanced migratory capacity, indicating its potential to support regenerative processes. Wound closure differences were statistically evaluated via two-way ANOVA (*$p < 0.001$). Representative micrographs are displayed; scale bar = 100 μm.

three independent experiments. Normality of distribution was assessed using quantile-quantile (Q-Q) plots. Based on the distribution characteristics and homogeneity of variances, comparisons among groups were carried out using either one-way ANOVA followed by Tukey's post hoc test or the non-parametric Mann–Whitney U test when appropriate. Statistical significance was established at $p < 0.05$.

3 | Results

3.1 | Flow Cytometry-Based Characterization of hDPSCs

Characterization analysis of the isolated dental pulp cells exhibited a typical mesenchymal stem cell (MSC) surface marker profile, exhibiting strong positivity for CD90, CD73, and CD105, whereas CD34 and CD45 were negative. This immunophenotypic profile confirms their identity as MSCs (Figure 1A).

3.2 | MTT Assay and Cell Cycle Analysis

Metabolic activity assay showed that undiluted THPT extracts significantly reduced cell viability at all evaluated time points compared to the control ($p < 0.001$). In contrast, lower concentrations (1:4 and 1:2 dilutions) induced only moderate reductions in metabolic activity, primarily at earlier time points, with partial recovery observed by 72 h. Reduced concentrations of THPT were less cytotoxic, allowing partial cellular recovery over time. In comparison, BD-XP maintained metabolic activity levels comparable to the control across all concentrations and time points (Figure 1B).

TABLE 2 | Analysis of migration of hDPSCs after treatment with different dilutions of tested materials by wound healing assays.

2way ANOVA				
Multiple comparisons test	**Mean diff**	**Significant?**	**Summary**	**Adjusted *p***
Control vs. Biodentine XP ND	−45,32	Yes	****	<0,0001
Control vs. TheraCal PT ND	−75,69	Yes	****	<0,0001
Control vs. TheraBase Ca ND	−39,68	Yes	****	<0,0001
Control vs. TheraBase Ca 1:2	−10,2	Yes	*	0,0233
Biodentine XP ND vs. Biodentine XP1:2	43	Yes	****	<0,0001
Biodentine XP ND vs. Biodentine XP1:4	46,39	Yes	****	<0,0001
Biodentine XP ND vs. TheraCal PT ND	−30,36	Yes	****	<0,0001
Biodentine XP ND vs. TheraCal PT 1:2	36,94	Yes	****	<0,0001
Biodentine XP ND vs. TheraCal PT 1:4	38,47	Yes	****	<0,0001
Biodentine XP ND vs. TheraBase Ca 1:2	35,12	Yes	****	<0,0001
Biodentine XP ND vs. TheraBase Ca 1:4	43,55	Yes	****	<0,0001
Biodentine XP1:2 vs. TheraCal PT ND	−73,37	Yes	****	<0,0001
Biodentine XP1:2 vs. TheraBase Ca ND	−37,36	Yes	****	<0,0001
Biodentine XP1:4 vs. TheraCal PT ND	−76,75	Yes	****	<0,0001
Biodentine XP1:4 vs. TheraCal PT 1:2	−9454	Yes	*	0,0479
Biodentine XP1:4 vs. TheraBase Ca ND	−40,74	Yes	****	<0,0001
Biodentine XP1:4 vs. TheraBase Ca 1:2	−11,27	Yes	**	0,0077
TheraCal PT ND vs. TheraCal PT 1:2	67,3	Yes	****	<0,0001
TheraCal PT ND vs. TheraCal PT 1:4	68,83	Yes	****	<0,0001
TheraCal PT ND vs. TheraBase Ca ND	36,01	Yes	****	<0,0001
TheraCal PT ND vs. TheraBase Ca 1:2	65,48	Yes	****	<0,0001
TheraCal PT ND vs. TheraBase Ca 1:4	73,91	Yes	****	<0,0001
TheraCal PT 1:2 vs. TheraBase Ca ND	−31,29	Yes	****	<0,0001
TheraCal PT 1:4 vs. TheraBase Ca ND	−32,82	Yes	****	<0,0001
TheraBase Ca ND vs. TheraBase Ca 1:2	29,47	Yes	****	<0,0001
TheraBase Ca ND vs. TheraBase Ca 1:4	37,9	Yes	****	<0,0001

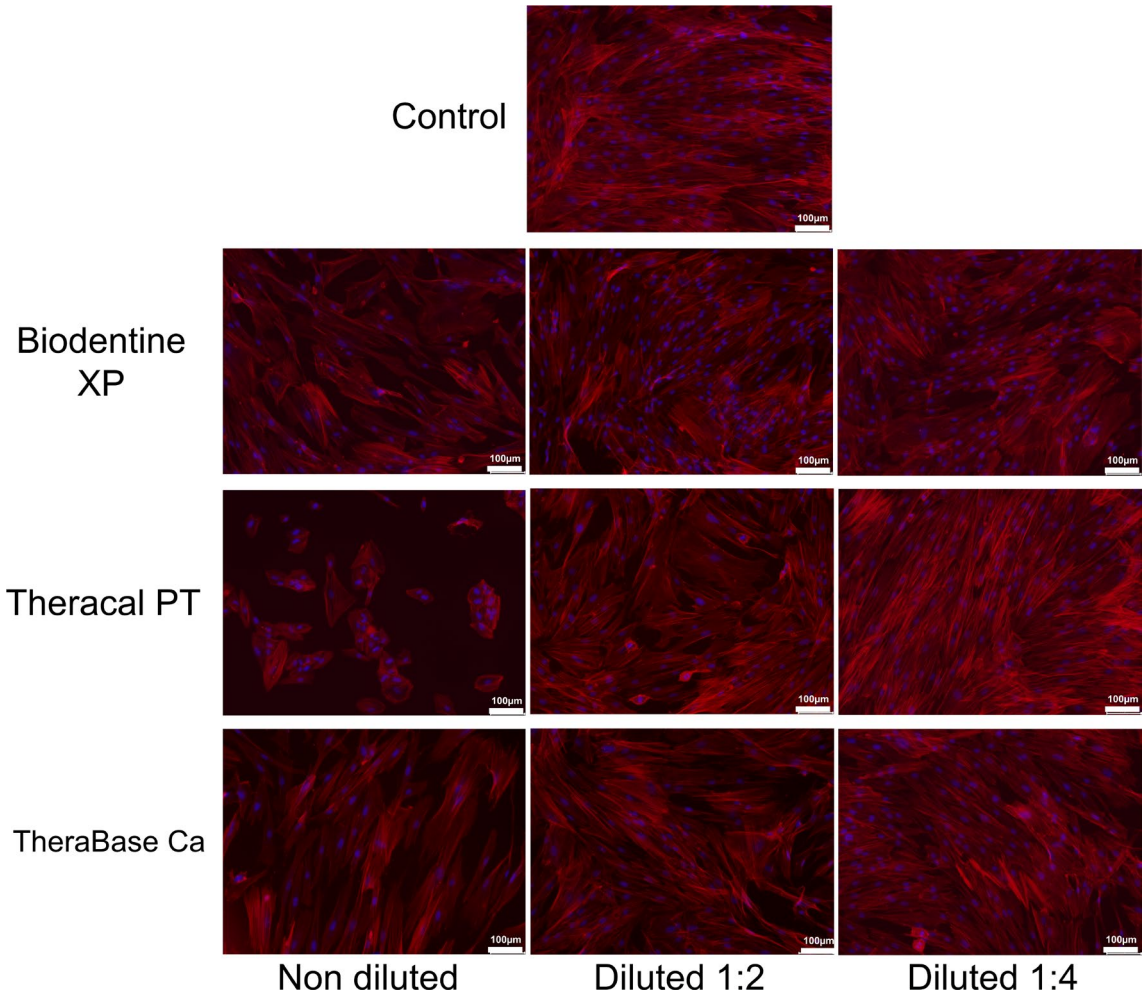

FIGURE 3 | Cytoskeleton staining of hDPSCs after 72 h of culture with undiluted eluates of BD-XP, THPT, and THB. The cytoskeleton of cells exposed to BD-XP remained well-structured, reflecting high biocompatibility and maintained cellular architecture. Scale bar = 100 µm.

