Original Article

Effect of Disinfection and Storage Media on the Fracture Strength of Teeth

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Abstract

Introduction: The aim of the study was to evaluate the effect of disinfection and storage solutions, and time periods on the fracture strength of whole teeth and tooth sections**. Method:** One hundred and sixty extracted teeth were divided into 16 groups based on disinfection methods, storage times and tooth types. Teeth samples were measured, and areas calculated. Specimens groups were 1. 10% buffered formalin, 2. 0.2% thymol-in-saline, 3. 5.25% sodium hypochlorite (NaOCl), 4. OHCWA disinfection protocol, 5. Distilled-water. Each group had storage subgroups of 14, 90 and 180 days. Group 6 (control) were frozen in distilled water for 14 days. Specimens were tested using an Instron Universal tester and load at fracture was analyzed for statistical significance. **Results:** The NaOCl group showed significantly lower loads at fracture compared to all other storage solutions at corresponding storage times. Distilled-water storage for 90 and 180 days had significantly lower fracture loads, except specimens stored in NaOCl for 14 and 90 days. The area of the specimen, was significantly associated with the magnitude of load at fracture. **Conclusions:** NaOCl storage significantly affected the fracture strength of teeth. Fracture resistance of teeth was inversely proportional to the storage time and directly proportional to the area of the specimen.

Keywords: Disinfection, Storage media, Demineralization, Fracture strength, Biomaterials, Materials Science.

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Introduction

Extracted teeth are ideal for *in vitro* studies but they need to be disinfected before use. Although most disinfection processes provide sterility, their impacts on structural and biomechanical properties of teeth are poorly understood. A literature review showed overwhelming evidence on the concentration and time dependent effects on enamel and dentine with commonly-used chemical disinfection and storage media (1-5). However, there is insufficient evidence regarding which disinfection method and storage medium is appropriate or least impacts tissue characteristics and to determine whether changes are severe enough to cause significant changes to test results. Hence, it seems sensible to question if a universal protocol of disinfection and storage can be followed for all test types and for whole teeth and tooth sections.

Currently used disinfection methods are classified as radiation (gamma rays), steam (autoclave), liquid chemicals (disinfection and storage solutions), and gaseous chemicals (ethylene oxide). Tests involving bond strength (6-13), permeability (13-15), optical properties (4) and surface toughness (3-5,16) have different parameters for the specimens being tested, some of which could be sensitive to disinfection processes, storage media or duration of storage.

As a lack of agreement exists, identifying variables that affect specimen characteristics becomes essential and a prerequisite in research so *in vitro* tests can be recognized as viable and clinically relevant.

Moisture and mineral depletion are the main concerns with prolonged storage in most storage solutions (17,18). This can alter the biomechanical properties of enamel and dentine with consequential detrimental effects on physical properties and bonding (8). This is especially evident when prepared specimens are stored over an extended period of time (19).

Tests of bond strengths to enamel and dentine are common and frequently conducted studies. They have proved to serve more as screening tools, rather than as true indicators of clinical efficacy (9). Despite enamel being highly mineralized and low in organic content, it is critically sensitive to disinfection solutions in *in vitro* bond strength studies. Extreme low bond strengths were found when enamel specimens were stored in ethanol (10% & 70%), 0.05% thymol in saline (thymol/saline) and 5.25% sodium hypochlorite (NaOCl) (6,7) compared to 10% formalin (8), 1% chloramine-T, isotonic saline, and distilled water (DW) (10,11,20).

Dentine bond strength showed extreme variations and low values when stored in thymol/saline, methanol, and glutaraldehyde (7). Higher bond strengths with 10% formalin were attributed to altered substrate owing to its ability to penetrate and fix the dentinal organic tissues (10,11), and the lower bond strengths with the use of 0.05% thymol/saline were due to the inhibitory effects of its phenolic compounds on resin polymerization. Neither reflects the true characteristics of the material or substrate, nor do they represent their expected *in vivo* test results (12,21). Similarly, 5.25% NaOCl diminished bond strengths due to its residual chlorine (6). Other critical parameters which impacted bond strengths were longer storage duration (over 6 months) (12,21), storage temperature, and non-standardized scoring criteria (10). Comparative studies with steam autoclave and ethylene oxide gas sterilization demonstrated little or no significant effects on dentine permeability or bond strength (13).

