HARDNESS AND PENETRATION OF GUTTA PERCHA WHEN EXPOSED TO TWO ENDODONTIC SOLVENTS

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DECLARATION

I, Ebrahim Patel, declare that this research report is my own work. It has been submitted for the degree of Master of Science in Dentistry in the Faculty of Health Sciences at the University of the Witwatersrand, Parktown, Johannesburg, South Africa. It has not been submitted before for any other degree or examination at this or any other University.

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This 4th day of July 2014

ABSTRACT

Purpose: Endodontic retreatment requires the removal of the obturation material from the root canal system. Gutta percha (GP), the most commonly employed obturation material, requires mechanical instrumentation coupled with a chemical adjunct to facilitate its removal. Xylene and Eucalyptus oil are recommended as endodontic solvents due to their dissolving capacity of GP. This study sought to test the changes in the hardness and penetrability of three types of GP (Conventional, Thermafil® and Guttacore™) when exposed to these solvents, utilizing distilled water as a control.

Method and materials: Textural analysis was performed to determine the hardness by testing for rigidity, and the penetrability by testing for deformation energy and resilience. These properties were tested on 81 GP cones prior to, and following solvent exposure. For each outcome variable, results were tabulated by group. Between-group differences were assessed by means of a General Linear Model, with the outcome variable as the dependent variable and the solvent, GP type and solvent-GP type interaction as the independent variables.

Results: A significant decrease in rigidity and deformation energy was observed across all groups. Resilience was observed to decrease with the thermoplastic GP, Thermafil and Guttacore, but increased with conventional GP. Thermoplastic GP was more amenable to a reduction in hardness and penetration when compared with Conventional GP. A greater reduction in the hardness of Thermafil was observed with Eucalyptus oil. Conventional GP was susceptible to a significant reduction in hardness with both solvents, however its penetrability may be reduced following exposure to Xylene. Guttacore was significantly altered by both solvents.

Conclusions: Considering the toxicity profile of Xylene, and the biocompatibility and antimicrobial effects of Eucalyptol, Eucalyptus oil is recommended for use during endodontic retreatment.

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To summarize, over the last few years I ventured far from my comfort-zone, I experienced, I made mistakes, I learned, I matured…The excitement does not stop here of course, for I still have a lot to learn…

"The real voyage of discovery consists not in seeking new landscapes, but in having new eyes."

- Marcel Proust (French novelist: 1871 - 1922)

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CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1. Background to the Study

Endodontic therapy is concluded with the filling of the root canal system to provide as perfect a seal as possible in order to facilitate periapical repair. This is a critical step which requires three-dimensional filling of the canals (Schilder, 1967; Gutmann et al, 2010). Since its introduction as a root filling material by Bowman in 1867, gutta-percha (GP) remains the material of choice for obturation, and has thus been synonymous with endodontic obturation (Prakash et al, 2005). While satisfying many of the requirements of an ideal obturation material (plasticity, ease of manipulation, minimal toxicity, radiopacity and ease of removal with solvents), GP lacks sufficient adhesiveness and rigidity, and easily displaces under pressure. However, these properties do not overshadow its advantages (Gutmann et al, 2010).

Successful endodontic therapy is dependent on multiple factors, some of which include adequate disinfection of the root canal system, correct preparation and adequate seal of the canals and the tooth. However, at times even meticulously performed endodontic therapies fail, resulting in the need for endodontic retreatment (Whitworth and Boursin, 2000; Martos et al, 2006).

The main objective of endodontic retreatment requires the removal of the GP filling material from the canal/s and to regain access to the apical foramen (Tasdemir et al, 2008). Whilst mechanical instrumentation serves as the primary method of GP removal, many studies have shown that this alone is insufficient due to the presence of residual GP material in the canal

(Hűlsmann and Bluhm, 2004; Ezzie et al, 2006). Therefore, chemical solvents have been proposed which then serve as an adjunct to mechanical removal (Magalhães et al, 2007). The solvent softens and partially dissolves the gutta-percha, rendering it more amenable to removal with hand instruments and thereby decreasing the risk of perforation (Kaplowitz, 1991).

1.2. Gutta-Percha in Endodontics

Gutta-Percha, first used in the Malaysian archipelago, is derived from the malay language as "Getah" meaning gum, and "Pertja" which refers to the tree (Prakash et al, 2005). GP is a *trans*-1,4-polyisoprene (Fig. 1.1). It is derived from *Palaquium gutta* bail obtained from the tree family Sapotaceae, by dried coagulated extracts (Gurgel-Filho et al, 2003). Whilst GP resembles natural rubber in many of its mechanical properties, it is harder, less elastic and more brittle due to its *trans*-isomer. This isomer differs from the *cis*-isomer of natural rubber (Fig. 1.2) in that it is more linear and crystallizes more easily (Metzger et al, 2011). The crystalline phase appears in two forms, the Alpha (α) phase and the Beta (β) phase. The transformation from the β-phase to the α-phase occurs at the endothermic peak between 42^{o}C and 49^{o}C. The forms differ only in the molecular repeat distance and single carbon-bond configuration (Schilder et al, 1974).

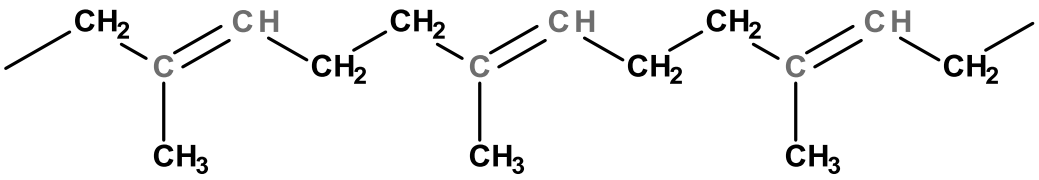


Fig. 1.1 Chemical structure of gutta-percha (trans-1,4-polyisoprene) (adapted from Roberts and Caserio, 1979).