Cell cycle analysis (Figure S1) demonstrated that exposure to higher concentrations of resin-modified calcium silicate led to an increased proportion of cells in the G0/G1 phase, suggesting a possible induction of cell cycle arrest. In contrast, at lower concentrations (25% and 50%), most cells remained in the G2/M phase, indicating minimal impact on cell cycle progression and preserving proliferative potential. These observations imply that while high concentrations of this material may transiently affect cell proliferation, more diluted forms are cytocompatible and do not interfere significantly with the normal cell cycle dynamics. All conditions were tested in triplicate and validated across three independent experiments.

3.3 | Cell Migration and Cell Morphology

Findings from the wound closure assay demonstrated that exposure to undiluted ThPT significantly affected the migratory capacity of hDPSCs in a concentration-dependent manner (Figure 2). While diluted extracts (25% and 50%) did not show notable differences compared to the control, undiluted material extracts was associated with a slower wound closure rate at both 48 and 72 h, with significant differences noted ($p < 0.01$ and $p < 0.001$) based on two-way ANOVA analysis (Table 2). Despite this, all conditions promoted partial wound closure over time, though at varying rates depending on the concentration. Phalloidin staining analysis (Figure 3) revealed clear differences in cytoskeletal organization in response to the material extracts. Cells exposed to higher concentrations of resin-modified calcium silicate evidenced altered cytoskeletal structure with disorganized F-actin filaments and impaired focal adhesion development, correlating with decreased cell spreading and confluence. In contrast, cells treated with diluted material extracts exhibited well-organized actin filaments and normal cytoskeletal architecture, comparable to control conditions. These results suggest that while higher concentrations may impair migration and cytoskeletal stability, lower concentrations maintain cellular morphology and functional integrity. All conditions were tested in triplicate and validated across three independent experiments.

3.4 | SEM and EDX Analysis

SEM observations at 1500× magnification revealed that hDPSCs exhibited well-developed cellular filopodia in contact with the surface of the tested VTP cements. These structures facilitated cell adhesion and spreading on the material surface, with clear evidence of cytoskeletal organization and anchorage within microenvironmental niches formed by the cement matrix (Figure 4). Cells displayed elongated morphology and close interaction with the

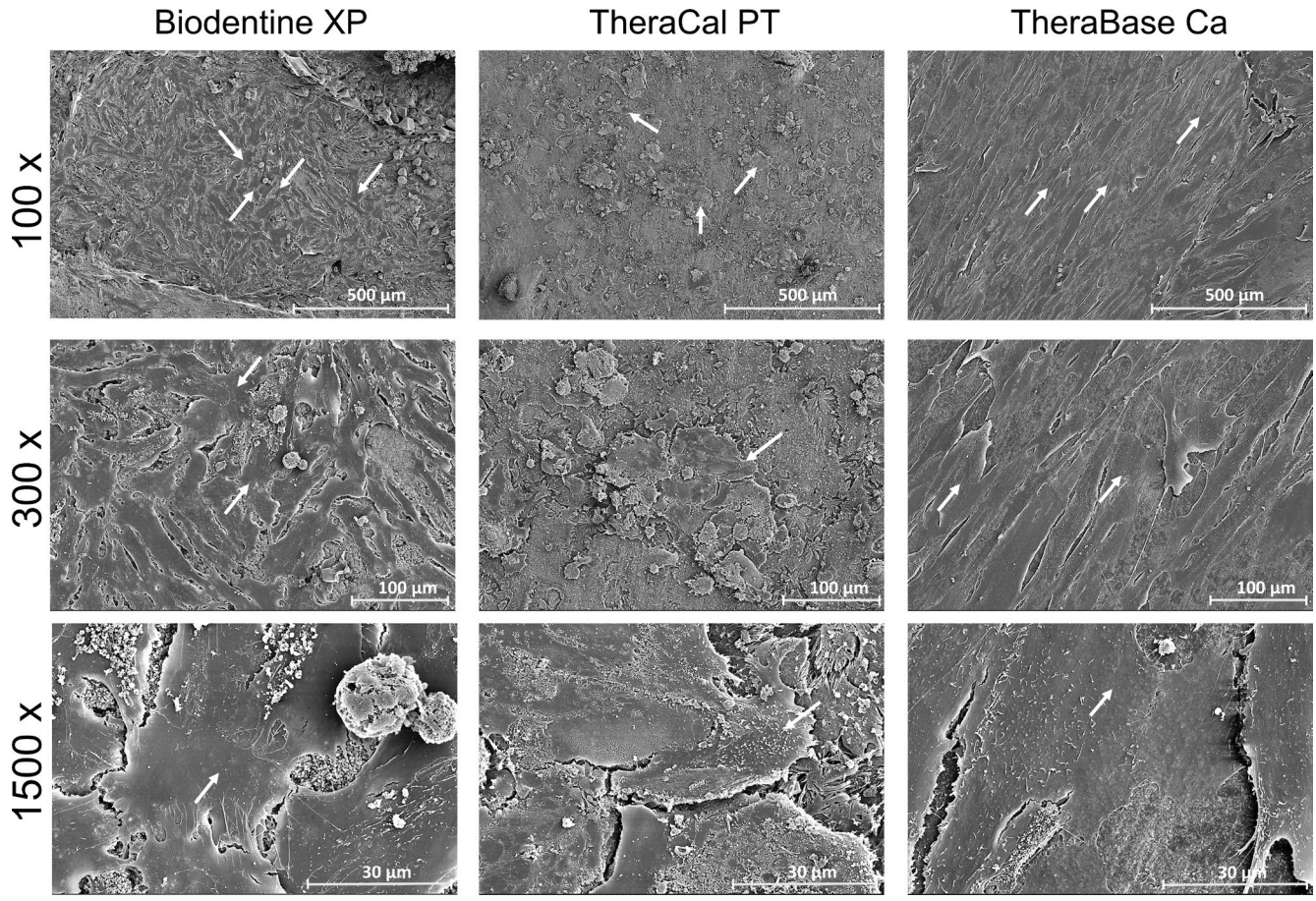

FIGURE 4 | SEM images showing cell adhesion on the surface of BD-XP, THPT, and THB after 72 h of culture. BD-XP presented better cellular anchorage and filopodia extension. Magnifications: 100X, 300X, 1500X. Scale bars = 400 µm, 100 µm, 20 µm.

substrate, indicating good biocompatibility and surface properties favorable for cell attachment. Complementary elemental analysis performed by EDX (Figure 5) verified the elemental composition of the VTP materials. In the calcium silicate-based material (A; Biodentine XP), EDX revealed high levels of calcium (35.13%), oxygen (48.57%), and carbon (8.00%), along with traces of silicon and chlorine. Conversely, the resin-modified calcium silicate material (B; TheraBase Ca) showed a composition dominated by oxygen (38.86%), carbon (25.03%), and silicon (13.23%), with moderate levels of aluminium (3.00%), strontium (4.47%), zirconium (4.72%), barium (3.79%), and ytterbium (4.66%), likely included as radiopacifying agents. The other resin-modified material (C; TheraCal PT) exhibited a different elemental profile, with major components including oxygen (42.48%), carbon (26.44%), silicon (9.54%), aluminium (4.30%), and strontium (3.77%), and smaller amounts of fluorine and barium. Notably, both resin-modified materials (B and C) demonstrated a markedly lower calcium content compared to Biodentine XP, which may limit their capacity for calcium ion release—a key factor in bioactivity and regenerative performance in pulp repair applications.

3.5 | Evaluation of Cell Apoptosis and Oxidative Ctress in hDPSCs

Cells were treated with BXP, THPT, and THB extracts at different dilutions (1:1, 1:2, and 1:4), labeled with FITC-conjugated Annexin-V and 7-AAD, followed by analysis via flow cytometry. As shown in Figure 6, all three compounds exhibited no cytotoxic effect at 1:4 and 1:2 dilutions. However, the undiluted (1:1) THB extract displayed the highest percentage of apoptotic and necrotic cells compared to the control and undiluted BXP and THPT extracts.

Flow cytometric analysis using the oxidative stress probe CM-H_2DCFDA was conducted to quantify intracellular reactive oxygen species (ROS) levels in hDPSCs exposed to the tested materials (Figure 7). All experimental groups exhibited a significant increase in ROS production compared to the untreated control group ($^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$), except for THPT at 1:4 and 1:2 dilutions, which did not significantly differ from baseline. Among the materials assessed, THB elicited the highest ROS levels, followed by BD-XP and THPT, particularly at the undiluted concentration (1:1). These results indicate that oxidative stress may contribute to the cytotoxicity associated with these calcium silicate-based materials.

