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ORIGINAL ARTICLE

Porous zirconia ceramic as an alternative to dentin for in vitro dentin barriers cytotoxicity test

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Abstract

Objectives This study assessed the potential of porous zirconia ceramic as an alternative to dentin via an in vitro dentin barrier cytotoxicity test.

Methods The permeability of dentin and porous zirconia ceramic was measured using a hydraulic-conductance system, and their permeability was divided into two groups: high and low. Using an in vitro dentin barrier test, the cytotoxicity of dental materials by dentin and porous zirconia ceramic was compared within the same permeability group. The L-929 cell viability was assessed by MTT assay.

Results The mean (SD) permeability of the high and low group for dentin was 0.334 (0.0873) and 0.147 (0.0377) $\mu l\ min^{-1}\ cm^{-2}\ cm\ H_2O^{-1}$ and for zirconia porous ceramic was 0.336 (0.0609) and 0.146 (0.0340) $\mu l\ min^{-1}\ cm^{-2}\ cm\ H_2O^{-1}$. The cell viability of experimental groups which are the low permeability group was higher than that of the high permeability group for both dentin and porous zirconia ceramic as a barrier except for Maxcem Elite™ by porous zirconia ceramic. There was no significant difference between dentin and porous zirconia ceramic in cell viability, within either the high or low permeability group for all materials. The SD for cell viability of the porous zirconia ceramic was less than that of the dentin, across all materials within each permeability group, except for Maxcem Elite™ in the high permeability group.

Conclusions Porous zirconia ceramic, having similar permeability to dentin at the same thickness, can be used as an alternative to dentin for in vitro dentin barrier cytotoxicity tests.

Clinical relevance In vitro dentin barrier cytotoxicity tests when a standardized porous zirconia ceramic was used as a barrier could be useful for assessing the potential toxicity of new dental materials applied to dentin before applying in clinical and may resolve the issue of procuring human teeth when testing proceeds.

Keywords Cytotoxicity · Dental materials · Dentin barrier test · Dentin permeability · Porous zirconia ceramic

Introduction

Recent advances have resulted in numerous new dental materials being available for use in clinical settings. However, when applied directly to dentin, many dental materials stimulate pulp cells [1]. The permeability of dentin and biocompatibility of dental materials are critical factors that must be considered [2, 3]. New dental materials should be evaluated for biocompatibility before they are utilized in clinical settings [4, 5]. The primary biocompatibility testing approaches are in vitro cytotoxicity tests, animal tests, and clinical tests [6, 7]. In vitro cytotoxicity tests for biocompatibility have several important technical advantages compared to animal and clinical tests. They can be standardized to yield the repeatable results as well as efficiently performed at a relatively low cost [8, 9]. In vitro cytotoxicity test results do not always correlate with in vivo test results, for example, zinc oxide-eugenol cement has shown a strong cytotoxicity reaction in vitro, but not when applied to dentin in vivo [10–12]. One possible explanation for the different test results may be the absence of a dentin barrier between the dental materials and target cells [13, 14]. When dentin disks were used as a barrier between dental materials and target cells in the in vitro cytotoxicity test, the

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results more closely resembled the response observed in the in vivo test [15, 16].

In vitro dentin barrier cytotoxicity tests most closely replicate the conditions of in vivo cytotoxic tests of the effects of dental material application after tooth preparation [17, 18], and have the potential to replace animal and clinical tests [14]. The International Organization for Standardization encourages the use of dentin as a barrier during in vitro cytotoxicity tests of dental restorative materials [5]. In addition to the chemical toxicity of a material, the permeability of dentin is a key factor determining the diffusion of toxic components, released from dental materials into the pulp via dentin, during in vitro dentin barrier cytotoxicity tests [2, 19]. Dentin permeability varies greatly, even among different areas of the same tooth [20]. In the process of human teeth growth, it will be affected by wear, decay of lesions, secondary dentin, sclerotic dentin, and other various factors [21]. These factors may lead to differences in permeability test results among dentin disks. Human teeth can be difficult to obtain for use in such tests, and it is necessary to find an appropriate alternative to dentin that has similar permeability and yields similar results, and can thus serve as a substitute for dentin for in vitro dentin barrier cytotoxicity tests. Polyurethane disks and Millipore filters have been used as dentin substitutes for such tests and showed similar results to bovine dentin. However, they have not been compared with the permeability of human dentin at the same thickness, and the substitute materials did not have the same mechanical properties as dentin [1, 22]. The pore size of porous ceramic can be adjusted by machining technology to obtained different permeability. This indicated that porous ceramic has the potential to be used as a dentin substitute material for in vitro dentin barrier cytotoxicity tests, but no further study has been carried out [23]. Porous zirconia ceramic is formed through high heat sintering, during which pore size can be controlled to obtain the similar permeability to human teeth [24].

