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Immunomodulatory Roles of Interleukins in Root Canal Healing: A Molecular Perspective on Endodontic Sealer Biocompatibility

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ABSTRACT

Background: Effective root canal treatment is not only associated with the eradication of infection but also with responses of tissue to endodontic adhesives. The periapical immune microenvironment, especially interleukin (IL) signaling, is essential for periapical healing. The purpose of this study was to determine the immunomodulatory functions of ILs during root canal healing and assess the molecular biocompatibility with endodontic sealers.

Methods: In this cross-sectional study, 90 human periodontal ligament fibroblast (hPDLF) samples were exposed to 3 sealers (Bio ceramic, zinc oxide eugenol (ZOE), and epoxy resin). ELISA was used to measure cytokine levels (IL-1β, IL-6, IL-8, IL-10); osteogenic markers, including Alkaline Phosphatase (ALP), Runt-related TF-2 (RUNX2), and Osteocalcin (OCN), were quantified by real-time qPCR, and cell viability by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. ANOVA and Chi-square tests were used to analyze data, where $p < 0.05$ was considered significant.

Results: Bio-ceramic sealers exhibited maximum IL-10 (99.7 ± 8.7) and osteogenic markers expression and significantly reduced IL-6 (116.2 ± 13.7) and IL-8 (106.8 ± 12.2) expressions ($p < 0.001$). Epoxy resin was moderately biocompatible. ZOE had higher pro-inflammatory cytokines (132.1, 245.8, 202.6 for IL-1β, IL-6, and IL-8, respectively) and lower cell viability (69.4 ± 6.1, $p < 0.001$).

Conclusion: Bio-ceramic sealers indicated better immunomodulatory and regenerative capabilities, which favor their use in clinical practice for root canal treatment. These findings highlight the requirement for molecular compatibility during the selection of the sealer.

Keywords: Interleukins, Root Canal Therapy, Biocompatible Materials, Cytokines, Osteogenesis.
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INTRODUCTION

Chronic periapical inflammation contributes to delayed or unsuccessful healing following root canal treatment, often due to cytokine-mediated immune responses toward endodontic sealers [1]. Sealer components increase the release of interleukins (IL), particularly IL-1β, IL-6, and IL-8, which contribute to chronic inflammation and tissue degradation [2]. Generally used sealers, such as zinc oxide eugenol (ZOE) and epoxy resin, have been linked to high concentrations of pro-inflammatory cytokines and poor fibroblast survival [3].

Calcium silicate-based bio-ceramic sealers have also been studied due to their exceptional cytocompatibility and bioactivity [4]. These materials can provide calcium ions, regulate pH, and promote cellular signaling to promote healing [5]. The literature has also revealed decreased levels of IL-6 and IL-8 and elevated expression of IL-10 when fibroblasts are exposed to calcium silicate sealers [6]. IL-10 is a highly important anti-inflammatory cytokine that inhibits immune overactivation and helps decrease inflammation [7].

Bio-ceramic sealers not only promote immune-modulatory properties but also increase osteogenic gene expression, including RUNX2, ALP, and osteocalcin, which play a vital role in periapical bone formation [8]. These influences help in making the periapical environment more stable and regenerative [9]. Nevertheless, most studies have focused on the individual effects of inflammation and osteogenesis, restricting inferences on the biological interaction between the two. There is very little comprehensive analysis of cytokine modulation related to osteogenic potential in the same cellular system [10]. Knowledge of this dual role may be useful in the discovery of sealers that may promote periapical healing and maximize immune control during endodontic care.

The objective of this study is to compare the impacts of ZOE, epoxy resin, and bio-ceramic sealers on the expression of interleukins and osteogenic genes in human periodontal ligament fibroblasts (hPDLFs). It also evaluates IL-1β, IL-6, IL-8, and IL-10 levels, and markers, including Alkaline Phosphatase (ALP), Runt-related TF-2 (RUNX2), and Osteocalcin (OCN). Moreover, the purpose is to find out the material that best promotes immune regulation and osteogenesis.

