Abstract

Introduction: The management of apical periodontitis involves the elimination of bacteria to achieve healing of the periapical tissues. To this end, understanding of the antimicrobial properties and cytotoxicity of root canal sealers over time is important. This study aimed to assess the cytocompatibility and antibacterial activity of leachates obtained from selected endodontic sealers.

Methods: Four sealers were used in this study; AH Plus, an epoxy resin-based sealer, and three hydraulic calcium silicate-based sealers: AH Plus Bioceramic, BioRoot RCS and BioRoot Flow. Sealer chemistry was assessed by scanning electron microscopy and energy dispersive spectrum analysis. Leachates of the tested sealers were prepared by immersing sealer discs in HBSS for 28 and 90 days. Element release was assessed using inductively coupled plasma mass spectroscopy. *Enterococcus faecalis* and *Fusobacterium nucleatum* were exposed to the leachates followed by CFU determination, and the human osteoblast-like cell line Saos-2 was assessed with regard to cell death, caspase expression and activity of alkaline phosphatase after stimulation with the leachates.

Results: Calcium leaching was detected in the tricalcium silicate-based sealers, which increased the leachate pH significantly ($p<0.0001$). AH Plus Bioceramic leachate displayed antimicrobial activity at 28 days against *E. faecalis* ($p<0.01$). Alkaline phosphatase levels were reduced in response to AH Plus Bioceramic and BioRoot RCS 1-day leachates ($p<0.05$). High cell viability was observed after exposure to all sealers.

Conclusions: The leachates of the tested sealers were cytocompatible, however, their antimicrobial activity as well as their potential to induce the bone formation marker alkaline phosphatase was minimal.

Introduction

Endodontic pathogens enter the root canal system progressively through caries, cracks or microleakage, leading to apical periodontitis (1). The root canal anatomy is often complex (2) making successful root canal treatment challenging. When treatment is carried out, reducing infection and encouraging healing is the primary aim (3), and is achieved by preparing the infected canal using chemo-mechanical methods and appropriate root filling materials (4).

Microbes in an infected pulp are diverse and different species are found at various stages of infections (1). Obturation and sealing of the canal space with an efficient material is crucial to suppress the growth of residual bacteria (5). Root canal sealers occupy the canal space and should possess antimicrobial properties capable of eliminating and preventing reinfection (6). The filling materials, when in contact with the surrounding oral tissues, can lead to complications and retard the healing process if not fully biocompatible (7). These materials should be non-irritant and cause no adverse effects (8). Leachates from obturation materials can pass through the dentinal tubules and into surrounding tissues. Therefore, the release from such materials should be beneficial to the treatment (9).

A wide range of endodontic sealers is available, which differ in their chemical composition. The epoxy resin based sealer AH Plus is a commonly used material with low solubility and aims to achieve a seal that prevents microleakage. However, it has been reported to show inferior biological properties (10,11). Hydraulic calcium silicate based cement sealers release calcium and hydroxyl ions and interact with the root dentine to achieve a biological seal. They are biocompatible (12), and exhibit better cell viability results when compared to conventional sealers (13). Their high pH is beneficial in inducing healing (14,15).

The antimicrobial and biological characteristics of hydraulic cements have been extensively reviewed and investigated (16). Most of the testing is undertaken on the materials *in vitro*. On the other hand, *in vivo* outcomes from endodontic treatment do not appear to be influenced by the type of sealer used, however, there is overall

conflicting evidence (17). Whether elution from the material is responsible for the healing outcome within the periapical tissues is currently unclear. At the same time, data on the biological characterization of leachates from endodontic sealers are scarce. Due to their direct contact with host tissues and microbes, they are likely to be an important factor to enhance antimicrobial activity of sealers and their effects on tissues and cells (18,19), highlighting the relevance of undertaking research into sealer properties.

Periapical tissues are composed of multiple different cell types involved in tissue repair (20). Amongst these, osteoblasts are crucial for bone regeneration through their production of the enzyme alkaline phosphatase (ALP) (21).The survival and function of these cells determines successful healing, and these parameters are strongly influenced by the presence of bacteria, which may cause ongoing inflammation and host cell death (22,23).The aim of this study was to investigate the leachates from four different endodontic sealers regarding their antimicrobial properties and cytocompatibility with osteoblast-like cells. The samples were investigated after immersion in Hank's balanced salt solution (HBSS) at different time points.