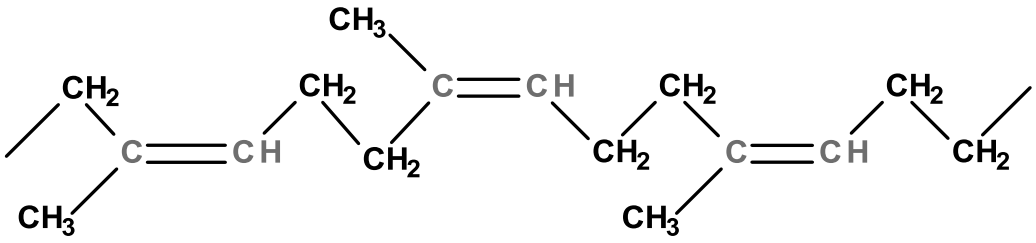


Fig. 1.2 Chemical structure of natural rubber (cis-1,4-polyisoprene) (adapted from Roberts and Caserio, 1979).

Conventionally, commercial Gutta Percha cones were composed of inorganic zinc oxide, barium sulphate, waxes, resins and organic gutta-percha. The percentage composition of these components differed between manufacturers (Marciano and Michailesco, 1989). However, Maniglia-Ferreira et al confirmed in 2005 that the absence of barium sulphate is not unusual in some of the more recent manufacturing forms of GP. Variations in the proportions of its inorganic components to the gutta-percha would lead to variations in the brittleness, stiffness, tensile strength and force, and thermal behaviour (Schilder et al, 1974).

Advances in technology have led to the advent of new types of GP. In addition to conventional GP utilised with cold lateral condensation obturation techniques, thermoplastic GP as well as the recently launched cross-linked GP have been developed, which permit warm vertical condensation. However, in 2004 Tsukada et al reported that the techniques using melted GP alone may not be favourable compared with conventional lateral condensation, because the melted GP undergoes a large amount of volumetric shrinkage in the crystalline polymer at phase-transition during setting. This shrinkage hinders the sealing ability of the GP and thus compromises successful endodontic therapy, although ironically it may favour retreatment due to greater penetration of the solvent in the potential spaces created.

1.2.1. Conventional Gutta-Percha

Conventional GP (Fig. 1.3) has pure gutta-percha and zinc oxide as its bulk constituents (Meyer et al, 2006). The percentage contribution of the gutta-percha ranges from 19 - 22, whereas the zinc oxide ranges from 59 - 79 (Metzger et al, 2011). During endodontic retreatment, solvents and files contact the surface layer of the coronal GP which enables them to uniformly soften this layer.

Fig. 1.3 A make of conventional GP cones size F3.

1.2.2. Thermafil Gutta-Percha

Thermafil® (Dentsply, York, USA) (Fig. 1.4) consists of warm α-phase gutta-percha wrapped around a central polysulfone core. When heated the α-phase gutta-percha becomes pliable and tacky and will flow when pressure is applied. It has been reported that solvents used during retreatment (with the exception of chloroform) are able to soften the circumferential GP only with little/no effect on the core (Ibarrola et al, 1993).

Fig. 1.4 Thermafil GP cones size F3.

1.2.3. Guttacore Gutta-Percha

Guttacore™ (Dentsply, York, USA) (Fig. 1.5) has an internal cross-linked gutta-percha core surrounded by an outer layer of α-phase gutta-percha (Gutmann, 2011). When heated, only the outer α-phase gutta-percha will flow whilst the inner core remains firm. Thus no shrinkage of the inner core occurs due to the absence of the transitional phase. According to the manufacturer, Guttacore is removed more easily than other carrier-based systems. However, the core behaves unlike gutta-percha in that it does not readily dissolve with solvents and it is not as amenable to plasticising with heat. During endodontic retreatment, the solvents coming into contact with the GP will only soften the circumferential α-phase gutta-percha with no change to the central gutta-percha core (Beasley et al, 2013).

Fig. 1.5 GuttaCore GP cones size F3.

1.3. Endodontic Solvents

Advances in endodontic retreatment have also lead to changes in the solvents used to soften and remove the GP from within the root canal system. Chloroform and Halothane were for many years the solvents of choice as they were the most effective in dissolving endodontic sealants (Wilcox, 1995; Ferreira et al, 2001). However, due to their related toxicity and carcinogenicity, and with the U.S. Food and Drug Administration prohibiting the use of chloroform, clinicians now seek a suitable alternative (Oyama et al, 1999; Karlović et al, 2001; Azar et al, 2011). Xylol (Xylene) and Eucalyptol are organic solvents which were introduced as they were not considered potential carcinogens (U.S Department of Health and Human Service, 1985).

1.3.1. Xylene

Xylene is an aromatic hydrocarbon with the chemical formula of $C_6H_4(CH_3)_2$ (Fig. 1.6) and is referred to as "dimethyl benzene" because it consists of a six-carbon ring to which two methyl groups are bound. It is a sweet smelling, colourless liquid occurring naturally in petroleum, coal and wood tar. Xylene is widely used in medical technology as a solvent (U.S Department of Health and Human Services, 1993). Dental histological laboratories use Xylene for tissue processing as its high solvency factor allows for maximum displacement of alcohol rendering the tissue transparent. Furthermore, it is utilised in staining and cover-slipping procedures as the Xylene allows for excellent de-waxing and clearing capabilities that contribute to exceptionally stained slides. Laboratory-grade xylene is composed of *m*-xylene (40–65%), *p*-xylene (20%), *o*-xylene (20%) and ethyl benzene (6–20%) with traces of toluene, trimethyl benzene, phenol, thiophene, pyridine and hydrogen sulphide (Kandyala et al, 2010).

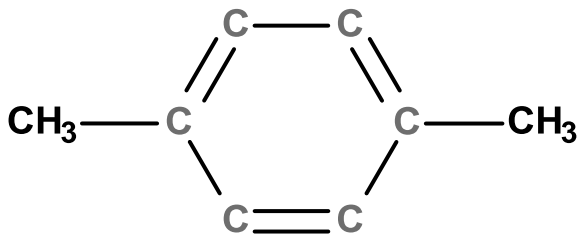


Fig. 1.6 Chemical structure of para-xylene (1,4-dimethylbenzene).