The aim of this study was to evaluate the porous zirconia ceramic as an alternative to dentin for in vitro dentin barrier cytotoxicity test when the ceramic with a similar permeability to dentin at the same thickness used. A standard alternative material providing similar results during in vitro dentin barrier cytotoxicity tests could resolve the difficulty of procuring human teeth for in vitro dentin barrier cytotoxicity testing.

Materials and methods

Preparation of human dentin and porous zirconia ceramic disks

A total of 100 third molars were collected from adult patients. Only complete crowns without subjecting to root canal therapy and obvious caries lesion were used. The molars were cleaned by removing soft tissue and debris and stored in a 0.5% chloramine T solution in deionized water at 4 °C. All molars were used within 2 months after extraction. Before the preparation of the dentin disks, the teeth were sterilized by soaking in 75% ethanol for 15 min [5].

Dentin disks $\Phi > 6$ mm and 0.5 ± 0.05 mm in thickness were obtained by cutting perpendicular to the long axis of the tooth with a low-speed saw (Isomet-Buehler, Lake Bluff, IL, USA). The dentin disk that was closest to the pulp cavity was selected. One dentin disk sample was obtained from each tooth. Porous zirconia ceramic disks $\Phi 13$ mm and 0.5 ± 0.05 mm in thickness require special processing and were supplied by the Beijing Key Laboratory of Fine Ceramics, Institute of Nuclear and New Energy Technology of Tsinghua University.

Permeability test of human dentin and porous zirconia ceramic disks

The permeability of the dentin and porous zirconia ceramic disks was measured prior to the in vitro dentin barrier cytotoxicity tests.

The equipment for the permeability experiment was assembled according to the hydraulic-conductance model of Outhwaite and Pashley [21, 25]. The experimental setup consisted of an iron support stand, a glass water tank, a stainless steel split-chamber, a micropipette, and some flexible rubber hose (used to connect the components; Fig. 1). To simulate normal pulp pressure, the glass water tank contained

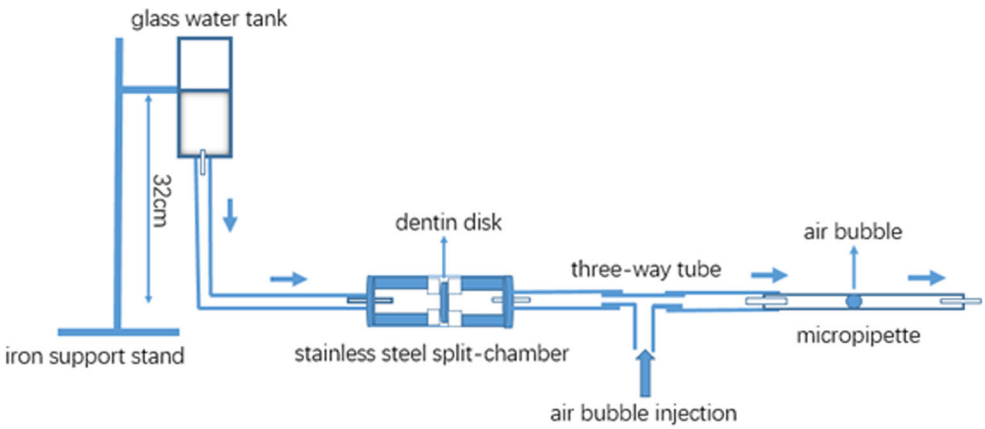

Fig. 1 The equipment used for measurement of dentin disks permeability

deionized water at a pressure of 32-cm H_2O (3.14 kPa) on the pulp side of the dentin disks [26].