Materials and methods

Materials and reagents

Four endodontic sealers were selected for this study: 1) AH Plus Jet (Dentsply Sirona, Charlotte, USA), 2) AH Plus Bioceramic,(Dentsply Sirona, Charlotte, USA), 3) BioRoot RCS (Septodont, Saint-Maur-des-Fosses, France)and 4) BioRoot Flow (Septodont, Saint-Maur-des-Fosses, France). Their composition and details are listed in **Table 1**. AH Plus was used in a double barrel system, where the two paste components were mixed in a 1:1 ratio. BioRoot RCS was hand mixed as instructed by the manufacturer, where 5 drops of the liquid were mixed with one scoop of powder. BioRoot Flow and the AH Plus Bioceramic sealers were presented in a single syringe and did not require mixing. Hanks' Balanced Salt Solution (HBSS) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Sealer disc preparation

AH Plus and BioRoot RCS were mixed on a sterile glass slab using a sterile spatula. AH Plus Bioceramic and BioRoot Flow were directly injected into the moulds. Cylindrical moulds measuring 10 mm in diameter and 2 mm in thickness were used to prepare the sample discs in three replicates per sealer type for each time point (0, 28 and 90 days). All sealers were prepared in in a laminar flow cabinet (Guardian MSC T1200, Monmouth Scientific, Bridgwater, UK). The moulded samples were placed in petri dishes with sterile gauze dampened with sterile water at the bottom. The dishes were sealed with cling film, then incubated at 37°C and 95% humidity, allowing the samples to set in these closed, humid conditions.According to the manufacturers the setting times indicated were as follows: AH Plus: 8 h, AH Plus Bioceramic: 4 h, BioRoot RCS: 5.4 h, BioRoot Flow: 8h. All materials set and their consistencies were similar after setting.

Material characterization by scanning electron microscopy

Subsequently, one group was characterized immediately without immersion, while the remaining discs were immersed in HBSS for 28 or 90 days after which they were retrieved, dried and processed. The sealer discs were embedded in epoxy resin (Epofix, Struers, Ballerup, Denmark). After setting, the resin blocks were refined using grinding discs (MD-Piano, Struers Ltd., Catcliffe Rotherham, UK) and polished with finer plates (MD-Largo, MD-Dac and MD-Nap, Struers Ltd.) and lubricants (DiaProDac 3 µm and OP-S, 0.04 µm, Struers Ltd.). The samples were left to dry overnight in a vacuum desiccator and gold coated (K550X Sputter Coater, Quorum Technologies Ltd., Laughton, UK) the following day. The specimens were imaged using scanning electron microscopy (SEM; EVO MA 10, Carl Zeiss Ltd., Cambridge, UK) to assess the microstructure in back scatter mode at 1k magnification and elemental analysis of the materials were performed by energy dispersive spectroscopy with settings fixed atWD = 8.5 mm, EHT = 20 kV, I probe = 1000 pA.

Leachate preparation

The immersion volume was calculated using ISO 10993-12:2021 for biological evaluation of medical devices and according to the surface area/volume ratio (3 cm^2/ml). The sealer discs were therefore immersed in 0.57 ml of HBSS within 5 mL bijou polystyrene tubes. The solution was replaced every 14 days for all samples. After

completion of immersion for the allocated periods of time, the samples were removed from the test tubes and the leachates were used for microbiological and cell assays.

pH measurements

The pH of the leachates was measured using a pH meter (Beckman Φ40, Beckman Coulter, Brea, USA) which was calibrated at pH levels of 4.00, 7.00 and 10.00 using standard calibrated solution. The pH of the leachates at different time points of 28, 90 days were recorded three times at each time point.