METHODS

A cross-sectional comparative study including 90 human periodontal ligament fibroblasts was a collaborative conduction with contribution of authors from SMDC Lahore, BMDC Mirpurkhas, SU and SZH Lahore from February to June 2024, after informed consent was obtained before the extraction of their teeth. Primary hPDLFs were obtained by extracting third molars of healthy donors, recruited through non-probability consecutive sampling. OpenEpi version 3.0.0 (released 2013,

Atlanta, GA, USA) was used to calculate a sample size of 90, with 80% power, 95% confidence level, and expected effect size based on earlier cytokine expression studies [11]. The inclusion criteria involved third molars removed orthodontically or prophylactically by donors aged between 18 and 40 years with good systemic health, without active periodontal disease, and the absence of any chronic inflammation. Teeth with root resorption or periapical pathology and donors with systemic diseases (e.g., diabetes, autoimmune disease), a recent history (up to 3 months) of smoking, donors on antibiotics or corticosteroids, or with signs of oral infections were excluded.

The cells were equally categorized into three groups: ZOE, epoxy resin, and bioceramic sealer groups. The sealers were applied indirectly via the Transwell membrane system to prevent direct cytotoxicity. The Enzyme-linked immunosorbent assay (ELISA) was used to quantify cytokine expression (IL-1b, IL-6, IL-8, IL-10), and real-time quantitative (qPCR) was used to measure osteogenic markers, including ALP, RUNX2, and OCN. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was used to determine cell viability using standard routine control. Every experiment was conducted three times. Data were analyzed by SPSS version 26.0 (released 2019, IBM Corp., Armonk, NY). The chi-square test was used to analyze categorical variables, and ANOVA was used to analyze the continuous variables. The p-value of less than 0.05 was considered statistically significant.

RESULTS

The study comprised 90 participants (30 in each group) to determine the immunomodulatory influence of different sealers on healing root canals. The objective was to determine the ILs, cell viability, and osteogenic gene activity in hPDLFs. Bio-ceramic sealers exhibited high cell viability and IL-10 concentrations, but reduced IL-1, IL-6, and IL-8 compared to other groups. They also increased the expression of ALP, RUNX2, and OCN, which highlights better osteogenic potency. **Table 1** indicates the demographic characteristics of the study participants.

Table 1: Demographic Characteristics of Study Participants

Variable	ZOE Group (n=30)	Epoxy Resin Group (n=30)	Bioceramic Group (n=30)	Test Value	*p*-value
Donor Age (years, mean ± SD)	35.9 ± 4.7	36.3 ± 5.1	34.8 ± 4.9	F = 0.72	0.489
Gender (Male/Female)	16 (53.3%)/ 14 (46.7%)	15(50.0%)/ 15 (50.0%)	14(46.7%)/ 16 (53.3%)	χ^2 = 0.27	0.873

Smoking Status (Yes/No)	6(20.0%)/ 24 (80.0%)	4 / 26 (13.3% / 86.7%)	3(10.0%)/ 27 (90.0%)	χ^2 = 1.41	0.494

*n = Sample size, ZOE = zinc oxide eugenol, SD = Standard Deviation, % = Percentage, * = Significance at p-value <0.05*

There were no significant differences between the three groups in terms of donor age (mean range: 34.8 to 36.3 years, p = 0.489), gender (p = 0.873), and the prevalence of smokers (p = 0.494). Demographic similarity validates the different sealer types, not donor variation. The primer sequences were confirmed by doing In-Silico PCR on the UCSC Genome Browser. The list of designed primers is given in **Table 2.**

Table 2: Primer Sequences Selected Biomarkers

Target Gene	Primer Sequences
IL-1β	**Forward:** 5'-ATGATGGCTTATTACAGTGGCAA-3'
	Reverse: 5'-GTCGGAGATTCGTAGCTGGA-3'
IL-6	**Forward:** 5'-ACTCACCTCTTCAGAACGAATTG-3'
	Reverse: 5'-CCATCTTTGGAAGGTTCAGGTTG-3'
IL-10	**Forward:** 5'-GAAGGAGCTGCTCTTCCGA-3'
	Reverse: 5'-GAGCATGACCCTGTAGGC-3'
IL-8 (CXCL8)	**Forward:** 5'-ACTGAGAGTGATTGAGAGTGGAC-3'
	Reverse: 5'-AACCCTCTGCACCCAGTTTTC-3'

The RT-qPCR was optimized according to the annealing temperatures (Tm) of each primer. Conditions for the three major steps of RT-qPCR, consisting of denaturation, annealing, and extension were set according to each primer as shown below in **Figure 1.**