Dentin disks were acid-etched on both sides with 35% phosphoric acid for 30 s and then rinsed with deionized water for 1 min to remove the smear layer. The dentin or porous zirconia ceramic disks were fixed in the middle of the stainless steel split-chamber, at a water pressure of 32 cm (from the pulp side to the occlusal side). The measurement area of the dentin and porous zirconia ceramic disks was 0.283 cm^2, demarcated by two rubber circle rings with an inner diameter of 6 mm. Only the area in the center of the dentin and porous zirconia ceramic disks was used to measure permeability. In the next stage of cytotoxicity testing, dental materials were each applied to the same area of the dentin and porous zirconia ceramic disks. The micropipette was used to introduce a small air bubble. Tracing the movement of the air bubble within the horizontal micropipette allowed calculation of the volume of deionized water filtering through the dentin or porous zirconia ceramic disks. The time required for the air bubble to move up to 10 μl after it showed stable motion for 1 min was recorded. The 100-μl micropipette had a precision of 1 μl. To ensure an appropriate seal for the permeability experiment equipment, a glass disk of similar size to the porous zirconia ceramic disks was used to test the equipment (under the same experimental conditions) before every dentin and porous zirconia ceramic disk permeability measurement. Each dentin and porous zirconia ceramic disk was measured three times and the average value obtained. All experiments were performed at room temperature.

The permeability of dentin and porous zirconia ceramic disks was calculated according to the following equation [27]:

$$\mathrm{Lp} = \mathrm{Jv}/(A \times t \times P)$$

where Lp is the permeability of dentin or porous zirconia ceramic disks (μl min^{-1} cm^{-2} cm H_2O^{-1}); Jv is the volume of deionized water filtering through the dentin or porous zirconia ceramic disks during the observation time (μl); A is the measurement area (cm^2); t is the observation time in minutes; and P is the deionized water pressure applied to the dentin or porous zirconia ceramic disks (cm H_2O). In this study, Jv = 10 μl, $A = 0.283$ cm^2, and $P = 32$ cm H_2O.

Forty dentin disks with high and similar permeability (Lp > 0.221 μl min^{-1} cm^{-2} cm H_2O^{-1}) and 40 with low and similar permeability (Lp ≤ 0.221 μl min^{-1} cm^{-2} cm H_2O^{-1}) were chosen from among the 100 dentin disks. And five disks which chosen from the remaining 15 disks were included in the blank group. The 40 high and 40 low permeability dentin disks were randomly divided into six experimental groups, one positive and one negative control group; so each group was further subdivided into high and low permeability subgroups, with five disks per subgroup. Out of 200 porous zirconia ceramic disks, 85 porous zirconia ceramic disks with a similar permeability to the 85 dentin disks were selected and divided into six experimental groups, one positive and one negative control group and one blank group. Prior to application of the dental cements and self-etching resin adhesives for the dentin barrier test, under the same pressure for 20 s, 400-grit sandpapers were used to reconstruct the smear layer of the selected dentin and the zirconia porous ceramic disks. The selected 85 dentin disks were stored at 4 °C in a 24-well cell culture plate with 0.9% sodium chloride solution and were used within 1 week.

L-929 cell culture

L-929 mouse fibroblasts (ATCC CCL1) were cultured in RPMI 1640 growth medium supplemented with 15% fetal bovine serum, 100 IU/ml penicillin, 150 μg/ml streptomycin, and 2.0 mg/ml sodium bicarbonate. The L-929 cells were used at the end of the exponential growth phase, and the L-929 cell density was 1.0 × 105 cells/ml [28].

In vitro dentin barrier cytotoxicity testing

Each well of the homemade cell culture dish was seeded with 100 μl of L-929 cell suspension (1.0 × 105 cells/ml) and 100 μl RPMI 1640 growth medium (Fig. 2).