3.6 | IL-6 Expression Analysis

The ELISA results for IL-6 secretion by hDPSCs in response to the VTP cements are shown in Figure 8. At day 3, all experimental groups (Biodentine XP, TheraCal PT, and TheraBase Ca) exhibited significantly lower IL-6 production compared to the

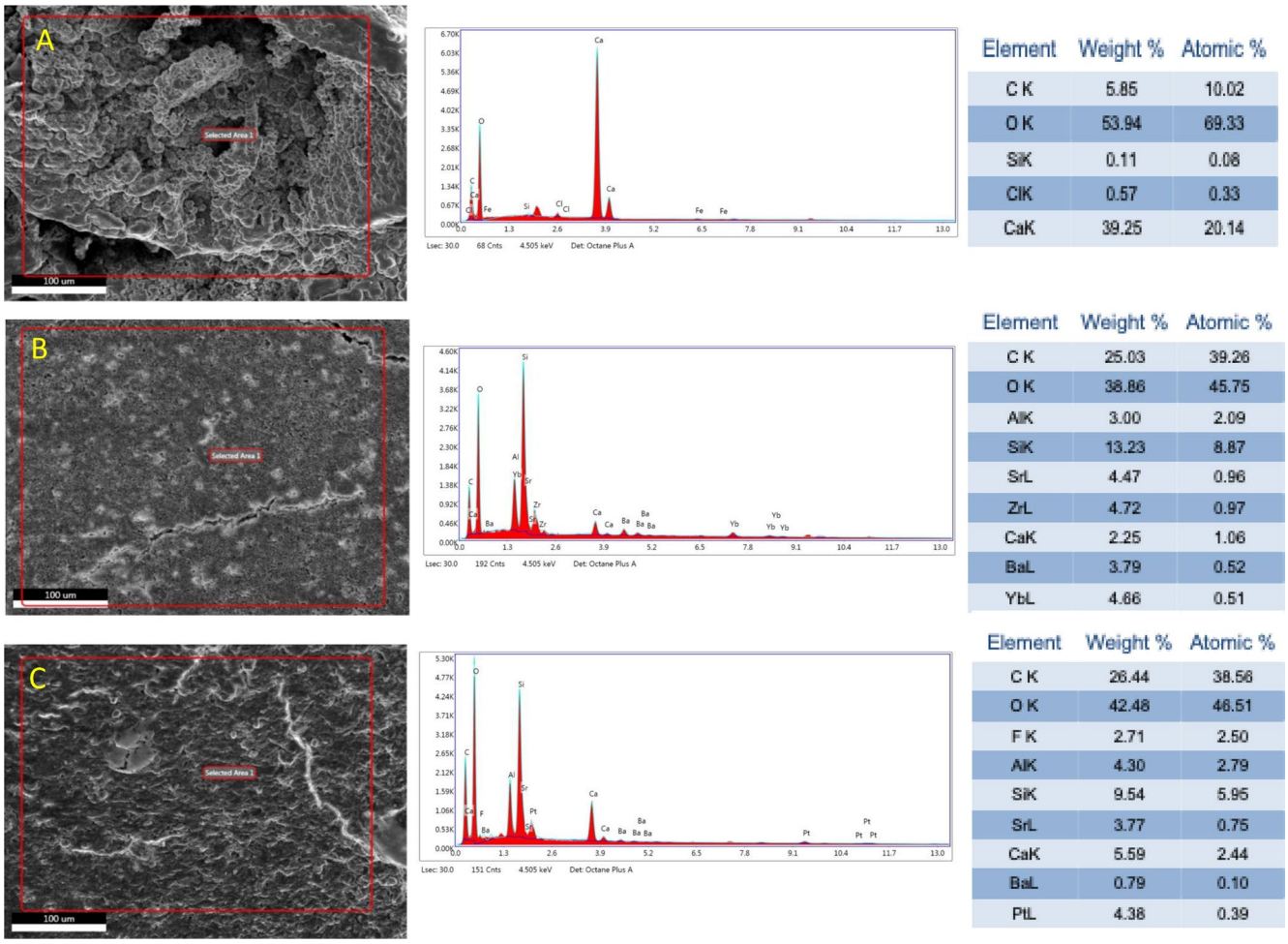

FIGURE 5 | SEM-EDS characterization of BD-XP (A), THB (B), and THPT (C). Column 1: Scanning electron microscopy (SEM) images of the materials (scale bar: 50 μm). Column 2: Energy-dispersive X-ray spectroscopy (EDS) profiles. Column 3: Elemental composition by weight. BD-XP showed high calcium levels, while THB and THPT had reduced calcium, which may influence bioactivity and regenerative potential.

control group (***$p < 0.001$), indicating an initial reduction of the proinflammatory response induced by these materials. This trend continued at day 7, with IL-6 concentrations in all experimental groups remaining significantly below those observed in the control group (**$p < 0.01$ for BD-XP and THB, *$p < 0.05$ for THPT). These findings indicate that all tested materials promote a reduction in IL-6 secretion over time, highlighting their potential role in modulating the early inflammatory response during pulp tissue healing.

3.7 | qRT-PCR and ARS Assays

Regarding the overexpression of genes involved in osteogenesis/cementogenesis, the OsteoDiff group (positive control) was the only condition to exhibit significant overexpression of the ALP gene at both day 14 and day 21, confirming its strong induction of early osteogenic activity. BD-XP, on the other hand, exhibited a sustained and significant overexpression of both RUNX2 and DSPP at day 14 and day 21, indicating its potent effect on osteo/odontogenic commitment and differentiation. Additionally, COL-1 expression in the BD-XP group was markedly increased by day 21. In contrast, THPT and THB induced lower and more variable expression levels of these genes, without reaching statistical significance when compared to the control (Figure 9).

Mineralization was further confirmed through ARS at day 21. The BD-XP group displayed the most intense staining, with the highest density and largest calcified nodules among all groups, showing a statistically significant difference compared to both THB and THPT-treated groups and the control (***$p < 0.001$). In contrast, THB and THPT induced only discrete calcium deposition, with less intense staining and smaller nodules (Figure 10). Taken together, these findings demonstrated that BD-XP is the most effective material in promoting osteogenic and odontogenic differentiation and mineralized matrix formation in hDPSCs.

4 | Discussion

The present study was conducted under the hypothesis that compositional variations among the evaluated vital pulp materials would elicit differential biological responses. This hypothesis was substantiated by the obtained results, which demonstrated statistically significant differences in the cytocompatibility of the bioactive pulp materials, thereby warranting the rejection of