The drawback of Xylene, however, is its toxicity to human tissue. This can occur via inhalation and/or direct contact, with an exposure limit of 100ppm before symptoms such as nausea and headaches are experienced. In cases of exposure to >200ppm weakness and vomiting occurs, with consequential loss of consciousness when levels exceed 10,000ppm (Uchida et al, 1993; Kandyala et al, 2010). However, its restricted use during endodontic retreatment, coupled with its removal by high-volume suction equipment, limits the exposure time to the clinician, provided universal infection control barriers such as gloves and masks are used.

Xylene was established as an efficient solvent during endodontic retreatment as early as the 1980s (Tamse et al, 1986; Wilcox et al, 1987). Its success as a solvent, probably due to destabilisation of the covalent bonds between the carbon atoms of the gutta-percha, has been further proven in studies conducted in the 1990s as well as more recent studies in the 2000s (Kaplowitz, 1990; Hansen et al, 1998; Oyama et al, 2002; Martos et al, 2006; Magalhães et al, 2007; Rubino et al, 2012).

1.3.2. Eucalyptus Oil

Eucalyptus belongs to the Myrtaceae family and comprises 900 species. This native genus from Australia comprises more than 300 species which contain volatile oils in their leaves. However, fewer than 20 of these have a content of greater than 70% of 1,8-cineole (Elaissi et al, 2012). 1,8-cineole (Fig. 1.7), more commonly known as Eucalyptol, is frequently used in the cosmetic and pharmaceutical industries. It increases the percutaneous penetration of drugs and may act as a nasal decongestant. It is also used in the treatment of bronchitis, sinusitis and asthma due to its related anti-inflammatory action which inhibits the production of tumour necrosis factor alpha (Juergens et al, 1998; Soares et al, 2005). Eucalyptol has also been demonstrated to be an anti-bacterial and anti-fungal agent (Takarada et al, 2004; Elaissi et al, 2012).

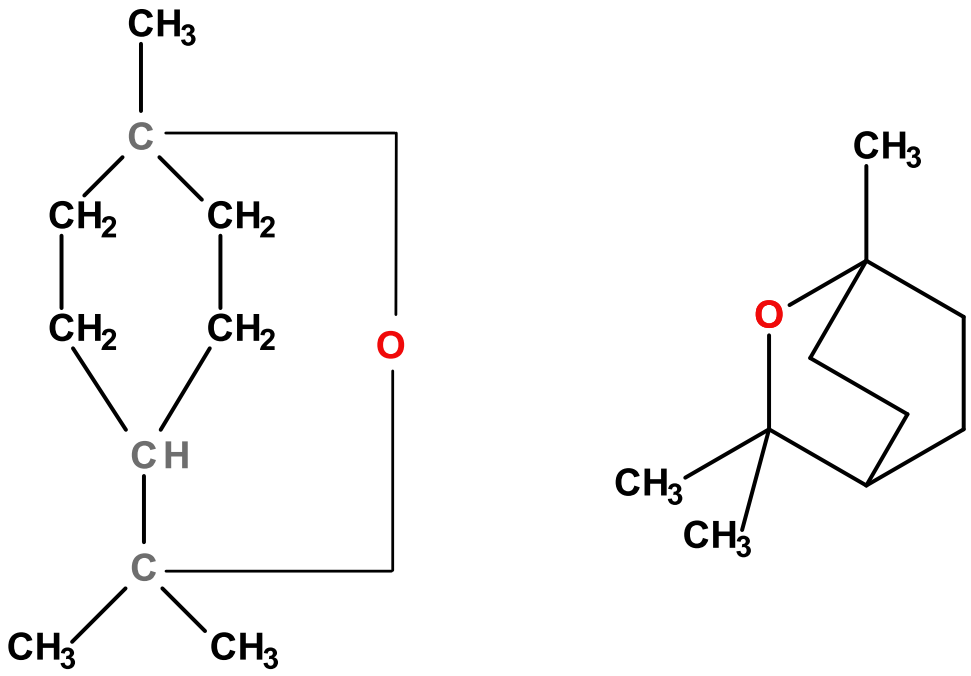


Fig. 1.7 Chemical structure of Eucalyptol (1,8-Cineole) (adapted from Mitchell, 1989).

Eucalyptol has been used as an endodontic solvent since the 1850s. However, its popularity was limited by the efficiency of chloroform in that era. When chloroform-induced cytoxicity and carcinogenicity became a concern, and the U.S Food & Drug Administration banned the use of chloroform in 1985, clinicians were forced to turn to other volatile and organic solvents. Eucalyptol gained popularity once again, and even though it proved to be effective together with other oils such as orange oil, was still not as effective as solvents such as Xylene (Oyama et al, 2002; Faria-Júnior et al, 2011). However, its favourable toxicity profile and biocompatibility lead to advocation for its use (Uemara et al, 1997; Tanomaru-Filho et al, 2010). Wourms et al (1990) attributed the reason for the decreased efficiency of Eucalyptol to the fact that it could not be heated within the root canals: they demonstrated that increasing the temperature of the solvent to 37^{o}C increases its penetrability. Johann et al (2006) confirmed in their review that both Xylene and Eucalyptol are acceptable endodontic solvents, being able to penetrate GP to at least 10mm within 70 seconds.

Eucalyptol is now an accepted alternative to chloroform, whereas Xylene has been described as a cytotoxic solvent that may even be carcinogenic (Hunter et al, 1991; Hansen et al, 1998; Schäfer and Zandbiglari, 2002; Magalhães et al, 2007). Additional commercial solvents such as Endosolv E, Endosolv R and DMS IV are available. However, their function is limited to dissolving endodontic sealers and thus they have little or no effect on GP.

To date, several authors have investigated the dissolving ability of multiple solvents on conventional GP and root canal sealants. Their studies quantified the dissolving capacity of a solvent by measuring the weight of the GP before and after exposure to it. The findings of these studies either validated or refuted solvents as capable of dissolving GP or sealants. None sought to test changes in the physical properties of the GP material (Tanomaru-Filho et

al, 2010; Faria-Júnior et al, 2011; Mushtaq et al, 2012). Changes in the physical properties following solvent exposure are important as they can render the material more easily removable by mechanical instrumentation; conversely, they make retreatment more difficult. Properties such as hardness and penetrability of the filling material are particularly important as mechanical files are required to engage the GP in the root canal to allow its removal

Therefore this study sought to measure and compare the hardness and penetrability of GP after exposure to solvents.