After incubation for 24 h in a humidified atmosphere at 37 °C and 5% CO_2, the liquid was removed and RPMI 1640 cell culture medium was added to each well. The dentin and selected porous zirconia ceramic disks were placed in contact with the RPMI 1640 cell culture medium, ensuring that the pulp side of the dentin disk was in contact with the medium. A circular ring with an outer diameter of 13 mm, inner diameter of 6 mm, and height of 2 mm was placed on each dentin disk and porous zirconia ceramic disk. The inner circle of the circular ring was filled with dental materials according to the manufacturer's instructions, to mimic actual clinical situations. The applied dental materials were Φ 6 mm × 2 mm, with the exception of the positive group, for which Φ 6-mm

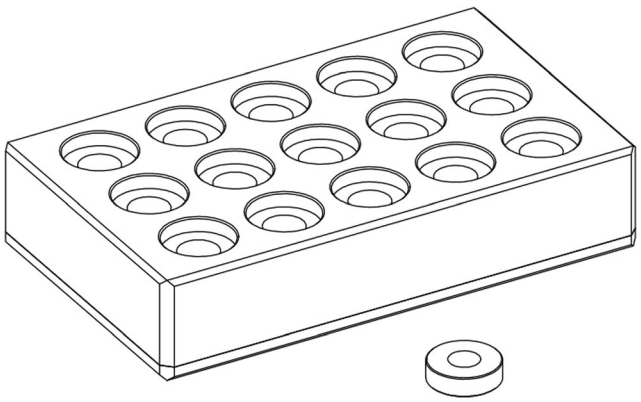

Fig. 2 Three-dimensional projection of the homemade cell culture dish

filter paper was dipped into the phenol solution. The cell culture dish was then incubated at 37 °C and 5% CO_2 for another 24 h.

The materials used in this study are listed in Table 1. Silicone rubber and 70 g/l phenol were used as the negative and positive control groups, respectively. The blank control group was formed of either dentin or porous zirconia ceramic disks [28].

Measuring cell viability using the MTT assay

MTT assay was used to assess the L-929 cell survival rate of dental materials when dentin or porous zirconia ceramic disks were used as a barrier in the in vitro dentin barrier cytotoxicity tests.

After 24 h of incubation, the dental materials, homemade circular rings, and dentin or porous zirconia ceramic disks were removed, and all of the liquid in the wells of the homemade cell culture dish was absorbed. Then, 100 μl of prewarmed MTT solution (Sigma, St. Louis, MO, USA; 1 mg MTT/ml in minimal essential medium [MEM] without phenol red) was placed in each well, and the culture dish was incubated at 37 °C with 5% CO_2 for another 2 h until all of the MTT solution was absorbed. After adding 200 μl of dimethyl sulfoxide, the blue formazan precipitate was extracted, and the culture dish was placed in an oscillator at room temperature for 20 min. The solution (200 μl) was then transferred to a 96-well plate, and the absorbance at 490 nm (OD490) was determined spectrophotometrically.

The mean optical densities of the dentin and porous zirconia ceramic disks were expressed as percentages and compared to the values of the blank control group.

Statistical analysis

Statistical analysis was performed using the nonparametric Mann-Whitney *U* test and one-way ANOVA ($P > 0.05$). A *P* value of < 0.05 was considered significant. SPSS software (ver. 20.0; SPSS Inc., Chicago, IL, USA) was used for all analyses.

Results

Permeability test of human dentin and porous zirconia ceramic disks

The mean (SD) permeability of the high and low group for dentin was 0.334 (0.0873) and 0.147 (0.0377) μl min^{-1} cm^{-2} cm H_2O^{-1} and for zirconia porous ceramic was 0.336 (0.0609) and 0.146 (0.0340) μl min^{-1} cm^{-2} cm H_2O^{-1}. There were no significant differences ($P > 0.05$)