Chemical analysis of leachates

Leachates extracted at 28 and 90 days from the samples were analysed for elements of interest, namely calcium, zirconium, aluminium, silicon. This was confirmed by energy dispersive spectroscopy. The analysis of the elemental release of the materials were then performed using inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 8000 ICP-OES, Perkin Elmer, Waltham, MA, USA). The leachates were acidified with 2% HNO_3 in a falcon tube and diluted 100-fold. Four calibration solutions were used and HBSS was also acidified with 2% HNO_3 which served as the blank sample.

Antibacterial testing of leachates

The bacterial species used for this test were *Fusobacterium nucleatum* subsp. *polymorphum* (*F. nucleatum*, ATCC 10953) and *Enterococcus faecalis* (*E. faecalis*, ATCC 29212). *F. nucleatum* was cultured in an anaerobic chamber (80% N_2, 10% CO_2, and 10% H_2; Don Whitley DG250 Anaerobic Workstation, Don Whitley Scientific, Bingley, UK) using Schaedler broth and agar (Sigma-Aldrich, St Louis, MO, USA), Loughborough, UK). *E. faecalis* was cultured at 37°C using brain heart infusion (BHI) broth and agar (Thermo Fisher Scientific). The optical density of the overnight cultures was measured in a spectrophotometer (Jenway 6300, Chelmsford, UK), and cultures were diluted with BHI to obtain an optical density of OD_{600} = 0.1. The culture was further diluted 100x with BHI to reach a final optical density of OD_{600} = 0.001 corresponding to 10^6 CFU/ml. The bacteria were assessed using Miles and Misra (24) with multiple dilutions to determine the CFU/ml. 50 µl of this bacterial culture was transferred to a 96-well plate and 50 µl of the leachates were added to each well. The

dilution was mixed thoroughly, plates were sealed and left overnight at 37°C in an incubator (*E. faecalis*) or in an anaerobic chamber (*F. nucleatum*). After 16 hours of incubation, samples were plated onto BHI agar and counted using the Miles and Misra method after 24h. Three biological repeats were performed for this test with the two selected species.

Cell culture of Saos-2 cells

The human osteoblast-like cell line Saos-2 (ATCC HTB-85) was grown in T-75 tissue culture flasks in McCoy's 5A medium (Thermo Fisher Scientific) supplemented with 15% fetal bovine serum (FBS) and 1% penicillin/streptomycin according to the ATCC standard protocol. Cells were cultured at 37°C in a humidified 5% CO_2 atmosphere.

Cell viability assay

Saos-2 cells were seeded at a density of 3 x 10^4 cells per well suspended in 0.5 mL, using a flat-bottom transparent 48-well cell culture plate (Corning™ Costar™, Thermo Fisher Scientific) and allowed to proliferate until they achieved 70-80% confluency. The leachates underwent dilution at a ratio of 1:4 (250 µl/well) using complete culture media, after which they were transferred into the designated wells. Subsequently, cells were incubated at 37°C in 5% CO_2 for 24h, followed by trypsinization and viability assessment by trypan blue staining at a ratio of 1:1. Both live and dead cells were counted using a hemocytometer, and the percentage of viable cells relative to the negative control was calculated. Cells treated with HBSS only were employed as the negative control.

Alkaline phosphatase (ALP) assay

ALP levels were determined using the colorimetric TRACP&ALP assay kit (Takara, Cat. #MK301) according to the manufacturer's instructions. In brief, Saos-2 cells were seeded into a flat-bottom transparent 96-well cell culture plate (Corning™ Costar™ 3596, Thermo Fisher Scientific) at a density of 1 x 10^4 cells per well in until reaching 70-80% confluence. The cells were exposed to leachate at a 5x dilution and subjected to incubation at 37°C in 5% CO_2 for 24h. Subsequently, the solution was removed and cells were washed with 0.9% sterile saline. Following this, the cells were lysed and ALP enzyme activity was evaluated. Absorbance was measured using a microplate

reader (Spark®, Tecan; software SparkControl, v. 2.3, Tecan Ltd., Männedorf, Switzerland) at a wavelength of 405nm.