CHAPTER 2
AIM AND OBJECTIVES

2.1. Aim

The aim of this study was to test the changes in the hardness and penetrability of three types of commercial GP used for endodontic obturation when exposed to two types of solvents, and to deduce whether these changes would benefit the operator during endodontic retreatment.

2.2. Objectives

1. To determine the hardness and penetrability of three types of gutta-percha (Conventional, thermoplastic Thermafil® or cross-linked Guttacore™), where hardness is inferred by rigidity and penetration by deformation energy and resilience.
2. To determine how the solvents Xylene and Eucalyptus oil will alter these physical properties relative to a control of distilled water.
3. To determine which manufacturing form of GP will be more resistant to changes in their physical properties.

2.3. Null Hypotheses

2.3.1 Neither of the solvents would alter the physical properties of GP significantly.

2.3.2 There will be no significant differences between the two solvents.

CHAPTER 3
METHOD AND MATERIALS

3.1. Sample Size Calculation

The sample size estimation, performed in G*Power (Buchner et al, 2009), was based on the combined influence of gutta-percha type (3 types) and solvent type (3 types) on each of the outcome variables (hardness and penetration). The effect of GP type and solvent type on each of the outcome variables was determined by a two-factor ANOVA with interaction. Sample size estimations were based on a significance level of 5%, a power of 80% and the effect sizes calculated from pilot data. From these data, the following sample sizes were determined:

Rigidity (9 groups): the sample size was estimated at 36 (4 per group).
Deformation Energy (9 groups): the sample size was estimated at 81 (9 per group)
Resilience (9 groups): the sample size was estimated at 45 (5 per group)

Due to each individual test yielding measurements on all three outcome variables, ultimately each group had 9 sets of data. Thus 81 cones were required in total

3.2. Method and Materials

The solvents were Xylene BP and Eucalyptus oil BP with distilled water serving as a control. Three different types of GP were chosen for the study and grouped as per table 2.1: conventional Protaper® size F3 GP cones, thermoplastic GP Thermafil® ISO 030 carrier and

cross-linked Guttacore™ 0.04 size 030. Each GP cone, from the respective GP type, used was from the same manufacturing batch to eliminate variations in physical properties.

Table 3.1 Group allocation of the three types of gutta-percha grouped with the respective solvents.

Gutta-percha	Solvents		
	Xylene	Eucalyptus oil	Distilled Water (control)
Conventional Gutta Percha	Group 1a	Group 1b	Group 1c
Thermoplastic Thermafil®	Group 2a	Group 2b	Group 2c
Crosslinked Guttacore™	Group 3a	Group 3b	Group 3c

The GP cones were placed in eppendorf vials (Fig. 3.1) and labelled according to their specific experimental group and were numbered from 01 – 81. These numbers were then tabulated on an Excel® spreadsheet and randomly arranged. Each experimental batch consisted of 27 vials.

Fig. 3.1 Two eppendorf vials

The gutta-percha from each group was texturally analysed using the TA.XT Plus® texture analyser (Stable Micro Systems, USA, and used with kind permission from the Department of Pharmacy under the guidance of Prof V Pillay) (Fig. 3.2A).