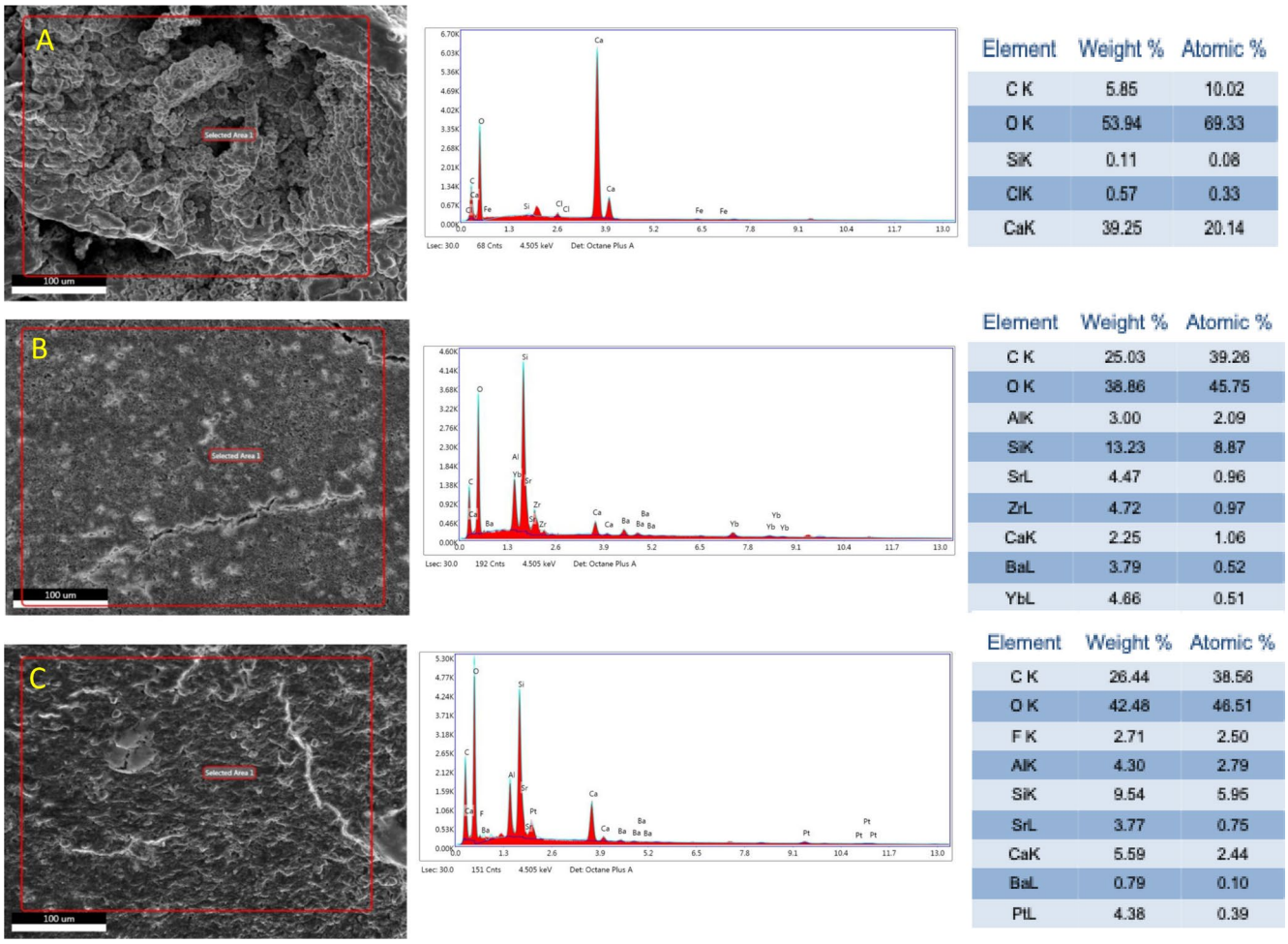

FIGURE 6 | Annexin V/7-AAD apoptosis assay of hDPSCs after 72 h exposure to BD-XP, THPT, and THB at 1:1, 1:2, and 1:4. Cell viability status, including apoptotic and necrotic fractions, is visualized in the dot plots. BD-XP maintained the highest cell viability. Representative of three experiments.

the null hypothesis. These variations were observed across several key parameters, including cell viability, migratory capacity, and cytoskeletal integrity. Such findings highlight the critical role of material composition in influencing cellular behavior, emphasizing the need for careful selection of vital pulp materials in clinical practice. A comprehensive understanding of the interactions between these biomaterials and hDPSCs that promote reparative dentin is essential for advancing regenerative endodontic strategies and informing the design of future bioactive formulations (Ayoub et al. 2025; El Hachem et al. 2024; Janini et al. 2025).

The biological behavior of new materials aimed at pulp repair and regeneration is fundamental to contribute to favorable outcomes in VTP (Rosa et al. 2025). Bioactive materials play a key role in determining the outcome of direct pulp capping (DPC). The main goal of applying a DPC material is to activate undifferentiated DPSCs, encouraging their differentiation into odontoblast-like cells that contribute to the formation of reparative dentin, thereby preserving and shielding the underlying pulp tissue (Pedano et al. 2021). Materials commonly used for this purpose include calcium hydroxide and calcium silicate-based cements, as they have demonstrated strong efficacy in maintaining pulp vitality and stimulating the formation of a dentin bridge as part of the reparative process. (Baldawa et al. 2024).

The in vitro assessment of cytocompatibility serves as a critical preliminary step in determining the potential clinical relevance and biological performance of vital pulp materials (Bueno et al. 2023; Sequeira et al. 2018), and the present work exhibited that these materials exhibit distinct patterns of cellular response. hDPSCs were selected for this study due to their ease of collection, minimal donor site morbidity, efficient isolation, and robust potential for differentiation. In addition, hDPSCs are known to interact well with various biomaterials, highlighting their relevance in tissue engineering and regenerative applications. Given these requirements, a comprehensive investigation into the cytocompatibility of materials applied in VPT is crucial for their successful clinical translation (Pedano et al. 2020; Rosa et al. 2025). Such materials should not only preserve but also enhance the viability and proliferation of hDPSCs, aligning with the innate regenerative capacity of the dental pulp tissue (Rosa et al. 2025).

In recent years, research has increasingly focused on resin-modified calcium silicate-based cements (RMCSCs) due to their promising bioactive characteristics (Tsuchiya et al. 2025).

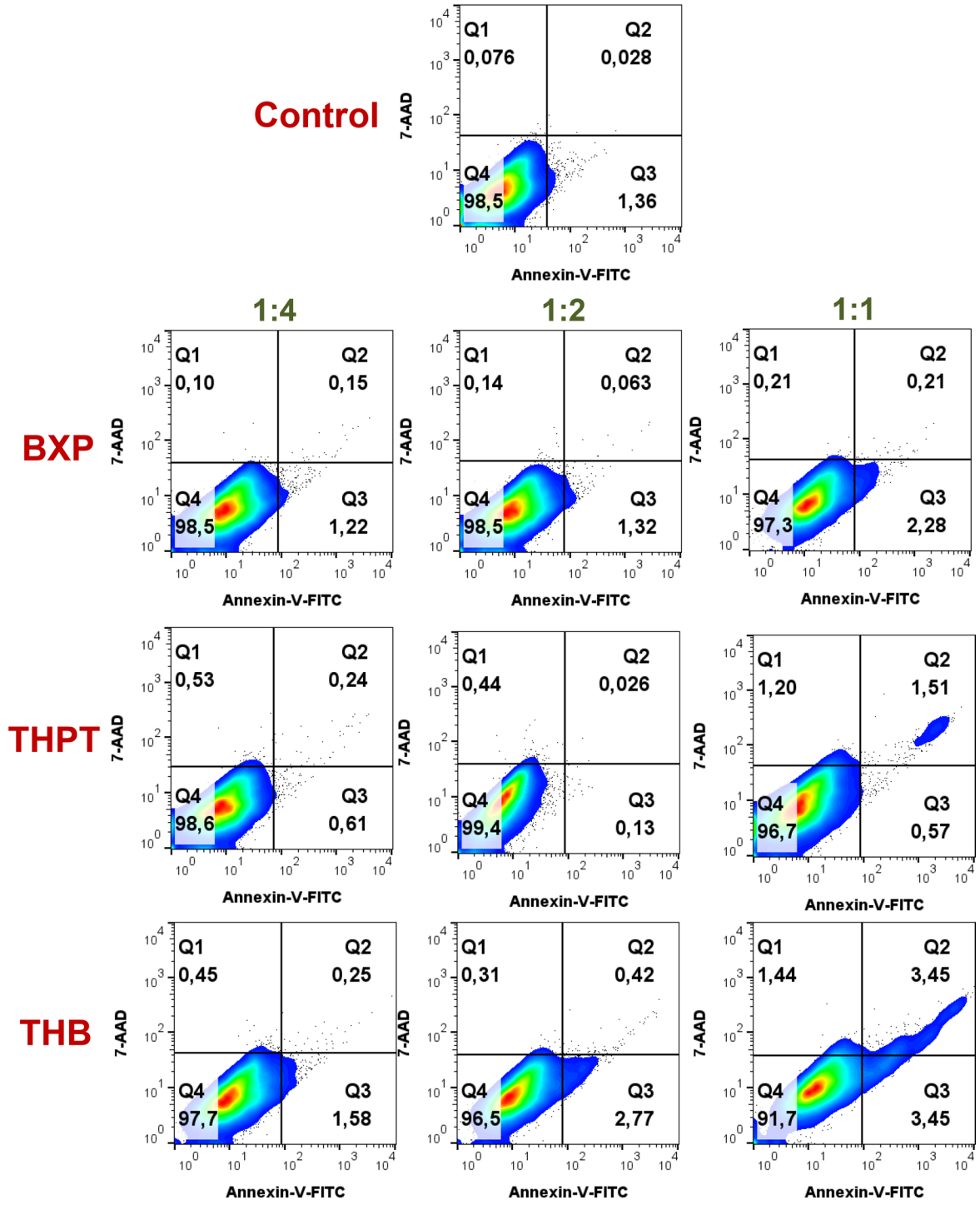

FIGURE 7 | Intracellular ROS production in hDPSCs measured by CM-H2DCFDA fluorescence after 72 h exposure to BD-XP, THPT, and THB. Bar graph shows percentage of ROS-positive cells. THB induced the highest ROS production. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (ANOVA).