Chemical disinfection and storage solutions have time and concentration dependent effects on the permeability and compositional structure of dental tissues (1,2). Solutions such as 70% ethanol, distilled-water with thymol, and phosphate-buffered thymol/saline showed significant mineral dissolution effects with biomechanical and bonding ability variations (14). Cryopreservation and steam autoclaving had the least effects on permeability and functional properties of dentine to effect bond strength tests, even though it caused some structural and compositional changes (15). Gamma irradiation has been proven to be effective and presents no detectable dentine structural or compositional changes (22).

Variations in enamel and dentine microhardness (to measure their physical properties) have been reported when stored in different disinfection and storage solutions for different times. Storage in deionized water and calcium chloride solutions showed progressive and rapid decrease in microhardness of enamel and dentine, even with short storage periods up to 2 weeks (3). This contrasted results for storage in de-ionized water, 0.2% glutaraldehyde, Hanks' Balanced Salt Solution (HBSS), 0.1% NaOCl and 0.1% thymol/saline, which had no significant changes for up to 2 months storage, but substantial changes after 12 months (16). Storage in NaOCl had a concentration dependent effect on dentine microhardness (4,5), while HBSS (23) and 10% formalin (24) imparted the least alteration in microhardness for the same storage duration.

Two definitive methods of sterilization of extracted teeth are autoclaving with high-pressure steam (20psi) and temperature (121℃) for 40 minutes, or immersion in 10% formalin for 14 days. These sterilization protocols for extracted teeth are accepted by the Centers for Disease Control and Prevention (CDC), USA and supported by several studies (24-28). Thymol/saline (0.02%) is a proven disinfectant and routinely used as the only solution for disinfection and storage of extracted teeth, particularly if teeth are stored for extended time periods (26,27). NaOCl is a strong oxidizer with a pH of 11 at a 5% concentration. It dissolves necrotic tissue at low concentrations (0.5% – 1%) and organic tissue and bacteria at higher concentrations. This renders NaOCl to be an appropriate irrigant for root canal treatment (26), but still a weak disinfection agent at most concentrations (1%, 2.6% and 5.25%) (26,28).

At the Oral Health Centre of Western Australia (OHCWA) and the UWA Dental School, University of Western Australia, the Infection Control Committee has a protocol for disinfection and storage of extracted teeth (OHCWA-Protocol). This involves disinfection in 5.25% NaOCl for two hours after gross debridement, thorough rinsing under running water and then immersion in 10% formalin for two weeks. Teeth are then stored in 0.02% solution of thymol/saline until used for teaching or research purposes. Although this protocol could satisfy disinfection standards, its effects on biomechanical properties of teeth are unknown.

The aim of this study was to compare the effects of four commonly used disinfection and storage media on fracture strength of whole and prepared teeth specimens when stored for different time periods, in order to identify a disinfection protocol which least affects specimen characteristics for use in *in vitro* fracture studies.

Materials and Methods

The University of Western Australia's Human Research Ethics Committee approved the use of extracted human teeth. Teeth were collected from participating dentists in the Perth region. Only teeth diagnosed and assessed as needing extraction for clinical reasons were collected from patients who consented to their teeth being used for

research. A detailed information guide was provided to the Dentists about the study's scope and the collection and storage procedure to use.

Participating Dentists were instructed to place teeth in DW and to store them in jars provided in a refrigerator until collected and transferred to the laboratory. Specimens were then rinsed in running DW to remove blood and attached tissues before being frozen in jars at -4ºC in moist conditions until further testing. Chemical disinfection of the teeth was performed after all specimens were collected so the disinfection processes and storage duration were standardized.