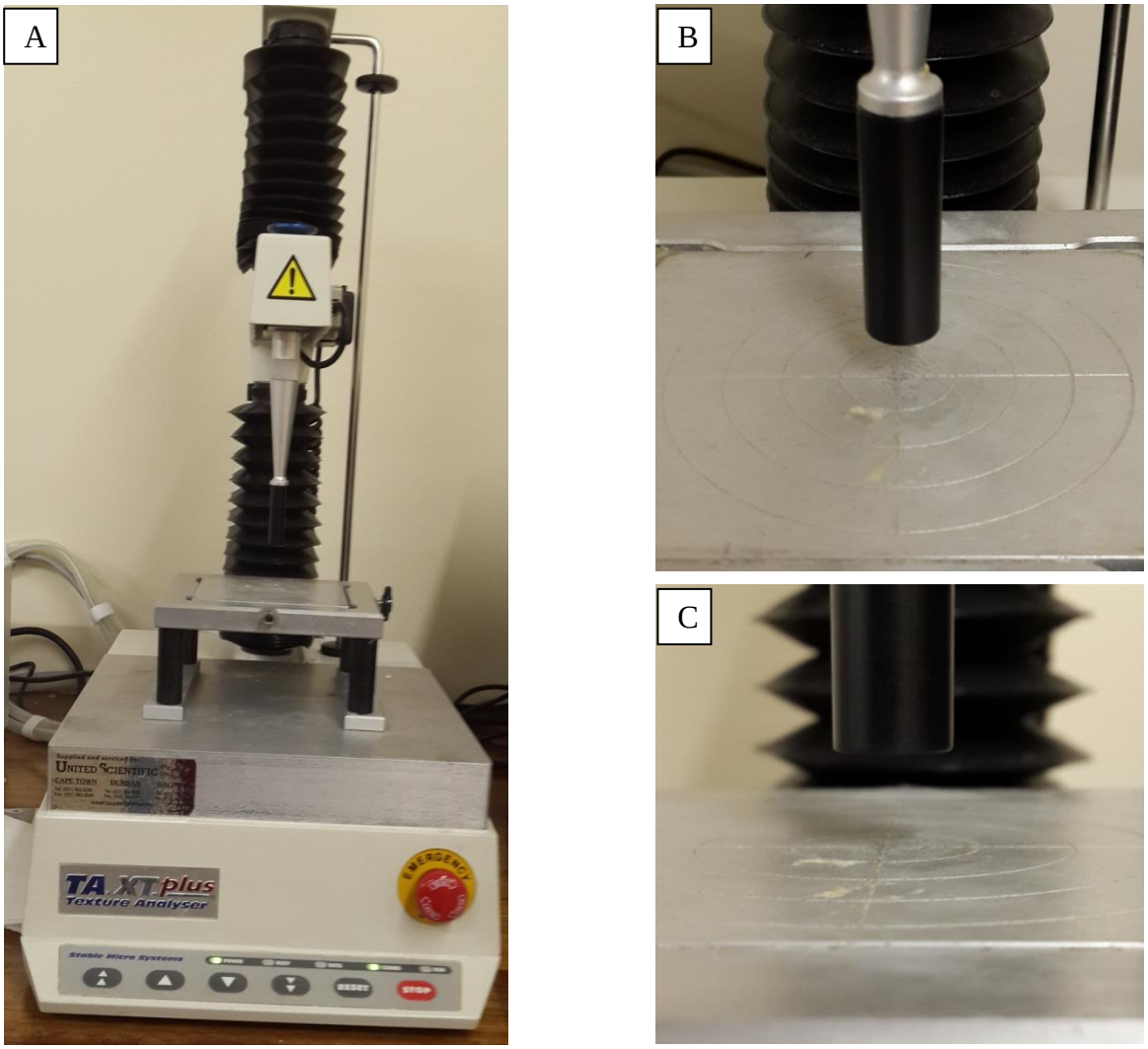


Fig. 3.2 A.The TA.XT plus texture analyser, **B**. Flat-ended probe above mounting plate with surface markings for positioning, and **C**. Probe in position 10mm above platform.

A flat-ended cylindrical probe was chosen which tested each cone against a fixed horizontal platform with graded markings (Fig 3.2B,C). These markings allowed for reproducible positioning of the GP cones. Prior to testing, the TA.XT was first calibrated for weight, force and distance. A force of 10N with a test speed of 5mm.s^{-1} was chosen for all tests. The handles of the Thermafil and Guttacore cones prevented the cones sitting flush on the measurement table and so these were cut off at a level above their respective rubber stops.

Each cone was analysed prior to solvent exposure for hardness (rigidity) and penetration (deformation energy and resilience) (Fig. 3.3).

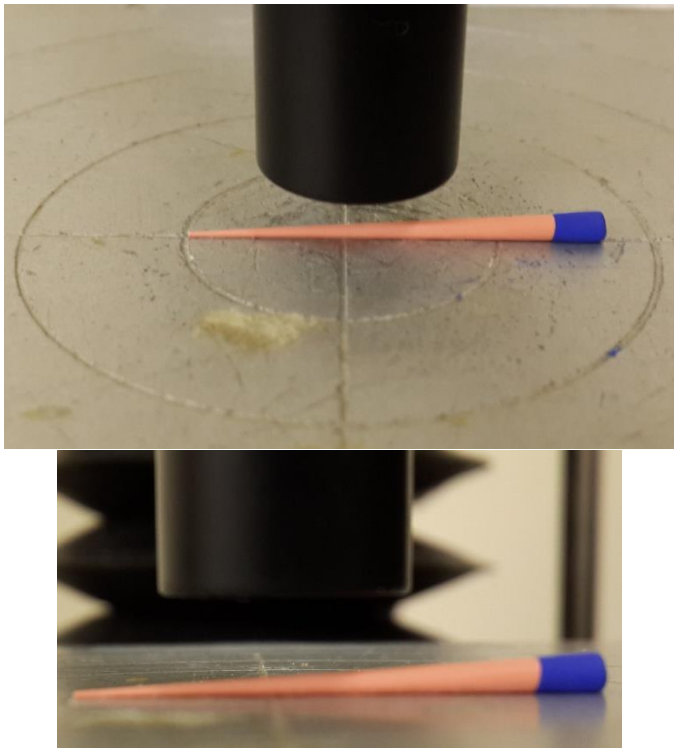


Fig. 3.3 A conventional GP cone positioned using the reference graded markings of the table, with the probe in position 10mm perpendicularly above.

The GP cones, according to their group allocation, were first seated in an Endo box® stand (Dentsply Maillefer, Switzerland). This allowed for a contact-free surface which facilitated solvent exposure around the entire cone circumference. The endo stand (with the GP cones) (Fig. 3.4) was then placed in its Endo box allowing for immersion into 160ml of the corresponding solvent at 24±1°C for 10 minutes, followed by immersion in 160ml of distilled water for 20 minutes to neutralise the solvent action. The cones were allowed to dry for 24h at room temperature 24±1°C. The details surrounding each batch including the ambient room temperature and the temperature of the solvent and distilled water were recorded. These GP cones were then texturally reanalysed for rigidity, deformation energy and resilience.

Fig. 3.4 GP cones seated in an endo stand, prior to batch allocation, demonstrating free contact of the entire circumference of the cone for solvent exposure.

The software, Exponent®, linked to this equipment captured data at 200pps and processed the data into the Force-Distance and Force-Time graphs illustrated in Figures 3.4 and 3.5 respectively. Rigidity is the gradient (1:2) of the curve on a Force-Distance graph and deformation energy is the area under the curve on a Force-Distance graph (Fig. 3.5). A Force-Time graph is used to measure resilience: it is the area from the peak of the curve to the end point (2:3) divided by the area from the beginning of the curve to its peak (1:2), with the resultant multiplied by 100 (Fig. 3.6).

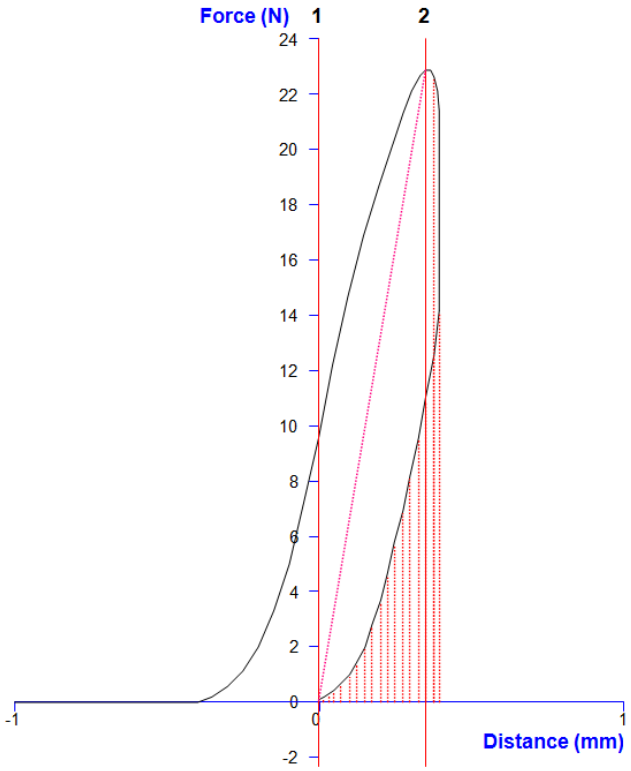


Fig. 3.5 Typical Force–Distance textural analysis graph illustrating the gradient and area used to calculate rigidity and deformation energy.