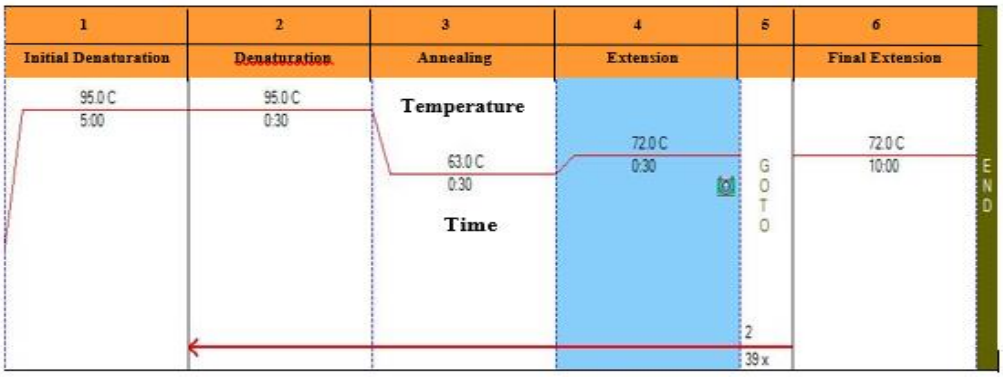

Figure 1: Major Steps of RT-qPCR

Figure 2: presents the cell viability (%) as outcome of the MTT assay, which indicates much better cell viability in hPDLFs in the presence of the bioceramic sealer in association with epoxy resin and ZOE sealers.

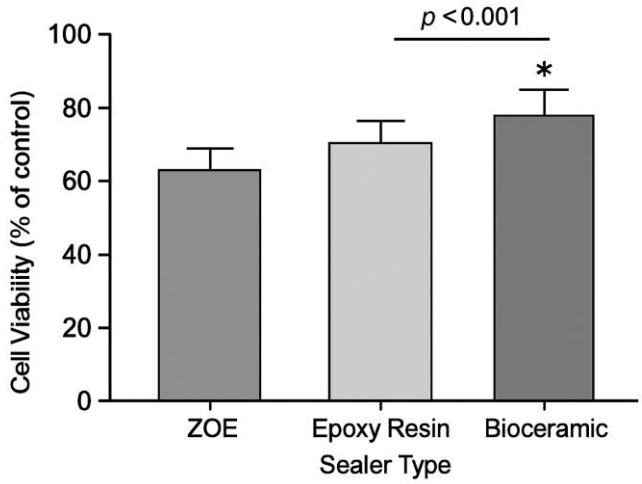

Figure 2: Cell viability (%) of compounds

The bioceramic group was the most metabolically active, which means that they are more biocompatible ($p < 0.001$). Error bars are measures of standard deviation. Cytokine expression and cell viability at 72 hours post-sealer exposure are illustrated in **Table 3.**

Table 3: Cytokine Expression and Cell Viability at 72 hours Post-Sealer Exposure

Parameter	ZOE Group	Epoxy Resin Group	Bioceramic Group	Test Value	*p*-value
Cell Viability (% of control)	69.4 ± 6.1	75.8 ± 5.6	91.8 ± 4.3	F = 92.73	<0.001
IL-1β (pg/mL, mean ± SD)	132.1 ± 14.9	112.7 ± 12.8	63.4 ± 10.2	F = 114.2	<0.001
IL-6 (pg/mL, mean ± SD)	245.8 ± 22.4	199.6 ± 18.5	116.2 ± 13.7	F = 121.4	<0.001
IL-10 (pg/mL, mean ± SD)	46.5 ± 7.1	58.6 ± 7.2	99.7 ± 8.7	F = 139.5	<0.001
IL-8 (pg/mL, mean ± SD)	202.6 ± 19.7	180.3 ± 17.2	106.8 ± 12.2	F = 106.1	<0.001

*n = Sample size, IL = Interleukin, SD = Standard Deviation, % = Percentage, * = Significance at p-value <0.05*

Cell viability was much higher with bio-ceramic sealers (91.8) compared to epoxy resin (75.8) and ZOE (69.4) ($p < 0.001$). Comparing all the groups, the pro-inflammatory cytokines IL-1β, IL-6, and IL-8 were lowest, whereas IL-10 was highest in the bio-ceramic group (63.4, 116.2, 106.8 pg/mL, respectively), with significance ($p < 0.001$). These findings demonstrate that bio-ceramic sealers show high immunomodulatory activity. Osteogenic gene expression is demonstrated in **Table 4.**