These materials are known to release calcium and hydroxide ions, promoting hydroxyapatite formation and supporting dentin bridge development. However, despite the growing interest in this class of biomaterials, there remains limited information regarding the biological behavior and regenerative potential of certain newer formulations, particularly THB. The lack of comprehensive data on its interaction with hDPSCs underscores the need for further investigation to assess its effects on cell viability, differentiation, and overall compatibility for clinical use in vital pulp therapy. The study showed an increase in cell viability with increasing concentrations of BXP, indicating adequate biocompatibility to hDPSCs. However, cell viability rates were lower in the case of RMCSCs materials. Although THPT (Bisco) showed improved in vitro cytocomatibility compared to TheraCal LC and exhibited a biological behavior comparable to Biodentine (Sanz et al. 2021), in our results, cell viability rates remained lower than those observed with BD-XP. Cell cycle regulation plays an essential aspect in preseving the proliferative and regenerative capacity of hDPSCs, as it is closely linked to their pluripotency and differentiation

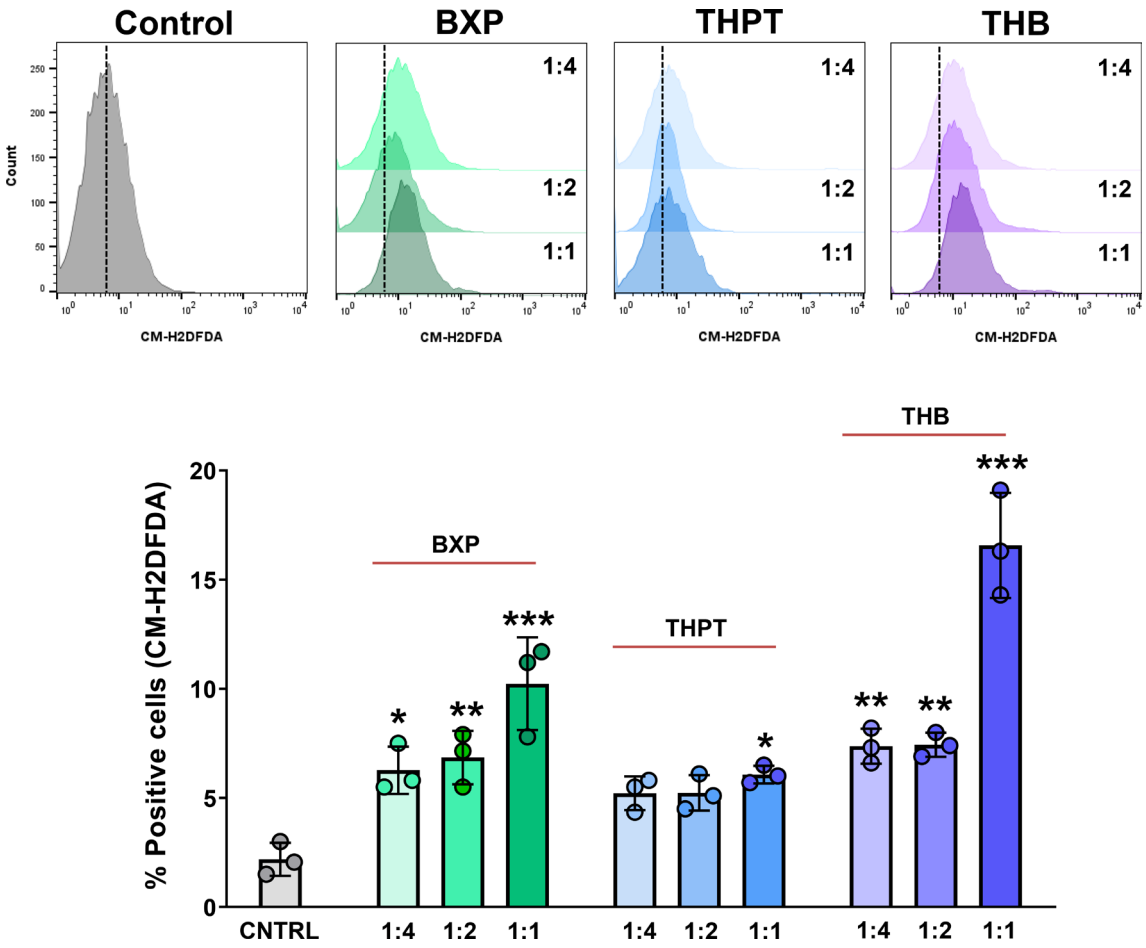

FIGURE 8 | ELISA quantification of IL-6 secretion by hDPSCs after 3 and 7 days of exposure to the VTP materials (BD-XP, THPT, and THB). At day 3, all material-treated groups showed a significant reduction in IL-6 levels compared to the untreated control group (***$p < 0.001$). This anti-inflammatory trend persisted at day 7, with IL-6 concentrations remaining significantly lower than the control (**$p < 0.01$ for BD-XP and THB; *$p < 0.05$ for THPT).

potential (Lopez-Garcia et al. 2021). As shown in Figure S1 exposure to higher concentrations of RMCSC materials led to an increased number of cells in the G0/G1 phase whereas the majority of DPSCs treated with BD-XP remained in the G2/M phase, suggesting minimal impact on cell cycle progression and preserving proliferative potential.

Effective reparation of pulp tissue not only relies on hDPSC proliferation but also on their directed migration and differentiation into odontoblast-like cells at the injury site. (Huang et al. 2024). Therefore, the capacity of pulp capping materials to support cell migration is critical for successful tissue regeneration. In this study, BD-XP promoted a measurable degree of wound closure at 24 h, indicating some level of support for cellular motility. However, when examining TheraBase Ca, the wound healing assay revealed a concentration-dependent effect on cell migration. Although the diluted extracts (1/4 and 1/2) groups did not exhibit significant differences compared to the untreated cells, the undiluted extracts groups resulted in a pronounced reduction in wound closure at both 48 and 72 h, with significant differences observed, as determined by two-way ANOVA (Figure 2, Table 2). These results suggest that, although lower concentrations of THB maintain cytocompatibility and allow for normal migratory behavior, higher concentrations may transiently hinder this process, potentially affecting early tissue regeneration dynamics. Among the experiments conducted, cytoskeletal changes were analyzed through immunocytofluorescence to assess the toxic effects of the materials used. In light of the findings reported by Rohr et al. [24], exposure of cells to cytotoxic components present in dental materials can compromise structural integrity, leading to morphological changes such as nuclear pyknosis and cytoskeletal disorganization. (Rohr et al. 2020). In the present study, RMRC-treated cells evidenced notable alterations in cytoskeletal architecture, including disorganized F-actin stress fibers and diminished focal adhesion complex formation, which correlated with reduced cell spreading and confluence. In contrast, cells treated with diluted material extracts retained well-organized actin filaments and preserved cytoskeletal integrity, resembling the morphology of control cells. Despite the potential for adverse effects at higher concentrations, no clear evidence of irreversible cytological damage was observed across conditions. These morphological observations align with the results obtained from the metabolic activity and wound healing assays.

The overproduction of reactive oxygen species (ROS) triggered by resin monomers present in dental materials has been linked to a range of adverse cellular outcomes, including cytotoxicity, DNA damage, and disturbances in the regulation of the cell cycle (Schweikl et al. 2006). Previous research by Sanz et al. (Sanz et al. 2021) investigated intracellular ROS levels in hDPSCs exposed to TheraCal LC, Biodentine, and TheraCal PT, reporting that Biodentine did not elicit a significant increase in