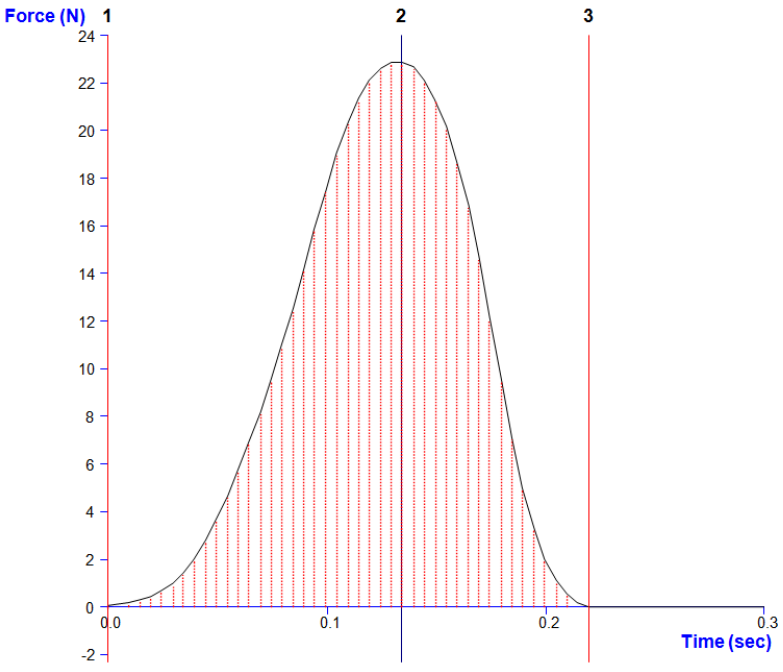


Fig. 3.6 Typical Force-Time textural analysis graph illustrating the area used to calculate resilience.

3.3. Data Analysis

The outcome variable for analysis, for each of the three measurements (rigidity, deformation energy and resilience), is the difference between the post- and pre-solvent exposure measurements. For each outcome variable, the results were tabulated by group, showing the mean and standard deviation of the data. Between-group differences were assessed by means of a General Linear Model (GLM) with the outcome variable as the dependent variable; independent variables were the solvent, GP type and solvent-GP type interaction; covariates were room and solvent temperatures. The 5% significance level was employed throughout the study. Data analysis was carried out using SAS software (SAS Instutute inc. USA).

CHAPTER 4
RESULTS

4.1. Background

The properties measured were rigidity, deformation energy and resilience.

There were 9 treatment combinations (3 levels of solvent tested at each of 3 levels of GP type). Based on a pilot study, a sample size of 9 per treatment combination was used, resulting in a total of 81 experiments. These were carried out in random order, over a period of 3 days (27 experiments per day).

Experiments were conducted by measuring each of the three properties on the sample cone (BEFORE measurement), followed by immersing the sample in the solvent for 10 min at 24±1°C. After rinsing the sample in demineralised water for 20 minutes, and drying at 24±1°C for 24 hours, the three properties were again measured (AFTER measurement).

Since each sample thus acts as its own control, the ultimate measurement of interest for each property was the difference between the AFTER and BEFORE measurements (DIFF=AFTER-BEFORE). The AFTER results are attached as Appendix A.

4.2. Univariate Data Analysis

The univariate statistics (mean, standard deviation, median, interquartile range) for the three dependent (outcome) variables (for BEFORE, AFTER, and DIFF measurements) are shown in Table 4.1, 4.2 and 4.3.

An example of the histogram plots for the dependent variables (for BEFORE, AFTER, and DIFF measurements) across all three GP types is depicted in Fig. 4.1.

The dependent variables (for BEFORE, AFTER, and DIFF measurements) were also plotted in run order to check for any trend with time. An example of the run plot graph is depicted in Fig. 4.2.

The following observations were established from these data:

- The histograms displayed no obvious outliers in the BEFORE, AFTER or DIFF data for any of the dependent variables.
- The distributions of the data were not markedly skewed.
- The plots of the data versus run order showed no obvious trends with time.

Table 4.1 Univariate statistics for the three dependent variables for Conventional GP.