Apoptosis detection

Cells were seeded at a density of $1x10^4$ cells per well into white 96-well plates with clear flat bottoms (Corning™ 3632, Thermo Fisher Scientific) and allowed to adhere overnight. Cells were then treated with the leachates at a 5x dilution and the induction of apoptosis was measured using the Caspase-Glo® 3/7 Activity Assay kit (Promega, Southampton, UK) according to the manufacturer's instructions. Briefly, the leachate-treated cells were mixed with the assay reagent at a 1:1 ratio. The well plate was placed immediately into a microplate reader (Spark®, Tecan) to measure luminescence at 37°C for 3h. HBSS-treated cells served as the negative control, while Fas ligand (Cat# 589402, BioLegend, London, UK) at 50nM served as the positive control.

Statistical analysis

Kolmogorov-Smirnov test was used to determine normal distribution of the data. One-way ANOVA and two-way ANOVA followed by Tukey post-hoc tests was applied to parametric data, while the Kruskal-Wallis test was done for non-parametric data. A p-value of less than 0.05 was considered statistically significant. For all statistical tests and for creating graphs, GraphPad Prism (version 10.1.1 for Windows, GraphPad Software, La Jolla, CA, USA) was used.

Results

Characterization of sealer surfaces and composition

The surface structure of the tested materials immediately after setting and after immersing the samples in HBSS for 28 and 90 days are shown in **Figure 1**. Bright dense particles were observed on AH Plus samples indicating $CaWO_3$, whereas the less bright particles were zirconium. The ziroconium particles were also seen in AH Plus Bioceramic, BioRoot RCS and BioRoot Flow. . After 28 days, AH Plus Bioceramic, BioRoot RCS and BioRoot Flow displayed isolated pores, whereas AH

Plus maintained a smooth surface structure. After 90 days, all sealers showed pores, indicating release of material components during immersion. The EDS analysis revealed the composition of the tested sealers, which were mainly composed of calcium, silicon, zirconium and, in case of AH Plus, tungsten (**Tables 1, 2**).

Leachate pH and calcium ion content

The measured pH values of the AH Plus Bioceramic, BioRoot RCS and BioRoot Flow leachates were ≥13 and significantly higher than the HBSS control and AH Plus leachates at both time points (28 and 90 days, $p<0.0001$) (**Figure 2A**). AH Plus and AH Plus Bioceramic leachates significantly decreased in pH over time ($p<0.001$ and $p<0.0001$, respectively), however, this change was minimal. The calcium ion release was assessed at 28 and 90 days, where the BioRoot RCS leachate exhibited the highest calcium release at both time points (**Figure 2B**), which was significantly higher than that of the other sealers' leachates ($p<0.0001$). A decrease in calcium release at 90 days in AH Plus Bioceramic and BioRoot RCS samples was observed ($p<0.05$ and $p<0.0001$, respectively). AH Plus exhibited the lowest calcium ion release and did not change over time.

Antimicrobial properties of leachates

The 28d AH Plus Bioceramic leachate significantly reduced CFUs of *E. faecalis* ($p<0.01$). However, none of the 90-day sealer leachates led to a reduction of *E. faecalis* CFUs (**Figure 3A, B**). None of the leachates showed antibacterial effects against *F. nucleatum*.

Alkaline phosphatase activity

AH Plus Bioceramic and BioRoot RCS 1-day leachates led to significantly decreased ALP activity compared to unstimulated control cells ($p<0.05$). This reduction was more pronounced in BioRoot RCS but was not sustained in the 28-day and 90-day samples (**Figure 4A**).

Saos-2 cell viability

None of the leachates affected cell viability (**Figure 4B**). Assessment of caspase 3/7 expression following exposure to the leachates showed significant inreases for AH

Plus Bioceramic in 28-day leachates compared to 1-day leaches ($p<0.05$), and for BioRoot RCS in 90-day leachates compared to 1-day leaches ($p<0.05$). At the same time, BioRoot RCS showed the lowest caspase 3/7 activation in day-1 leachates. None of the leachates enhanced caspase activity compared to the negative control (**Figure 4C**).