The 160 teeth were randomly distributed into 16 groups (n=10) based on disinfection methods and storage times (Table I). Teeth were kept frozen until specimens were prepared. Each group had an equal and random distribution of tooth types with a combination of single root lower premolar whole teeth (two lower premolars – PM) and tooth root sections (four upper molar palatal roots - UM-P and four lower molar distal roots - LM-D). Roots were sectioned horizontally apical to the furcation with a diamond bur (High Speed Diamond Bur 881, Komet, Gebr Brasseler, Lemgo, Germany) under air/water spray.

Each tooth was rinsed in DW and debrided using an ultrasonic piezo scaler (Satelec P5XS Newtron, Acteon, France) to remove adhered soft tissue or bone. They were measured (mesio-distally and bucco-lingually) using Vernier calipers at the mid-root level for roots and at the cemento-enamel junction for whole teeth. These measurements were used to assess the area of the tooth or root being tested. The measurement sites were marked, and these were the points of load application while testing. Specimens were kept hydrated by holding them with a moist gauze while being handled and the time that specimens were not immersed in the solution was minimized.

Specimens were prepared and stored in their respective disinfection solutions for specified periods of times (Group 1 - 10% buffered formalin (Perrigo, Australia), Group 2 - 0.2% thymol/saline (Sigma – Aldrich, USA), Group 3 – 5.25% NaOCl (Chem-Supply Pty Ltd, Australia), Group 4 - OHCWA-Protocol and Group 5 – DW (control group). Storage times were 14, 90 and 180 days. Group 6 (Control) constituted of freshly extracted and frozen teeth, stored for 14 days under moist conditions, to simulate teeth in *in vivo* conditions. Stringent infection control protocols were followed when handling and testing these non-sterile specimens in this group.

At the end of each storage period, specimens were tested by applying compressive forces until fracture using an Instron Universal testing machine (ElectroPuls™ E3000 All-Electric Dynamic Test Instrument, Instron Engineering Corporation, USA). Specimens were loaded on the test platform so the point of loading was at the same point where size measurements were taken. Specimens were subjected to load with a blunt indenting point until fracture at a crosshead speed of 0.5mm/min. Load at fracture was recorded in Newtons. Data was analyzed for statistical significance using linear regression by comparing the three-way interaction of the disinfection solutions, number of days in the solution, and area of specimen.

Statistical Analysis

Data were collated in a spreadsheet (Microsoft Excel 2016) and the statistical analysis was performed with IBM SPSS STATISTICS, version 28 software (IBM Corp. USA). The data were normally distributed and therefore they were analyzed for statistical significance ($P < 0.05$) using linear regression by comparing the three-way interaction of the disinfection solutions, the number of days in the solution, and the area of the specimen. Linear regression was used to provide a P-value for each comparison in order to obtain the estimate of the effect of each variable.

Table I: Summary statistics for load at break and area broken down by Solution and Storage Time (Days)

Solution	No. of Days (Groups)	No	Label	Mean	Std. Dev
10% Formalin	14	10	Load at Break	1007.85	295.26
	(Group 1a)		Area	28.42	7.11
	90	10	Load at Break	922.26	316.08
	(Group 1b)		Area	27.43	5.23
	180	10	Load at Break	1180.18	247.15
	(Group 1c)		Area	28.89	4.1
0.2% Thymol in saline	14	10	Load at Break	1094.67	274.78
	(Group 2a)		Area	29.65	7.29
	90	10	Load at Break	1042.03	339.66
	(Group 2b)		Area	27.76	4.17
	180	10	Load at Break	1225.28	207.7
	(Group 2c)		Area	33.67	6.94
5.25% Sodium Hypochlorite	14	10	Load at Break	1033.25	337.84
	(Group 3a)		Area	29.56	8.7
	90	10	Load at Break	761.67	191.08
	(Group 3b)		Area	29.06	5.17
	180	10	Load at Break	861.05	330.48
	(Group 3c)		Area	28.08	3.83
OHCWA disinfection	14	10	Load at Break	1141.18	292.91
	(Group 4a)		Area	31.48	4.79
	90	10	Load at Break	970.91	162.1
	(Group 4b)		Area	27.07	4.59
	180	10	Load at Break	1102	311.18
	(Group 4c)		Area	27.02	5.31
Distilled water	14	10	Load at Break	933.79	355.58
	(Group 5a)		Area	27.94	5.34
	90	10	Load at Break	960.22	317.6
	(Group 5b)		Area	28.51	6.86
	180	10	Load at Break	945.15	174.36
	(Group 5c)		Area	27.53	5.59
Control	14	10	Load at Break	811.03	155.96
	(Group 6)		Area	25.51	4.9