Table 1 Dental materials applied to the dentin and porous zirconia ceramic disks

Materials	Description	Manufacturer	Lot. no.	Main components
MI FLOW II nano	Light-cured hybrid flowable composite resin	GC, Tokyo, Japan	1412031	Diurethane dimethacrylate, poly(oxy-1,2-ethanediyl) BPO, triethylene glycol dimethacrylate
Aura	Light-cured composite resin	SDI, Victoria, Australia	140585	Bisphenol A diglycidyl methacrylate, urethane dimethacrylate, BPO
Filtek™ Z350XT	Light-cured composite resin	3M ESPE Dental Products, St. Paul, USA	N458562	Silane ceramic, silane oxidation zirconium, oxide silicon, ERGP-DMA, dimethacrylate
Maxcem Elite™	Self-adhesive resin cement	Kavo, Biberach, Germany	5849496	Two-ethyl methacrylate, HEMA, glyceryl polymethacrylate, glycerol dimethacrylate
Ketac™ Universal	Glass ionomer cement	3M ESPE Dental Products, St. Paul, USA	600264	Lanthanum calcium aluminosilicate glass, acrylic acid, tartaric acid
Zinc oxide-eugenol cement	Zinc oxide-eugenol cement	Shanghai Rongxiang Dental Material Company, Shanghai, China	170101	Zinc oxide, rosin, zinc acetate, zinc stearate, eugenol, olive oil
Negative control	Silicone rubber	Kavo, Biberach, Germany	3-2009	Polydimethylvinyl siloxane, chloroplatinic acid catalyst
Positive control	Phenol	Beijing Chemical Factory, Beijing, China	0100106	Phenol

between the permeability of porous zirconia ceramic and dentin disks.

Cell viability on in vitro dentin barrier cytotoxicity test

The mean (standard deviation; SD) OD490 readings for the six dental materials, one positive control, one negative control, and one blank control, when dentin or selected porous zirconia ceramic disks were used as a barrier, are shown in Tables 2 and 3 according to the permeability of dentin or selected porous zirconia ceramic. The SD for cell viability among the low permeability was higher than that for high permeability across all materials, except for zinc oxide-eugenol cement when dentin disks were used as a barrier. The SD for cell viability among the high permeability was higher than that for the low permeability across all materials, except for MI FLOW II, Aura, and 70 g/l phenol when porous zirconia ceramic disks were used as a barrier. In addition, the difference in SD for cell viability among all materials, when porous zirconia ceramic disks were used as a barrier, was less than that when dentin disks used, except for Maxcem Elite™ (within the high permeability group).

The cytotoxicity results of the six dental materials, one positive control, one negative control, and one blank control, when dentin or selected porous zirconia ceramic disks were used as a barrier, are summarized in Fig. 3 according to the permeability of dentin or selected porous zirconia ceramic. The cell viability of the low permeability was higher than that of the high permeability across all materials, except for silicone rubber and 70 g/l phenol when dentin disks were used as a barrier ($P < 0.05$). The cell viability of the low permeability materials was higher than that of the high permeability materials, except for Maxcem Elite™ when porous zirconia ceramic disks were used as a barrier ($P < 0.05$). Within the high permeability group of dentin, Maxcem Elite™ showed the highest cell viability ($P < 0.05$). While among the other materials, except for the positive and negative controls, there was no significance difference in cell viability in high and low permeability groups, with use of both the dentin and zirconia porous ceramic as a barrier ($P > 0.05$). There was no significant difference in cell viability of materials, for all of the high and low permeability group, according to whether dentin or porous zirconia ceramic disks were used as a barrier ($P > 0.05$).

Table 2 The mean (SD) OD (optical density) and RCV (relatively cell viability) values when human dentin disks were used as a barrier

Material	Permeability	OD	RCV (%)
MI FLOW II	High	0.3178 (0.0342)	67.82 (7.30)
	Low	0.3932 (0.0395)	83.91 (8.43)
Aura	High	0.3156 (0.0324)	67.35 (6.91)
	Low	0.3750 (0.0469)	80.03(10.01)
Filtek™ Z350XT	High	0.3366 (0.0378)	71.83 (8.07)
	Low	0.3848 (0.0389)	82.24 (8.30)
Maxcem Elite™	High	0.3706 (0.0246)	79.09 (5.25)
	Low	0.3998 (0.0268)	85.32 (5.72)
Ketac™ Universal	High	0.3500 (0.0315)	74.69 (6.72)
	Low	0.3882 (0.0325)	82.84 (6.94)
Zinc oxide-eugenol cement	High	0.3214 (0.0320)	68.59 (6.83)
	Low	0.3826 (0.0318)	81.64 (6.79)
Negative control (silicone rubber)	High	0.4462 (0.0200)	95.22 (4.27)
	Low	0.4596 (0.0257)	98.08 (5.48)
Positive control (70 g/l phenol)	High	0.0294 (0.0162)	6.27 (3.46)
	Low	0.0550 (0.0246)	11.74 (5.25)
Blank control		0.4686(0.0200)	100 (4.27)