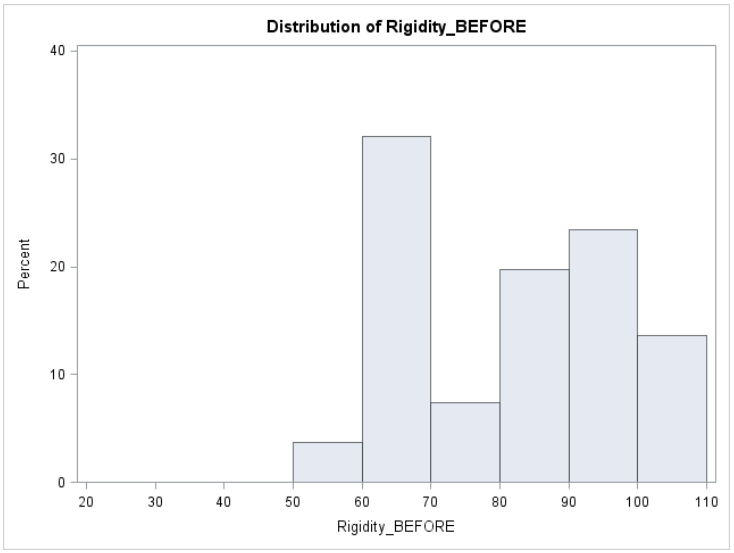
GP type	Solvent	n	Variable	Mean	Standard Deviation	95% Confidence Limits for Mean		Minimum	Maximum	Median	Inter-quartile Range	
Conventional	Distilled Water	9	Rigidity_BEFORE	65.19	2.34	63.39	67.00	61.77	68.26	65.73	63.69	66.92
			Rigidity_AFTER	62.17	2.33	60.38	63.96	58.30	65.31	62.81	59.94	63.80
			Rigidity_DIFF	-3.02	3.10	-5.41	-0.64	-8.62	1.81	-2.97	-3.75	-1.38
			Deformation_Energy_BEFORE	3.93	0.22	3.76	4.10	3.57	4.26	3.95	3.81	4.11
			Deformation_Energy_AFTER	3.78	0.23	3.60	3.96	3.36	4.07	3.83	3.62	3.94
			Deformation_Energy_DIFF	-0.15	0.34	-0.41	0.11	-0.79	0.42	-0.17	-0.29	-0.03
			Resilience_BEFORE	96.65	1.61	95.41	97.88	94.63	99.95	96.65	95.43	97.41
			Resilience_AFTER	94.81	1.83	93.40	96.22	92.24	97.86	94.67	93.57	96.18
			Resilience_DIFF	-1.84	2.69	-3.90	0.23	-6.77	3.23	-1.67	-2.51	-1.23
	Eucalyptus Oil	9	Rigidity_BEFORE	62.02	4.21	58.78	65.25	55.66	67.72	60.81	58.66	65.26
			Rigidity_AFTER	54.17	2.06	52.59	55.76	52.10	58.87	53.50	52.82	54.69
			Rigidity_DIFF	-7.84	4.89	-11.60	-4.08	-15.17	-1.31	-8.36	-10.32	-4.66
			Deformation_Energy_BEFORE	3.80	0.24	3.62	3.98	3.50	4.22	3.77	3.67	3.86
			Deformation_Energy_AFTER	3.29	0.16	3.16	3.41	3.07	3.59	3.23	3.20	3.32
			Deformation_Energy_DIFF	-0.51	0.35	-0.78	-0.24	-1.03	0.05	-0.61	-0.69	-0.28
			Resilience_BEFORE	88.92	9.08	81.94	95.90	73.75	100.28	91.78	82.26	94.92
			Resilience_AFTER	91.94	3.17	89.50	94.38	87.86	98.54	91.35	89.88	93.43
			Resilience_DIFF	3.02	9.60	-4.36	10.40	-12.42	18.72	1.65	-4.33	9.27
	Xylene	9	Rigidity_BEFORE	65.02	2.43	63.15	66.88	60.15	68.35	65.31	64.24	65.84
			Rigidity_AFTER	40.56	10.41	32.56	48.57	30.73	57.86	36.89	31.15	46.24
			Rigidity_DIFF	-24.45	10.88	-32.81	-16.09	-35.01	-5.46	-27.88	-33.96	-19.09
			Deformation_Energy_BEFORE	3.92	0.13	3.82	4.02	3.63	4.13	3.95	3.89	3.95
			Deformation_Energy_AFTER	2.02	0.82	1.38	2.65	1.17	3.28	1.59	1.38	2.52
			Deformation_Energy_DIFF	-1.90	0.92	-2.61	-1.20	-2.77	-0.35	-2.48	-2.57	-1.33
			Resilience_BEFORE	84.73	6.10	80.04	89.42	80.95	100.84	82.80	82.23	83.41
			Resilience_AFTER	96.28	6.37	91.38	101.17	84.60	106.37	96.99	91.98	99.01
			Resilience_DIFF	11.55	11.61	2.62	20.47	-16.24	23.57	14.78	8.60	16.03

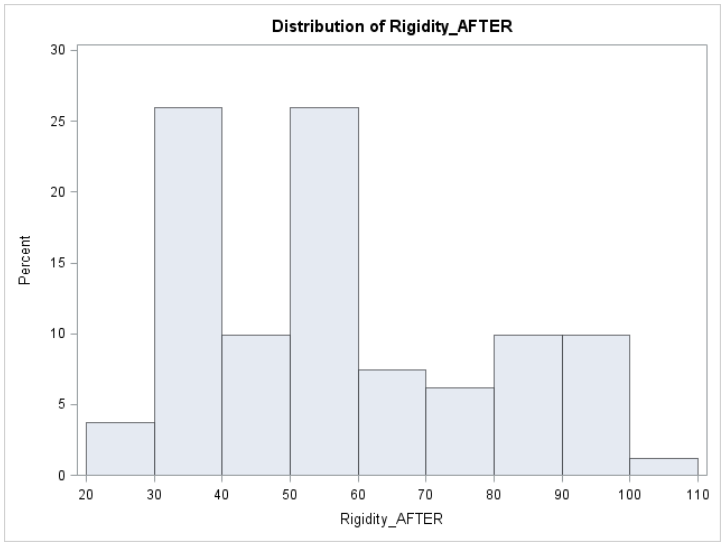
Table 4.2 Univariate statistics for the three dependent variables for Guttacore.