Results

The statistical summary of load at fracture of all sub-groups in relation to specimen area, utilized solutions, storage period and controls is presented in Table I. Teeth stored in NaOCl had significantly lower breaking loads for all samples regardless of their storage time (Table II). Load at fracture among specimens stored in 0.2% thymol/saline, 10% formalin and the OHCWA-Protocol were not significantly different during different storage periods.

Table II: P-values for comparisons of solutions. Significant comparisons (P<0.05) are highlighted (*)

SOLUTIONS	0.2% Thymol in saline	10% Formalin	5.25% Sodium Hypochlorite	OHCWA Disinfection	Distilled Water
0.2% Thymol in saline		0.678	0.004*	0.899	0.120
10% Formalin			0.018*	0.6048	0.269
5.25% Sodium Hypochlorite				0.005*	0.183
OHCWA Disinfection					0.107
Distilled Water					

The NaOCl group had a significantly lower load at fracture compared to the OHCWA-protocol at all storage periods - 14 days (P=0.005), 90 days (P=0.019), 180 days (P=0.001). Compared to 0.2% thymol/saline, significantly lower loads at fracture were found at all storage periods - 14 days (P=0.008), 90 days (P=0.008), 180 days (P=0.001). In comparison to 10% formalin, lower fracture loads were recorded between all storage periods - 14 days (P=0.03), 90 days (P=0.039), 180 days (P=0.000). Teeth stored in NaOCl for 90 days had breaking loads significantly lower than those stored in DW for 90 and 180 days, and in NaOCl for 14 days.

Specimens stored in DW for 90 and 180 days had significantly lower fracture loads compared to all other solutions and time periods, except specimens stored in 5.25% NaOCl for 14 and 90 days. No significant differences in fracture loads were observed between the DW and control group at 14 days storage (P=0.866) (Tables III, IV). Summary statistics for load at break and area, in relation to solutions and tooth type are presented in Table V. The area of specimen tested was significantly associated with load at fracture (P=0.005). An increase in tooth area resulted in a greater required load to fracture the tooth or root. Regarding the type of tooth specimen, lower molar distal roots (LM-D) in 5.25% NaOCl or DW had significantly lower fracture loads than the same specimen type stored in 10% formalin or the OHCWA-Protocol (Table VI).

Table III: P-values for all comparisons of solution by day. Significant comparisons (P<0.05) are highlighted (*)