Table 3 The Mean (SD) OD (optical density) and RCV (relatively cell viability) values when porous zirconia ceramic disks were used as a barrier

Material	Permeability	OD	RCV (%)
MI FLOW II	High	0.3614 (0.0292)	75.89 (6.13)
	Low	0.4088 (0.0329)	85.85 (6.91)
Aura	High	0.3322 (0.0226)	69.76 (4.75)
	Low	0.3976 (0.0232)	83.49 (4.87)
Filtek™ Z350XT	High	0.3576 (0.0203)	75.09 (4.26)
	Low	0.3870 (0.0182)	81.27 (3.82)
Maxcem Elite™	High	0.3564 (0.0278)	74.84 (5.84)
	Low	0.3692 (0.0239)	77.53 (5.02)
Ketac™ Universal	High	0.3594 (0.0280)	75.47 (5.88)
	Low	0.3892 (0.0224)	81.73 (4.70)
Zinc oxide-eugenol cement	High	0.3468 (0.0280)	73.25 (5.88)
	Low	0.3826 (0.0190)	80.34 (3.99)
Negative control (silicone rubber)	High	0.4564 (0.0111)	95.84 (2.33)
	Low	0.4808 (0.0056)	100.97 (1.18)
Positive control (70 g/l phenol)	High	0.0258 (0.0090)	5.42 (1.89)
	Low	0.0514 (0.0122)	10.79 (2.56)
Blank control		0.4762 (0.0110)	100 (2.31)

Discussion

This study investigated the use of porous zirconia ceramic, which has a similar permeability to human dentin at the same thickness (0.5 ± 0.05 mm), as an alternative to dentin during in vitro dentin barrier cytotoxicity tests. In vitro dentin barrier cytotoxicity testing is an effective method to evaluate the cytotoxicity of dental materials when they are applied directly to dentin [3]; the permeability of human dentin also plays a key role [2]. Previous studies have investigated various dentin alternative materials, such as bovine dentin disks, pressed dentin powder disks, Millipore filters, and polyurethane disks [1, 22, 29, 30]. This study focused on porous zirconia ceramic as a dentin alternative material, because it is possible to control permeability of the ceramic according to