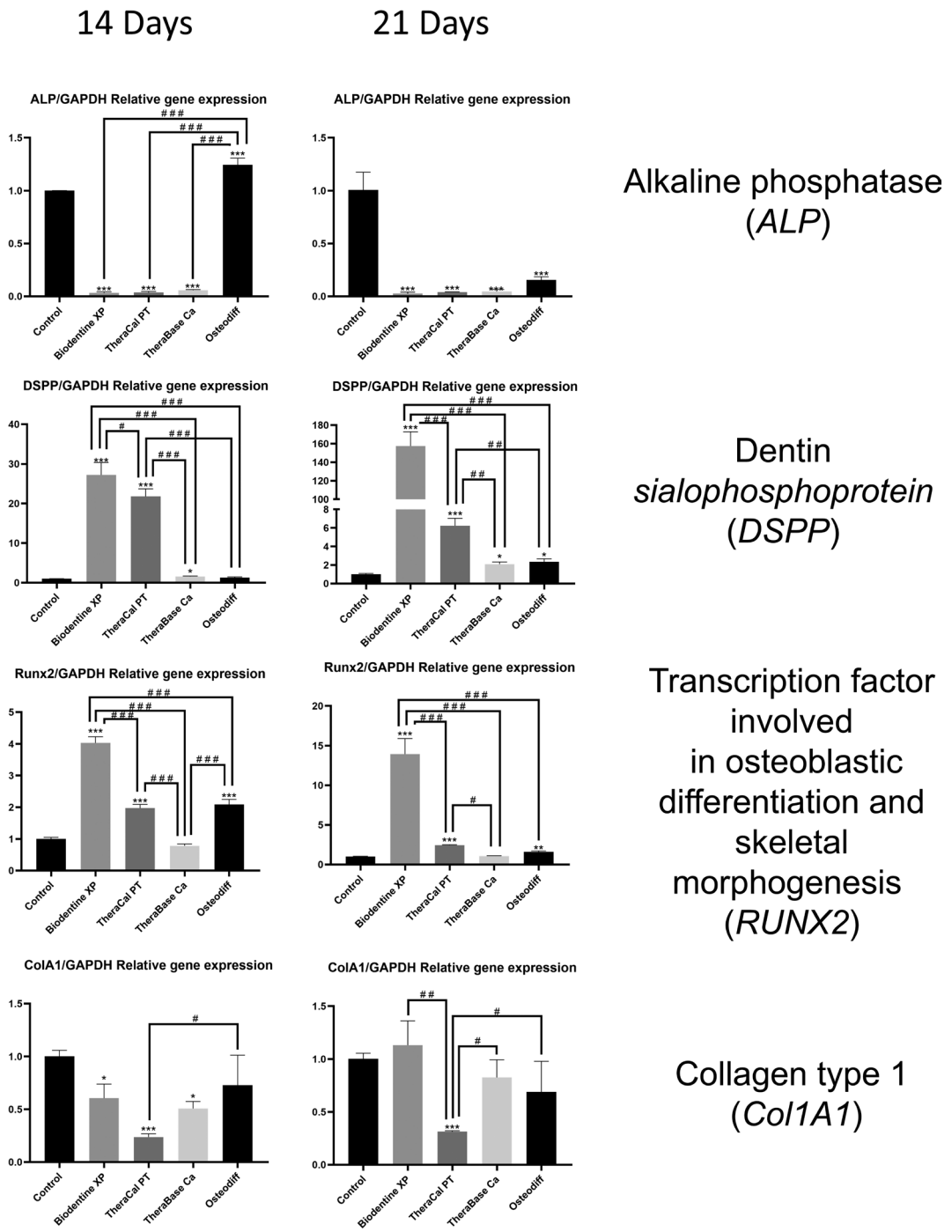

FIGURE 9 | RT-qPCR results of odontogenic/osteogenic markers (ALP, DSPP, COL1A1, RUNX2) in hDPSCs cultured with BD-XP, THPT, and THB at 14 and 21 days. Expression levels of DSPP and RUNX2 were significantly increased in response to BD-XP. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (two-way ANOVA).

ROS across different dilutions compared to untreated hDPSCs. In contrast, TheraCal LC was associated with markedly elevated ROS levels, although this effect diminished with increasing dilution. The high ROS generation observed in TheraCal LC has been attributed to the release of reactive additives during light curing, which may trigger cell damage and compromise cell membrane integrity (Chen and Suh 2017). These outcomes are supported by earlier reports indicating that methacrylate-based components in resin-modified materials can impair DPSC function, reduce cellular metabolism, and result in incomplete tissue bridge formation (Bakhtiar et al. 2017). In line with this evidence, our study demonstrated that THB induced the highest ROS production among the materials tested, followed by BD-XP and THPT, with the effect being most pronounced at the undiluted concentration (1:1).

The role of inflammation in pulp tissue repair is increasingly recognized as a fundamental aspect of the healing process, with cytokines such as interleukin-6 (IL-6) playing a central role in regulating early immune responses (Chen et al. 2021). IL-6, secreted by both macrophages and hDPCs, contributes to cellular recruitment, differentiation, and immunomodulation (Galler

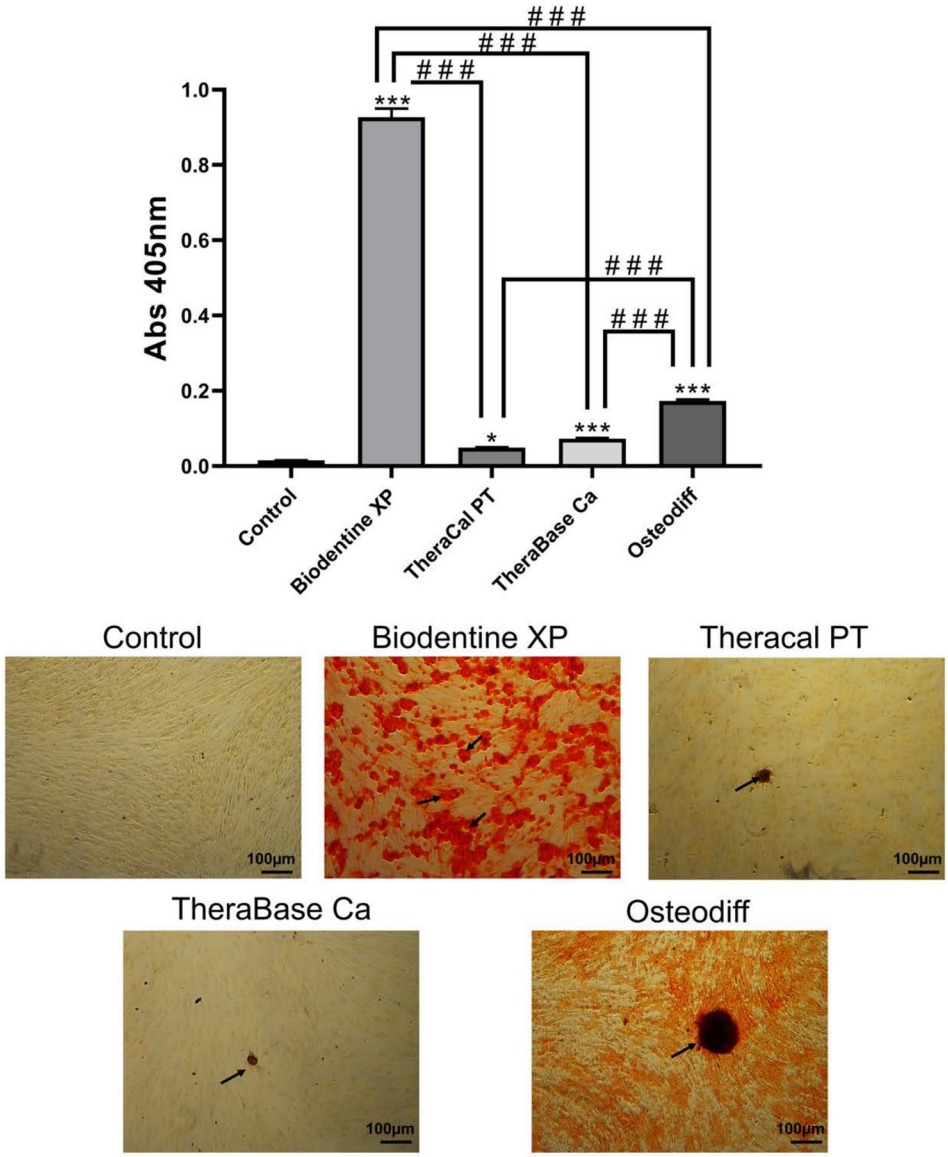

FIGURE 10 | ARS staining of hDPSCs following 21 days of incubation with BD-XP, THPT, and THB. BD-XP showed the highest mineral deposition, with larger and more numerous nodules. Representative micrographs along with quantitative data are shown. Statistical differences were determined using two-way ANOVA (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

et al. 2021). Prior investigations have indicated that calcium silicate-based materials like Biodentine can transiently modulate IL-6 levels, promoting a favorable inflammatory profile that supports regeneration. For instance, da Fonseca et al. (2016) observed a transient increase in IL-6 expression in vivo, which diminished over time, while Eraković et al. (2020) reported reduced pro-inflammatory cytokine expression in vitro in response to Biodentine (da Fonseca et al. 2016; Erakovic et al. 2020). Consistent with these findings, our results demonstrate that all tested materials—BXP, THPT, and THB—reduced IL-6 secretion in hDPSCs, an early anti-inflammatory response. Clinically, this modulation could help reduce postoperative inflammation and favor reparative dentin formation during the early healing phase (Esposito et al. 2024; Iaculli et al. 2022; Spagnuolo 2022).