SOLUTION AND DAY	0.2% Thymol-saline 90 days	0.2% Thymol-saline 180 days	0.2% Thymol-saline 14 days	10% Formalin 90 days	10% Formalin 180 days	10% Formalin 14 days	5.25% NaOCl 90 days	5.25% NaOCl 180 days	5.25% NaOCl 14 days	OHCWA protocol 90 days	OHCWA protocol 180 days	OHCWA protocol 14 days	DW 90 days	DW 180 days	DW 14 days
0.2% Thymol in saline 90 days		0.51	0.99	0.70	0.40	0.61	0.01*	0.22	0.67	0.81	0.54	0.64	0.49	0.63	0.37
0.2% Thymol in saline 180 days			0.50	0.33	0.86	0.25	0.001*	0.06	0.28	0.39	0.96	0.91	0.18	0.26	0.13
0.2% Thymol in saline 14 days				0.71	0.40	0.62	0.01*	0.22	0.68	0.82	0.53	0.64	0.50	0.63	0.37
10% Formalin 90 days					0.25	0.93	0.04*	0.46	1.00	0.88	0.35	0.44	0.81	0.95	0.66
10% Formalin 180 days						0.18	0.001*	0.04*	0.20	0.29	0.82	0.78	0.12	0.18	0.08
10% Formalin 14 days							0.03*	0.47	0.93	0.80	0.26	0.36	0.86	0.98	0.70
5.25% NaOCl 90 days								0.14	0.02*	0.02*	0.001*	0.01*	0.04*	0.03*	0.07
5.25% NaOCl 180 days									0.41	0.34	0.06	0.12	0.58	0.45	0.74
5.25% NaOCl 14 days										0.87	0.30	0.40	0.79	0.95	0.63
OHCWA protocol 90 days											0.40	0.51	0.68	0.82	0.53
OHCWA protocol 180 days												0.94	0.19	0.27	0.13
OHCWA protocol 14 days													0.29	0.37	0.21
DW 90 days														0.84	0.83
DW 180 days															0.67
DW 14 days															

Table IV: P-values for all comparisons of solution by tooth type. Significant comparisons (P<0.05) are highlighted (*)

SOLUTION AND TOOTH TYPE	.2% Thymol-saline for LM-D	0.2% Thymol-saline for UM-P	0.2% Thymol-saline for PM	10% Formalin for LM-D	10% Formalin for UM-P	10% Formalin for PM	5.25% NaOCl for LM-D	5.25 % NaOCl for UM-P	5.25 % NaOCl for PM	OHCWA protocol for LM-D	OHCWA protocol for UM-P	OHCWA protocol for PM	DW for LM-D	DW for UM-P	DW for PM
0.2% Thymol in saline for LM-D		0.33	0.61	0.60	0.91	0.95	0.08	0.27	0.38	0.52	0.92	0.40	0.08	0.95	0.76
0.2% Thymol in saline for UM-P			0.76	0.66	0.35	0.49	0.004*	0.03*	0.08	0.77	0.46	0.94	0.04*	0.28	0.61
0.2% Thymol in saline for PM				0.95	0.68	0.72	0.04*	0.15	0.22	0.96	0.72	0.75	0.04*	0.58	0.86
10% Formalin for LM-D					0.67	0.73	0.02*	0.10	0.18	0.90	0.73	0.67	0.02*	0.56	0.89
10% Formalin for UM-P						0.98	0.05*	0.17	0.32	0.58	0.99	0.42	0.05*	0.84	0.83
10% Formalin for PM							0.15	0.35	0.43	0.66	0.98	0.52	0.15	0.91	0.84
5.25% NaOCl for LM-D								0.53	0.53	0.01*	0.11	0.02*	0.98	0.09	0.06
5.25% NaOCl for UM-P									0.94	0.08	0.26	0.07	0.54	0.25	0.20
5.25% NaOCl for PM										0.15	0.39	0.13	0.54	0.42	0.29
OHCWA for LM-D											0.65	0.76	0.02*	0.48	0.81
OHCWA for UM-P												0.50	0.11	0.87	0.85
OHCWA for PM													0.02*	0.35	0.62
DW for LM-D														0.09	0.07
DW for UM-P															0.71

DW for PM					

Table V: Summary statistics for load at break and area broken down by solution and tooth type.