Discussion

Success of root canal therapy depends on the elimination of bacteria, but also on the healing of the periapical tissues. These processes may be impaired if the materials used are not adequately antimicrobial, allow microbial ingress or are cytotoxic to the periapical tissues. To be able to assess this, both the antimicrobial and biological properties of four root canal sealers were assessed in this study, which were selected based on their similar chemistry.

During root canal treatment, material-tissue interactions are likely to occur, hence biocompatibility plays a vital role. When sealers come in contact with the surrounding tissue fluid, release of material components occurs (25). From a clinical point of view, it is important to understand how the leachate could impact the treatment outcome. Few studies have biologically tested leachates from endodontic materials (18,19), and a recent review paper highlighted the need to characterize and study leachates of these materials in more depth (26).

AH Plus is a resin-based sealer whereas the other three are derivatives of tricalcium silicate cements. The manufacturers list the presence of calcium, zirconium, aluminium and silicon (**Table 1**) in the composition of AH Plus, AH Plus Bioceramic, BioRoot RCS and BioRoot Flow, as well as tungsten in AH Plus. This was confirmed by EDS analysis in our study. Regardless of the similar chemistry of the hydraulic cement sealers, it has been shown that the quantities of the respective components and the different delivery systems may affect the sealer properties (27). In the present study, AH Plus Bioceramic displayed pore formation at an early stage and all calcium silicate-based sealers showed pores by the 28th day indicating displacement of

particles from the set structure overtime. This pore formation could lead to decrease in material strength and therefore suboptimal sealing, enabling surrounding tissue fluid to enter the canal space, and potentially encourage growth of bacteria. From the surface microstrcucture study, it was also observed that the radiopacifiers in AH Plus samples are larger and more prevalent as compared to the calcium silicate based sealers.

The buffer added to the sealer discs to obtain the leachates was changed every two weeks. This was done to avoid oversaturation of the buffer and to prevent microbial contamination. Changing the solution likely affected the pH and leaching profile, however, this effect was intentional, because *in vivo*, constant tissue fluid and ion exchange processes prevent accumulation of leachate components (28–30). Tricalcium silicate-based sealer leachates in our study showed overall high pH levels. An alkaline pH contributes to the antibacterial and biocompatibility properties (11,31) and is closely related to bacterial inhibition through the release of hydroxyl ions (32,33). In our study, BioRoot RCS, BioRoot Flow and AH Plus Bioceramic, which are tricalcium silicate based cements, led to higher pH in the 28-day and 90-day leachates. Tricalcium silicate based cements have been found to maintain an alkaline state even after a long period of time (34). In theory, this should not only promote the antibacterial effect but also allow ALP to become activated hence increasing bone formation (35). AH Plus produced a lower pH comparable to that of the negative control, indicating that AH Plus may not exert the same antibacterial and osteogenic effects.

F. nucleatum and *E. faecalis* were selected as they are associated with root canal infections, where the latter is found in post treatment lesions (16). Contrarily to previously reported antibacterial effects by alkaline pH environments, CFU reductions were only observed in 28-day AH Plus Bioceramic leachates when tested with *E. faecalis*, however, albeit statistically significant, these reductions were minimal. All other groups displayed no significant reduction in CFUs. In the context of our *in vitro* study, these results indicate no clinically relevant antibacterial effects of the leachates. This could be due to the decrease in pH when the leachates were further diluted with the microbial suspension in our experiments. In addition, the broth/medium used could

have acted as a pH buffer (18). *In vivo*, such buffer effects may also occur through periapical interstitial fluids.

Extensive research has been dedicated to hydraulic cements within the field of dentistry due to their diverse chemical compositions, which can vary considerably depending on the manufacturer (36). The main objective of this inquiry was to examine the hydration reactions leading to the release of calcium hydroxide. The phenomenon of pH elevation is widely acknowledged to be a consequence of this chemical process (37). An elevation in pH induces the expression of alkaline phosphatase (ALP) (38), a marker of hard tissue formation, which is thought to enhance the healing process in the periapical area (39). However, evidence regarding the pH optimum for ALP enzymes is inconclusive, and a number of reports have demonstrated a pH optimum between pH 8.2 and 10.7, or even at physiological pH (40–42).