A set of analytical methods was utilized to evaluate the biological effects of various vital pulp materials. These included the evaluation of odonto/osteogenic gene expression via RT-PCR and quantification of calcium accumulation based on Alizarin Red staining intensity (Sultan et al. 2025). Dentin sialophosphoprotein (DSPP) and dentin matrix protein-1 (DMP-1) are widely recognized as are widely acknowledged as pivotal indicators of odontoblastic differentiation (Martin-Gonzalez et al. 2022). Our results demonstrated that BD-XP consistently and significantly increased DSPP expression at days 14 and 21, highlighting its strong influence on odontogenic lineage commitment. This trend was accompanied by upregulated expression of additional differentiation-associated genes, such as RUNX2 and COL1A1, particularly at later time points, suggesting a progressive enhancement of the osteo/odontogenic phenotype. By contrast, different resin-containing calcium silicate materials, such as THPT and THB produced lower and more inconsistent expression levels of these genes, failing to reach statistical significance compared to the control. These observations underscore the superior bioinductive potential of BXP in promoting hDPSC differentiation through the activation of critical gene pathways. Finally, Alizarin Red S staining at day 21

revealed that BD-XP induced significantly greater mineralization than THB, THPT, and the control (***$p < 0.001$). These results indicate superior bioactivity of BD-XP, potentially exceeding that of MTA Angelus (Mora et al. 2025). However, limited existing data on hDPSC responses to BD-XP hinder direct comparison. Further studies are needed to clarify its mechanisms and clinical relevance.

This study presents several strengths, including compliance with ISO standards and high reproducibility across experimental replicates. Nonetheless, some limitations should be considered. As an in vitro study, the findings may not fully reflect the complex biological environment encountered in clinical scenarios. Although the controlled laboratory setting enables sample standardization and the isolation of specific variables, It does not accurately mimic the physiological conditions of the oral cavity or the tissue–material interactions in vivo. Therefore, while the data provide valuable insight into the early biological behavior of pulp capping materials, the results should be interpreted with caution. Ongoing confirmation through animal studies and clinical research is necessary to support the translational relevance of these outcomes.

5 | Conclusions

This study demonstrated that BD-XP exhibits superior biocompatibility, migratory support, and mineralization potential in hDPSCs compared to THB and THPT. Significant differences were observed in cell viability, oxidative stress, and gene expression profiles among the materials. BDXP promoted higher odontogenic marker expression and mineral nodule formation. These findings highlight the critical role of material composition in biological outcomes. Furthers in vivo investigations are warranted to validate the clinical significance of these findings.

Author Contributions

Investigation and methodology: Sergio López-García, Francisco Javier Rodríguez-Lozano, Nuria Pérez-Guzmán; supervision, visualization, conceptualization, and data curation: Paula García-Rios, Adrián Lozano; investigation, methodology, and writing – original draft: Francisco Javier Rodríguez-Lozano; conceptualization, formal analysis, project administration, supervision, validation, and writing – review and editing: Adrián Lozano, David García-Bernal; investigation, methodology, project administration, resources, writing – original draft, and writing – review and editing: Nuria Pérez-Guzmán, Francisco Javier Rodríguez-Lozano. All authors have read and agreed to the published version of the manuscript.

Funding

This work has been supported by the Spanish Network of Advanced Therapies (TERAV+), RICORS project (RD24/0014/0040) funded by the Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union—NextGeneration EU. Recovery, Transformation and Resilience Plan. This publication is also part of the project 22629/PI/24, funded by FSRM/10.13039/100007801.

Ethics Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Committee for Clinical Research of the Universidad de Murcia (ID: 4930/2024). Likewise, permission was obtained from the Health Department authorities to use the information contained in the CDHs, which had previously been anonymized by one of the investigators belonging to the medical staff of the Health Department in order to protect patient confidentiality. All the information was processed in accordance with the confidentiality regulations defined under Act 15/1999 referred to personal data protection.

Consent

Written informed consent was obtained from the parents of all individual participants included in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Ayoub, K. M., M. M. Nagy, R. M. Aly, G. N. El Deen, and K. El-Batouty. 2025. "Effect of Bio MTA Plus & ProRoot MTA Pulp Capping Materials on the Regenerative Properties of Human Dental Pulp Stem Cells." *Scientific Reports* 15, no. 1: 4749. https://doi.org/10.1038/s41598-025-88816-5.

Bailleul, L., L. Ceballos, S. Domejean, and V. Fuentes. 2025. "Deep Caries and Pulp Exposures Management Preferences in Permanent Teeth: A Survey Amongst Spanish Dentists." *International Endodontic Journal* 58, no. 2: 239–256. https://doi.org/10.1111/iej.14159.

Bakhtiar, H., M. H. Nekoofar, P. Aminishakib, et al. 2017. "Human Pulp Responses to Partial Pulpotomy Treatment With TheraCal as Compared With Biodentine and ProRoot MTA: A Clinical Trial." *Journal of Endodontics* 43, no. 11: 1786–1791. https://doi.org/10.1016/j.joen.2017.06.025.

Baldawa, H., V. Ravindran, G. Jeevanandan, et al. 2024. "Histopathological Assessment of Tricalcium Aluminate-Free Mineral Trioxide Aggregate and Two Antibacterial Enhanced Mineral Trioxide Aggregates as Pulpotomy Agents in Rat Model." *European Endodontic Journal* 9, no. 4: 344–351. https://doi.org/10.14744/eej.2024.53315.

Bueno, C. R. E., J. L. Sarmiento, A. M. V. Vasques, et al. 2023. "Biocompatibility, Biomineralization and Induction of Collagen Maturation With the Use of Calcium Hydroxide and Iodoform Intracanal Dressing." *J Funct Biomater* 14, no. 10: 507. https://doi.org/10.3390/jfb14100507.

Chen, J., H. Xu, K. Xia, S. Cheng, and Q. Zhang. 2021. "Resolvin E1 Accelerates Pulp Repair by Regulating Inflammation and Stimulating Dentin Regeneration in Dental Pulp Stem Cells." *Stem Cell Research & Therapy* 12, no. 1: 75. https://doi.org/10.1186/s13287-021-02141-y.

Chen, L., and B. I. Suh. 2017. "Cytotoxicity and Biocompatibility of Resin-Free and Resin-Modified Direct Pulp Capping Materials: A State-Of-The-Art Review." *Dental Materials Journal* 36, no. 1: 1–7. https://doi.org/10.4012/dmj.2016-107.

da Fonseca, T. S., G. F. da Silva, M. Tanomaru-Filho, E. Sasso-Cerri, J. M. Guerreiro-Tanomaru, and P. S. Cerri. 2016. "In Vivo Evaluation of the Inflammatory Response and IL-6 Immunoexpression Promoted by Biodentine and MTA Angelus." *International Endodontic Journal* 49, no. 2: 145–153. https://doi.org/10.1111/iej.12435.

El Hachem, C., S. Sauro, A. Eid, et al. 2024. "Novel Temporary Endodontic Medication Based on Calcium Silicate Strategy: A Biological and Physicochemical Study." *Frontiers in Dental Medicine* 5: 51275. https://doi.org/10.3389/fdmed.2024.1451275.

Elbahary, S., B. R. Johnson, Y. Nimer, et al. 2024. "Vital Pulp Therapy Outcomes in Children's First Permanent Molars Under General Anesthesia: A Retrospective Study." *Journal of Endodontics* 51: 3. https://doi.org/10.1016/j.joen.2024.12.003.

Erakovic, M., M. Duka, M. Bekic, et al. 2020. "Anti-Inflammatory and Immunomodulatory Effects of Biodentine on Human Periapical Lesion Cells in Culture." *International Endodontic Journal* 53, no. 10: 1398–1412. https://doi.org/10.1111/iej.13351.

Esposito, L., A. Niccolò Giuseppe, M. Cernera, M. Simeone, C. Rengo, and F. Iaculli. 2024. "Vital Pulp Therapy of Permanent Mature Teeth." *Giornale Italiano di Endodonzia* 38, no. 2: 13. https://doi.org/10.32067/GIE.2024.38.01.13.

Galler, K. M., M. Weber, Y. Korkmaz, M. Widbiller, and M. Feuerer. 2021. "Inflammatory Response Mechanisms of the Dentine-Pulp Complex and the Periapical Tissues." *International Journal of Molecular Sciences* 22, no. 3: 480. https://doi.org/10.3390/ijms22031480.