Group	Tooth type	No	Variables	Mean	Std Dev
10% Formalin	LM-D	11	Load at Break	1124.1	306.62
			Area	31.12	6.46
	PM	5	Load at Break	1020.83	224.72
			Area	30.59	4.59
	UM-P	14	Load at Break	973.84	315.72
			Area	25.15	2.89
0.2% Thymol in saline	LM-D	10	Load at Break	1079.36	262.07
			Area	32.06	3.8
	PM	6	Load at Break	1140.84	256.21
			Area	31.96	3.37
	UM-P	14	Load at Break	1141.51	317.72
			Area	28.46	8.63
5.25% Sodium Hypochlorite	LM-D	12	Load at Break	885.79	325.26
			Area	31.87	5.54
	PM	6	Load at Break	996.17	432.48
			Area	32.43	5.86
	UM-P	12	Load at Break	829.43	214.54
			Area	24.16	3.08
OHCWA disinfection	LM-D	13	Load at Break	1151.29	293.44
			Area	31.16	2.9
	PM	6	Load at Break	1131.63	198.17
			Area	28.3	5.75
	UM-P	11	Load at Break	944.02	229.41
			Area	25.54	5.72
Distilled water	LM-D	12	Load at Break	859.22	274.93
			Area	30.8	3.91
	PM	6	Load at Break	1070.16	121.46
			Area	30.6	7.08
	UM-P	12	Load at Break	971.66	333.97
			Area	23.89	4.37
Control	LM-D	2	Load at Break	746.28	103.08
			Area	22.69	5.64
	PM	4	Load at Break	758.77	128.78
			Area	28.51	4.27

	UM-P	4	Load at Break	895.67	191.97
			Area	23.92	4.74 continues

Group	Tooth type	No	Variables	Mean	Std Dev
Thymol 0.2%	LM-D	2	Load at Break	928.84	11.02
			Area	33.18	4.76
	PM	1	Load at Break	992.47	.
			Area	26.04	.
	UM-P	2	Load at Break	986.04	59.42
			Area	19.69	1.54

(Legend: LM-D Lower molar distal root, PM-Premolar root, UM-P Upper molar palatal root)

Table VI: P-values for DW and Control at 14 days

Group	N	Mean	Std Dev	Min	Median	Max	P-value	
							Group	Area
Distilled water at 14 days	10	933.79	355.58	317.49	967.16	1372.69	0.866	0.435
Control at 14 days	10	811.03	155.96	615.44	794.08	1144.98		

Discussion

An ideal storage solution or method should disinfect and preserve tissue properties so that *in vitro* test results are standardized and reproducible. However, the literature suggests that disinfection and storage protocols can alter specimen characteristics and thereby influence test results. Teeth specimens are thought to be more susceptible to alterations when tooth or root sections are used instead of whole teeth (2,6). This study compared the effects on fracture resistance of whole teeth to root sections when treated with four disinfection solutions over three time periods and discovered some significant correlations.

Overall, there were no statistically significant relationships between fracture resistance of specimens in the solutions, number of days and tooth types. However, teeth stored in 5.25% NaOCl for 90 days (Group 3b) tolerated significantly lower fracture loads. In addition, this group exhibited significantly lower fracture resistance than teeth stored for 14 (Group 3a) and 180 (Group 3c) days in the same solution. Specimens stored in DW for 90 and 180 days also demonstrated significantly lower fracture loads compared to all other solutions and time periods, except those stored in NaOCl for 14 and 90 days.

Exposure to NaOCl has been reported to aggravate the mechanical properties of enamel and dentine, due to its demineralizing effect (4,5,16,24,26). Fracture resistance of teeth in this study when stored in 5.25% NaOCl had the lowest values compared to other solutions for the same storage duration. The effects of NaOCl were concentration and duration dependent, as demonstrated by Slutzky-Goldberg *et al* (2004). Exposure to a higher concentration of NaOCl (6%) and longer duration, induced a significant decrease in dentine microhardness (4,5). These effects were greater in superficial dentine layers where there are wider dentinal tubules and thinner peritubular dentine, which allowed a greater amount of collagen to be exposed to the solution (2). This could be another reason for lower fracture strength of root sections apart from their size, as observed in this study.