In our experiments, the 1-day leachates of BioRoot RCS and, to a lesser extent, AH Plus Bioceramic, decreased ALP activity, whilst all other test conditions showed no effect. This finding is in contrast to the concept of ALP induction by alkaline pH. On the other hand, they are in line with those previous reports supporting a lower pH optimum for ALP. Hence, in our assay, optimum ALP activity may have already been present in control samples. Further *in vitro* research should be conducted to clarify pH optima of ALP in both cell lines and primary osteoblasts.

In this study, a trypan blue exclusion assay was conducted. According to ISO 10993-5 guidelines, cell viability percentages exceeding 80% are indicative of non-cytotoxicity, while viability rates within the range of 80%–60% are considered weak (43). All sealers in this study exhibited an acceptable level of viability based on observed viability rates. Most studies have demonstrated that hydraulic calcium silicate cements generally exhibit favourable biocompatibility, with minimal cytotoxic effects on human cells (35,44), while few have shown a contrary result (45). A recent literature review pertaining to the biocompatibility of root canal sealers has revealed that AH Plus demonstrates cytotoxic effects in the majority of the studies included (46).

Furthermore, it has been observed that the presence of toxicity is primarily limited to the initial 24-hour period when freshly mixed (47,48). It has also been reported that

the cytotoxicity resulting from direct contact with epoxy resin based sealers is significantly more pronounced compared to indirect contact. This may be attributable to the lower concentration of cytotoxic components in the leachates, due their low solubility (49). Therefore, the indirect contact method used in our study may explain the low cytotoxicity as determined by cell death observation. However, other parameters of cytotoxicity such as the MTT assay, which was commonly employed in numerous studies, alongside alternative methodologies such as XTT, CCK-8/WST-8, and LDH release assays (13,46), were not assessed in the present study and could be included in future experiments using leachates.

Caspases are key enzymes in the complex regulation of cellular survival and apoptosis. Caspase-3 and caspase-7 play a crucial role in the final stages of apoptosis (50). Our results indicate that 1-day BioRoot RCS leachate treatment may have decreased these caspases, however, this was not significant compared to the negative control. At the same time, 28-day and 90-day leachates showed an overall increase in caspase 3/7 levels compared to day 1, which was significant in case of AH Plus Bioceramic and BioRoot RCS. Caspase activation may be attributed to a rise in alkalinity, however, research investigating the impact of increased pH on apoptosis is scarce (51). Cell viability was largely unaffected by the leachates, which could be explained by the fact that during apoptosis without secondary necrosis, the cell membrane remains intact (52) This may have caused these cells not to be stained by trypan blue. Furthermore, it is possible that an equilibrium between apoptosis and cell proliferation may have existed in our cell culture.

The primary discovery of this study pertains to the apparent absence of cytotoxicity, but also to the inability to increase ALP levels or to exert relevant antimicrobial effects of any of the tested sealer leachates and conditions *in vitro*. Future studies may reveal approaches to enhance antimicrobial activity in sealer leachates and to support osteoblastic activity whilst maintaining biocompatibility.

Declaration

I affirm that we have no financial affiliation (e.g., employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the

subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest is disclosed.

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Author contributions

JC and JH conceptualised this research. JHC and VR carried out the experiments, acquired and analysed the data. VR and JC wrote the manuscript. SAK, JC and JH supervised and conceptualized the study. JH and JC reviewed the manuscript, JH edited the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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Figure captions

Fig. 1. Scanning electron images of the tested sealers at 0, 28 and 90 days taken with back scatter mode at 1,000 x magnification. The white arrows indicate the bright zirconium particles, and the black arrows display pore formation observed over time. Cracks observed in some images are artefacts due to placement of the saturated materials into a vacuum during the SEM procedure.

Fig. 2. pH of the leachates (**A**) and calcium ion release from sealers into the leachates (**B**) at the different time points. Mean values and SD are shown, n=3 in triplicate, 2-way ANOVA, **p<0.01, ****p<0.0001 , $^{\#}$p>0.05 , $^{\#\#\#}$p<0.001, $^{\#\#\#\#}$p<0.0001.