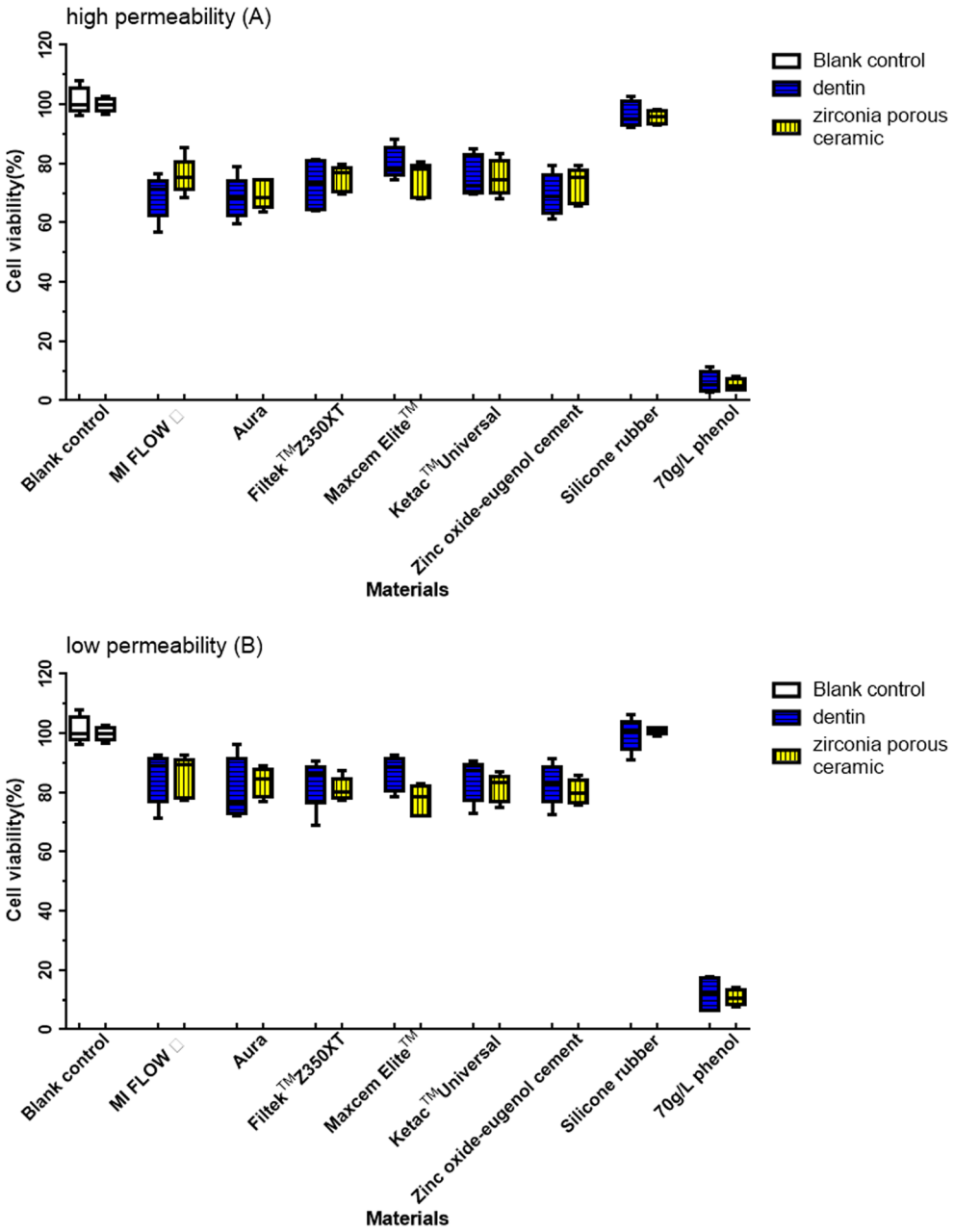

Fig. 3 Cell viability of different materials when dentin or porous zirconia ceramic disks were used as a barrier, for the high and low permeability groups. The figure illustrates the cytotoxicity test results of different materials, for the high (**a**) or low (**b**) permeability group, when dentin and porous zirconia ceramic disks were used as a barrier. Cell viability is expressed as a percentage relative to the blank control group (100%); error bars indicate standard deviations of five replicate disks for each material/barrier combination. Symbol (*) indicates a statistically significant difference between groups ($P < 0.05$)

the amount of pore-forming agent used and the sintering technology applied [24]. Porous zirconia ceramic has an appropriate mechanical function and good biocompatibility and is biologically inert [23, 31, 32]. Hydroxyapatite porous ceramic is biologically active, which can affect the growth of cells during in vitro dentin barrier tests [33, 34].

Dentin permeability varies [20], so in this study, the dentin was chosen and divided into high and similar permeability ($Lp > 0.221$ μl min^{-1} cm^{-2} cm H_2O^{-1}) and low and similar permeability ($Lp \leq 0.221$ μl min^{-1} cm^{-2} cm H_2O^{-1}) groups to reduce the effect of permeability differences among dentins. The mean (SD) of the high and low permeability group of human dentin disks was 0.334 (0.0873) and 0.147 (0.0377) μl min^{-1} cm^{-2} cm H_2O^{-1}, respectively. In this study, the mean permeability of human dentin for the high permeability group was higher than that reported in previous studies and lower than that when compared to the low permeability group [35]. One possible explanation for this difference may be that we divided dentin into high and low permeability types. Compared to the results obtained by Ozok et al. [26], the mean permeability of our human dentin was higher, even in the low permeability group. This difference may be attributable to a difference in measurement area, which was 0.07 cm^2 in the previous study and 0.283 cm^2 in the current study. Compared to the permeability reported by Isable et al. [36], the mean permeability of our result was also higher. This can be

explained by the fact that the disks were obtained in the middle of the coronal dentin in previous study. While in our study, the dentin disks, with a thickness of 0.5 ± 0.05 mm, was closest to dental pulp, which increased its permeability.