The surface area of specimens had a significant effect on overall strength of the specimen being tested, with whole teeth showing higher fracture resistance compared to root sections. The area of the specimen tested was significantly and directly associated with load at fracture,

Within the root types, the smaller lower molar distal roots showed lower fracture strengths compared to upper molar palatal roots, signifying that dentine thickness plays a role in fracture resistance. Apart from dentine thickness, the permeability and area of the dentine exposed to disinfection solutions, contributes to the demineralizing effects of solutions, especially with higher concentrations and prolonged exposures (3-5).

The control group (Group 6) contained freshly extracted non-sterile teeth frozen at -4°C in DW for 14 days, to simulate *in vivo* conditions for teeth rather than of dentine after disinfection or any chemical storage. Contrary to what would be thought, specimens of this group (Group 6) had no significant differences in fracture resistance compared to other groups stored for the same duration, including those stored in DW at room temperature (Group 5a, 5b, 5c). This is comparable to a study by Habelitz *et al*. (2002) where there was a rapid and substantial decrease in hardness and elastic modulus of enamel and dentine after 2 weeks of storage in deionized water, especially when dentine sections were used. However, minimal biomechanical and compositional variations can be expected from teeth specimens stored in HBSS for more than 2 months owing to its high mineral ion concentration, which is comparable to the mineral phases of enamel and dentine (16).

Sterilization by autoclaving is a protocol recommended by the CDC (26). However, sterilization involving heat (moist or dry) deteriorates the biomechanical properties of dental tissues, thus its use is best reserved for tests not involving physical properties, materials that are not heat sensitive and teeth that do not contain amalgam (26). Sterilization by immersion in 10% buffered formalin for 2 weeks is the recommended method, as it is known to fix and maintain the compositional structure of dental tissues (26). Autoclaving was found to produce consistent and comparable bond strengths to dentine when compared to chemical disinfection procedures (29).

Since dentine specimens are prone to moisture loss, increased brittleness and decreased strain at fracture were noticed when stored for more than 2 months, even when stored in DW (18). Dehydration was more evident in sectioned specimens than whole teeth (19). Although dentine brittleness secondary to moisture loss was reversible on rehydration (17), compositional changes from mineral leach-out was significant in most solutions when stored for 45 days, except in when using HBSS (2,18).

As per the CDC guidelines, immersion of whole teeth in 10% formalin for 14 days or autoclaving and minimal storage time for prepared specimens are the ideal disinfection protocols for effective tissue fixation and minimal structural alterations of dentine (24,26). The sterilization potency of NaOCl and that of thymol/saline were shown to be ineffective. Newer products such as Gigasept-PA (Schülke UK, Sheffield, UK) are safer and effective alternatives to formalin for sterilizing extracted teeth (26).

The results of this study show a direct correlation of disinfection and storage methods to the biomechanical properties of dental tissues to the extent that choosing a compatible disinfection protocol forms an important prerequisite for research methodology to obtain clinically relevant test results. However, the limitations of specimen numbers, physiological variations, donor ages, developmental and acquired defects must be considered when designing studies on fracture strength.

Conclusion

NaOCl (5.25%) is not recommended as a disinfection and storage solution for specimens that are planned to be subjected to assessment of the physical properties of teeth. Formalin (10%) is a potential disinfection solution and preserves the compositional structure of teeth so it is the best solution for *in vitro* testing of fracture strength. Fracture resistance of specimens stored in 0.2% thymol/saline, 10% formalin and the OHCWA-Protocol for different time periods were not significantly different. Longer periods of storage, irrespective of the solution, jeopardized the physical properties of enamel and dentine. The area or size of the specimen tested was significantly associated with load at fracture, with increased area corresponding to increased load required to fracture the tooth or the root.

Conflict of Interest

The authors deny any conflicts of interest related to this study.

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Author 1: Contributed to conception, design, data acquisition and interpretation, performed all statistical analyses, drafted and critically revised the manuscript. Author 2: Contributed to conception, design, data interpretation and critically revised the manuscript.

All authors gave their final approval and agree to be accountable for all aspects of the work.

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