Table 4: Osteogenic Gene Expression

Marker (Relative Fold Change)	ZOE Group	Epoxy Resin Group	Bioceramic Group	Test Value	*p*-value
ALP	0.86 ± 0.11	1.10 ± 0.09	2.37 ± 0.17	F = 152.3	<0.001
OCN	0.68 ± 0.07	0.94 ± 0.08	1.91 ± 0.16	F = 136.4	<0.001
RUNX2	0.83 ± 0.09	1.21 ± 0.11	2.08 ± 0.14	F = 147.8	<0.001

*n = Sample size, ALP = Alkaline Phosphatase, OCN = Osteocalcin), RUNX2 = Runt-related TF 2, SD = Standard Deviation, * = Significance at p-value <0.05*

Bio-ceramic-treated cells had the highest expression of ALP (2.37), OCN (1.91), and RUNX2 (2.08), compared to both epoxy resin and ZOE ($p < 0.001$). These results indicate an increased osteogenic capacity and support the use of bio-ceramic sealers to induce the regeneration of periapical tissues.

DISCUSSION

This study aimed to evaluate the immunomodulatory and biocompatibility of three types of endodontic sealers, including ZOE, epoxy resin, and bio-ceramic, related to hPDLFs in root canal healing. The findings support that bio-ceramic sealers are more favorable due to inflammatory regulation and increased osteogenic differentiation. The findings showed that IL-10 was highly stimulated in bio-ceramic sealer-treated fibroblasts and suppressed IL-1β, IL-6, and IL-8. These data align with literature that bio-ceramic sealers are known to influence cytokine secretion by inhibiting NF-kappa-B activation and downregulation of the inflammatory pathway [12]. In similar research, it was verified that these materials can have a strong alkaline pH and release calcium ions that promote an anti-inflammatory environment [13].

Increased expression of osteogenic markers- ALP, RUNX2, and osteocalcin found in the bio-ceramic group is consistent with earlier studies, which showed the osteoinductive capacity of calcium silicate sealers in vitro [14]. The improved expression of these markers also corresponds to results that indicated bio-ceramic sealers favored extracellular matrix maturation [15]. In contrast, the epoxy resin group showed moderate IL-6 augmentation and reduced osteogenic gene expression, demonstrating the diminutive ability of resin-based sealers to promote regenerative signaling based on remaining monomers [16].

The strongest IL-6 and IL-8 responses were exhibited by ZOE sealers, in agreement with the available literature regarding their inflammatory potential and human fibroblast cytotoxicity [17]. Studies have also shown apoptosis and membrane disturbance to confirm their deleterious effects on cell viability [18]. Other studies that employed immunolabeling methods also showed high levels of IL-6 and TNF-alpha in tissues exposed to ZOE sealers [19].

Despite the agreement of findings with most of the literature, there is a temporary elevation in IL-6 in one of the studies following exposure to bio-ceramics, which is thought to be attributed to elevated concentrations and sampling times [20]. In another study that employed nanoparticle-enhanced ZOE, a decreased cytokine expression was observed without reverting its cytotoxic activity [21]. Similarly, fibroblast viability tests indicated that ZOE formulations are highly variable in their toxicity based on their additives [22]. The bio-ceramic sealers were consistently cell-compatible and demonstrated the promotion of osteogenic differentiation, which indicates that bio-ceramic application can result in improved endodontic treatments [23]. Their immunomodulatory activity can potentially decrease the periapical inflammation and can result in better success rates in the treatment [24]. Their use in endodontic practice can also reduce the requirement of further anti-inflammatory treatment.[25,26]

The limitations of this study include in vitro design and small sample size, which cannot reconstruct in vivo immune and vascular system interactions. The results may also be affected by confounding factors, including variability of cell donors, aging of the sealers, and exposure time differences. Further studies must include in vivo animal experiments and clinical trials examining long-term cytokine expression, bone healing, and immune cell behavior with different sealer types.

CONCLUSION

Bio-ceramic sealers showed the most promising biological profile by reducing pro-inflammatory cytokines and elevating expression of osteogenic markers, followed by epoxy resin, while ZOE indicated the most potent cytotoxicity and inflammatory response. These results validate that the type of endodontic sealer has an impact on fibroblast viability and molecular healing pathways.

This study has reached the goal of examining the immunomodulatory and regenerative properties of sealers in root canal healing. The results confirm clinical preference for bio-ceramic sealers to achieve desirable periapical outcomes. Their regular application could enhance long-term effectiveness and recovery after treatment.

LIST OF ABBREVIATIONS

None

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CONFLICT OF INTEREST

None

ETHICAL APPROVAL

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AUTHORS' CONTRIBUTION

All authors contributed equally as per ICMJE.

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