Fig. 3. Antibacterial activity testing of the leachates by assessing CFU/mL of *E. faecalis* (**A**) and *F. nucleatum* (**B**) after treatment with the leachates. Mean values and SD are shown, n=3 in triplicate, 2-way ANOVA, **p<0.01, $^{\#}$p<0.05.

Fig.4. Assessment of ALP release and cell death in response to leachates. A: ALP activity in response to sealer leachates based on absorbance measured at 405 nm wavelength. **B**: Cell viability carried out using trypan blue-based cell counting. **C**: Caspase 3/7 activity indicating apoptosis relative to the negative control which was set

at 100%. Mean values and SD are shown, n=3 in triplicate, 2-way ANOVA, *p<0.05, #p<0.05, ##p<0.01.

Tables

Name	Composition	Lot number	Manufacturer
AH Plus Jet	**Paste A**: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments. **Paste B**: dibenzyldiamine, aminoadamantane, calcium tungstate, tricyclodecane-diamine, zirconium oxide, silica, silicone oil.	2207000903	Dentsply Sirona, Tulsa OK, USA
AH Plus Bioceramic	Zirconium dioxide (50%-75%), tricalcium silicate (5%-15%), dimethyl sulfoxide (10%-30%), lithium carbonate (<0.5%), thickening agent (<6%).	KS220303	Dentsply Sirona, Tulsa, OK, USA
BioRoot RCS	**Powder**: tricalcium silicate, zirconium dioxide, povidone. **Liquid**: calcium chloride, polycarboxylate.	B27782	Septodont, Saint-Maur-des-Fosses, France
BioRoot Flow	Tricalcium silicate, zirconium oxide, calcium carbonate, propylene glycol, povidone, aerosol(silica), acrylamide/ sodium, acryloyldimethyltaurate copolymer, isohexadecane, polysorbate.	B29973AAA	Septodont, Saint-Maur-des-Fosses, France

Table 1. Composition of the sealers used in the study, according to the manufacturer's information.

Sealer	AH Plus	AH Bioceramic	BioRoot RCS	BioRoot Flow
Ca	6.02 (5.66-7.98)	1443.18 (1297.01-1651.80)	5746.6 (5516.16-5874.10)	1870.58 (1627.05-2130.83)
Si	5.69 (3.39-25.51)	15.40 (14.14-27.99)	32.10 (28.3-91.2)	12.54 (9.10-13.94)
P	0.116 (0-1.81)	BDL	7.95 (2.93-14.57)	BDL
Li	0.35 (0.27-0.411)	38.56 (27.07-45.27)	0.34 (0.24-0.36)	0.16 (0.10-0.27)

Zr	0.52 (0.268-0.29)	10.62 (2.91-80)	3.30 (1.83-3.47)	0.46 (0.34-2.38)
W	8.36 (7.15-9.87)	BDL	BDL	0.5 (0.07-1.2)

Table 2. Medians and ranges of calcium, silicon, phosphorus, lithium, zirconium and tungstate ion concentrations (in mg/l) of the sealer leachates after 28 days as determined by ICP analysis. BDL= below detection limit.

Sealer	AH Plus	AH Bioceramic	BioRoot RCS	BioRoot Flow
Ca	6.23 (5.98-9.03)	663.7 (519-678)	3165.9 (2511.8-3247)	2256.9 (2178-2620)
Si	0.52 (0.50-3.3)	10.51 (9-12.14)	13.1 (6.4-17.2)	7.18 (6.9-7.2
P	BDL	BDL	BDL	BDL
Li	BDL	1.8 (1.7-1.9)	0.2 (0.01-0.13)	0.02 (0-0.3)
Zr	0.16 (0.12-0.39)	16.5 (8.5-32.6)	2.37 (1.24-4)	0.4 (0.4-0.7)
W	8 (6.8-10.8)	BDL	BDL	0.9 (0.8-1)

Table 3. Medians and ranges of calcium, silicon, phosphorus, lithium, zirconium and tungstate ion concentrations (in mg/l) of the sealer leachates after 90 days as determined by ICP analysis. BDL= below detection limit.