In this study, the test materials included flow resin, composite resin, self-adhesive resin, glass ionomer cement, and zinc oxide-eugenol cement, which are the most commonly used in clinical. They can be used as a typical representative of dental restorative materials that are directly applied to dentin. The cell viability of the low permeability materials was higher than that of the high permeability materials, except for silicone rubber and 70 g/l phenol when dentin disks were used as a barrier ($P < 0.05$). This is consistent with the results of Abou et al. [2] and supports the conclusion that the permeability of dentin plays a key role during in vitro dentin barrier cytotoxicity tests. No significant effect of the positive control group (70 g/l phenol) was observed, because a too high quantity of toxic materials was released not to distinguish the difference between the high and low permeability groups.

The cell viability of the materials in the low permeability group was higher than that of the high permeability group, for all materials except Maxcem Elite™ when porous zirconia ceramic disks were used as a barrier ($P < 0.05$). This suggests that the permeability of porous zirconia ceramic also plays a key role when this ceramic were used as a dentin substitute during in vitro dentin barrier cytotoxicity tests. The difference in SDs for cell viability, of all materials when porous zirconia ceramic disks were used as a barrier, was less than that when dentin disks were used, for all materials except Maxcem Elite™ within the high permeability group. In this study, dentin disks were separated by permeability and randomly divided into all groups, which may cause dentin disks with a permeability near 0.221 μl min^{-1} cm^{-2} cm H_2O^{-1} all being sorted into the high and low group of one material. This may explain why the cell viability of Maxcem Elite™ for the high permeability group was similar to that for the low permeability group when dentin disks were used as a barrier. It may also explain why the difference in SD for cell viability for Maxcem Elite™ was smaller when porous zirconia ceramic disks were used as a barrier instead of dentin (within the high permeability group).

The cell viability for all materials when porous zirconia ceramic were used as a barrier was similar to that for dentin during the in vitro dentin barrier cytotoxicity tests, for both the high and low permeability groups ($P > 0.05$). This indicates that porous zirconia ceramic disks with a similar permeability to that of human dentin disks of the same thickness (0.5 ± 0.05 mm) could be used as a dentin alternative material for in vitro dentin barrier cytotoxicity tests.

Porous zirconia ceramic has a different composition, but similar permeability, to human dentin. The cell viability of all materials when porous zirconia ceramic was used as a barrier was similar to that when dentin disks were used during our in vitro dentin barrier cytotoxicity tests, for both the high and low permeability groups. In this study, the biocompatibility evaluation was reliable, and a high correlation was obtained between in vivo and in vitro evaluation methods. It addressed the issue of procuring human teeth for in vitro dentin barrier cytotoxicity testing. Future studies should focus on defining the permeability range of porous zirconia ceramic takes the place of dentin to standardize in vitro dentin barrier cytotoxicity tests. Three-dimensional cell cultures grown under dynamic culture conditions can be used to mimic pulp cell environment with blood flow in vivo, which also will study in the next step.

Conclusion

Porous zirconia ceramic disks, having a similar permeability to human dentin disks at the same thickness (0.5 ± 0.05 mm), are a viable alternative to dentin for in vitro dentin barrier cytotoxicity tests, which are used to evaluate the toxicity of dental materials applied to dentin. Porous zirconia ceramic disks could aid in standardizing dentin barrier cytotoxicity tests in the future. Furthermore, our dental material biocompatibility results were reliable and a high correlation between in vivo and in vitro methods. Use of porous zirconia ceramic could also address the difficulty of procuring human teeth for in vitro dentin barrier cytotoxicity testing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study, formal consent is not required.

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