Huang, X., X. Ge, W. Fu, Z. Zhang, K. Xiao, and H. Lv. 2024. "Effects of Novel Nanoparticulate Bioceramic Endodontic Material on Human Dental Pulp Stem Cells in Vitro." *International Dental Journal* 74, no. 3: 482–491. https://doi.org/10.1016/j.identj.2023.12.008.

Iaculli, F., F. J. Rodríguez-Lozano, B. Briseño-Marroquín, T. G. Wolf, G. Spagnuolo, and S. Rengo. 2022. "Vital Pulp Therapy of Permanent Teeth With Reversible or Irreversible Pulpitis: An Overview of the Literature." *Journal of Clinical Medicine* 11, no. 14: 4016. https://doi.org/10.3390/jcm11144016.

Janini, A. C. P., B. F. Moraes, L. E. Pelepenko, et al. 2025. "Physicochemical Properties and Biological Interaction of Calcium Silicate-Based Sealers - In Vivo Model." *Clinical Oral Investigations* 29, no. 1: 86. https://doi.org/10.1007/s00784-025-06150-6.

Koutrouli, A., F. Machla, K. Arapostathis, M. Kokoti, and A. Bakopoulou. 2024. "Biological Responses of Two Calcium-Silicate-Based Cements on a Tissue-Engineered 3D Organotypic Deciduous Pulp Analogue." *Dental Materials* 40, no. 5: e14–e25. https://doi.org/10.1016/j.dental.2024.02.024.

Lopez-Garcia, S., J. Guerrero-Girones, M. P. Pecci-Lloret, M. R. Pecci-Lloret, F. J. Rodriguez-Lozano, and D. Garcia-Bernal. 2021. "In Vitro Biocompatibility of CPP-ACP and Fluoride-Containing Desensitizers on Human Gingival Cells." *Operative Dentistry* 46, no. 6: E264–E275. https://doi.org/10.2341/20-245-L.

López-García, S., J. L. Sanz, L. Murcia, et al. 2024. "Assessment of the Anti-Inflammatory and Biological Properties of Bioroot Flow: A Novel Bioceramic Sealer." *Tissue and Cell* 88: 102391. https://doi.org/10.1016/j.tice.2024.102391.

Lozano-Guillen, A., S. Lopez-Garcia, F. J. Rodriguez-Lozano, et al. 2022. "Comparative Cytocompatibility of the New Calcium Silicate-Based Cement NeoPutty Versus NeoMTA Plus and MTA on Human Dental Pulp Cells: An in Vitro Study." *Clinical Oral Investigations* 26, no. 12: 7219–7228. https://doi.org/10.1007/s00784-022-04682-9.

Martin-Gonzalez, J., J. J. Segura-Egea, A. Perez-Perez, D. Cabanillas-Balsera, and V. Sanchez-Margalet. 2022. "Leptin in Dental Pulp and Periapical Tissues: A Narrative Review." *International Journal of Molecular Sciences* 23, no. 4: 984. https://doi.org/10.3390/ijms23041984.

Mora, A., D. Garcia-Bernal, F. J. Rodriguez-Lozano, J. Ghilotti, A. Lozano, and S. Lopez-Garcia. 2025. "Biocompatibility and Osteogenic Potential of Novel Tricalcium Silicate-Based Materials in Human Dental Pulp Stem Cells: Advancing Vital Pulp Therapies." *Dental Materials* 41: 644–657. https://doi.org/10.1016/j.dental.2025.03.011.

Osorio, R., F. J. Rodriguez-Lozano, M. Toledano, et al. 2024. "Mitigating Lipopolysaccharide-Induced Impairment in Human Dental Pulp Stem Cells With Tideglusib-Doped Nanoparticles: Enhancing Osteogenic Differentiation and Mineralization." *Dental Materials* 40, no. 10: 1591–1601. https://doi.org/10.1016/j.dental.2024.07.012.

Pedano, M. S., X. Li, K. Yoshihara, K. V. Landuyt, and B. Van Meerbeek. 2020. "Cytotoxicity and Bioactivity of Dental Pulp-Capping Agents Towards Human Tooth-Pulp Cells: A Systematic Review of in-Vitro Studies and Meta-Analysis of Randomized and Controlled Clinical Trials." *Materials (Basel)* 13, no. 12: 670. https://doi.org/10.3390/ma13122670.

Pedano, M. S., K. Yoshihara, X. Li, B. Camargo, K. Van Landuyt, and B. Van Meerbeek. 2021. "Experimental Resin-Modified Calcium-Silicate Cement Containing N-(2-Hydroxyethyl) Acrylamide Monomer for Pulp Tissue Engineering." *Materials Science & Engineering. C, Materials for Biological Applications* 126: 112105. https://doi.org/10.1016/j.msec.2021.112105.

Rodriguez-Lozano, F. J., S. Lopez-Garcia, D. Garcia-Bernal, et al. 2021. "Cytocompatibility and Bioactive Properties of the New Dual-Curing Resin-Modified Calcium Silicate-Based Material for Vital Pulp Therapy." *Clinical Oral Investigations* 25: 5009–5024. https://doi.org/10.1007/s00784-021-03811-0.

Rohr, N., C. Bergemann, J. B. Nebe, and J. Fischer. 2020. "Crystal Structure of Zirconia Affects Osteoblast Behavior." *Dental Materials* 36, no. 7: 905–913. https://doi.org/10.1016/j.dental.2020.04.017.

Rosa, V., B. N. Cavalcanti, J. E. Nor, et al. 2025. "Guidance for Evaluating Biomaterials' Properties and Biological Potential for Dental Pulp Tissue Engineering and Regeneration Research." *Dental Materials* 41, no. 3: 248–264. https://doi.org/10.1016/j.dental.2024.12.003.

Saber, S. M., S. M. Gomaa, M. M. Elashiry, A. El-Banna, and E. Schafer. 2023. "Comparative Biological Properties of Resin-Free and Resin-Based Calcium Silicate-Based Endodontic Repair Materials on Human Periodontal Ligament Stem Cells." *Clinical Oral Investigations* 27, no. 11: 6757–6768. https://doi.org/10.1007/s00784-023-05288-5.

Sanz, J. L., A. Soler-Doria, S. Lopez-Garcia, et al. 2021. "Comparative Biological Properties and Mineralization Potential of 3 Endodontic Materials for Vital Pulp Therapy: Theracal PT, Theracal LC, and Biodentine on Human Dental Pulp Stem Cells." *Journal of Endodontics* 47, no. 12: 1896–1906. https://doi.org/10.1016/j.joen.2021.08.001.

Schweikl, H., G. Spagnuolo, and G. Schmalz. 2006. "Genetic and Cellular Toxicology of Dental Resin Monomers." *Journal of Dental Research* 85, no. 10: 870–877. https://doi.org/10.1177/154405910608501001.

Sequeira, D. B., C. M. Seabra, P. J. Palma, A. L. Cardoso, J. Peca, and J. M. Santos. 2018. "Effects of a New Bioceramic Material on Human Apical Papilla Cells." *J Funct Biomater* 9, no. 4: 74. https://doi.org/10.3390/jfb9040074.

Spagnuolo, G. 2022. "Bioactive Dental Materials: The Current Status." *Materials (Basel)* 15, no. 6: 16. https://doi.org/10.3390/ma15062016.

Spagnuolo, G., B. Codispoti, M. Marrelli, C. Rengo, S. Rengo, and M. Tatullo. 2018. "Commitment of Oral-Derived Stem Cells in Dental and Maxillofacial Applications." *Dent J (Basel)* 6, no. 4: 72. https://doi.org/10.3390/dj6040072.

Sultan, N. A., H. H. Hamama, M. E. Grawish, R. I. El-Toukhy, and S. H. Mahmoud. 2025. "Impact of Different Capping Materials Extracts on Proliferation and Osteogenic Differentiation of Cultured Human Dental Pulp Stem Cells." *Scientific Reports* 15, no. 1: 11140. https://doi.org/10.1038/s41598-025-93759-y.

Tsuchiya, K., S. Sauro, H. Sano, et al. 2025. "Clinical Applications and Classification of Calcium Silicate-Based Cements Based on Their History and Evolution: A Narrative Review." *Clinical Oral Investigations* 29, no. 4: 187. https://doi.org/10.1007/s00784-025-06274-9.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Flow-cytometry analyses of hDPSCs after 72 h of continuous treatment with different concentrations of Biodentine XP, TheraBase Ca and Theracal PT were conducted to monitor potential interference with cell-cycle progression.