GP type	Solvent	n	Variable	Mean	Standard Deviation	95% Confidence Limits for Mean		Minimum	Maximum	Median	Inter-quartile Range	
Guttacore	Dist Water	9	Rigidity_BEFORE	90.41	10.67	82.22	98.61	74.45	103.09	92.14	81.81	98.80
			Rigidity_AFTER	87.76	8.56	81.17	94.34	73.11	99.28	89.86	82.61	91.69
			Rigidity_DIFF	-2.66	17.55	-16.15	10.83	-24.54	23.44	-9.53	-13.30	12.90
			Deformation_Energy_BEFORE	5.21	0.92	4.50	5.92	3.92	6.47	5.58	4.47	5.88
			Deformation_Energy_AFTER	5.11	0.66	4.60	5.62	4.13	6.02	5.22	4.60	5.54
			Deformation_Energy_DIFF	-0.10	1.42	-1.19	0.99	-1.76	1.93	-0.93	-1.04	1.30
			Resilience_BEFORE	95.98	3.61	93.21	98.75	92.01	102.78	95.78	93.15	97.57
			Resilience_AFTER	93.31	2.49	91.39	95.22	89.96	97.03	93.06	91.88	95.05
			Resilience_DIFF	-2.67	3.10	-5.06	-0.29	-7.90	2.92	-3.18	-4.08	-1.87
	Eucalyptus Oil	9	Rigidity_BEFORE	90.17	9.22	83.08	97.26	75.48	102.25	92.57	82.16	96.48
			Rigidity_AFTER	42.25	8.90	35.41	49.09	31.66	57.49	38.69	36.89	43.39
			Rigidity_DIFF	-47.92	13.52	-58.31	-37.53	-70.59	-31.20	-47.06	-57.91	-36.92
			Deformation_Energy_BEFORE	5.28	0.83	4.64	5.92	3.97	6.38	5.40	4.72	5.90
			Deformation_Energy_AFTER	2.05	0.37	1.76	2.33	1.75	2.69	1.84	1.80	2.10
			Deformation_Energy_DIFF	-3.24	0.95	-3.96	-2.51	-4.54	-1.86	-2.92	-3.92	-2.52
			Resilience_BEFORE	93.73	3.51	91.03	96.42	89.90	99.67	93.53	91.15	94.12
			Resilience_AFTER	92.48	7.98	86.35	98.62	79.71	105.55	91.40	86.39	96.85
			Resilience_DIFF	-1.25	9.44	-8.50	6.01	-12.72	15.65	-2.72	-8.34	4.91
	Xylene	9	Rigidity_BEFORE	85.83	12.70	76.07	95.60	62.42	99.34	90.67	80.29	92.45
			Rigidity_AFTER	34.73	4.64	31.17	38.30	27.53	40.22	34.75	32.20	39.86
			Rigidity_DIFF	-51.10	14.33	-62.11	-40.09	-66.17	-22.43	-56.34	-61.13	-45.55
			Deformation_Energy_BEFORE	4.53	0.82	3.90	5.17	3.22	5.46	4.76	3.78	5.19
			Deformation_Energy_AFTER	1.58	0.43	1.25	1.91	0.97	2.06	1.85	1.23	1.91
			Deformation_Energy_DIFF	-2.95	0.92	-3.66	-2.24	-3.79	-1.26	-3.31	-3.59	-2.55
			Resilience_BEFORE	93.87	3.87	90.89	96.84	87.43	98.62	94.51	92.44	96.59
			Resilience_AFTER	83.84	6.44	78.89	88.79	73.21	94.98	85.78	80.36	87.44
			Resilience_DIFF	-10.02	6.40	-14.94	-5.10	-24.26	-3.64	-7.39	-9.15	-7.07

Table 4.3 Univariate statistics for the three dependent variables for Thermafil.

GP type	Solvent	n	Variable	Mean	Standard Deviation	95% Confidence Limits for Mean		Minimum	Maximum	Median	Inter-quartile Range	
Thermafil	Distilled Water	9	Rigidity_BEFORE	89.90	13.92	79.20	100.60	70.24	107.08	86.60	82.54	102.78
			Rigidity_AFTER	90.91	7.06	85.49	96.34	80.26	103.34	92.82	85.34	94.61
			Rigidity_DIFF	1.01	12.05	-8.26	10.27	-20.74	14.52	5.93	-6.62	8.01
			Deformation_Energy_BEFORE	5.29	1.14	4.42	6.17	3.85	6.71	5.03	4.41	6.38
			Deformation_Energy_AFTER	5.37	0.61	4.90	5.84	4.53	6.53	5.41	4.86	5.67
			Deformation_Energy_DIFF	0.08	1.09	-0.76	0.91	-1.79	1.28	0.64	-0.71	0.78
			Resilience_BEFORE	93.48	3.43	90.84	96.11	87.50	99.13	93.03	92.11	94.65
			Resilience_AFTER	92.84	1.40	91.76	93.92	90.64	94.29	93.29	91.56	94.02
			Resilience_DIFF	-0.63	4.05	-3.75	2.48	-8.48	4.06	1.01	-3.62	2.11
	Eucalyptus Oil	9	Rigidity_BEFORE	91.38	9.93	83.75	99.02	71.53	100.99	95.57	84.96	99.28
			Rigidity_AFTER	42.23	10.42	34.22	50.24	29.55	56.07	36.44	35.38	50.68
			Rigidity_DIFF	-49.15	14.93	-60.63	-37.68	-71.44	-26.24	-49.36	-59.54	-41.29
			Deformation_Energy_BEFORE	5.39	0.80	4.78	6.00	3.82	6.35	5.50	5.01	5.94
			Deformation_Energy_AFTER	2.25	0.54	1.83	2.67	1.62	3.02	2.13	1.81	2.65
			Deformation_Energy_DIFF	-3.14	0.90	-3.83	-2.44	-4.22	-1.78	-3.14	-3.89	-2.51
			Resilience_BEFORE	93.13	2.29	91.37	94.89	90.01	96.51	93.52	91.03	94.80
			Resilience_AFTER	91.00	8.42	84.53	97.48	78.38	105.14	88.55	86.41	97.60
			Resilience_DIFF	-2.12	7.22	-7.68	3.43	-11.95	10.07	-4.02	-6.26	1.08
	Xylene	9	Rigidity_BEFORE	91.42	8.33	85.02	97.83	81.12	101.64	90.36	84.97	99.03
			Rigidity_AFTER	59.94	15.50	48.03	71.85	39.53	81.50	55.99	48.68	72.75
			Rigidity_DIFF	-31.48	13.81	-42.09	-20.86	-58.04	-14.52	-28.89	-34.02	-22.82
			Deformation_Energy_BEFORE	5.82	0.42	5.50	6.14	4.84	6.35	5.81	5.77	6.02
			Deformation_Energy_AFTER	2.60	1.31	1.60	3.61	1.04	4.36	2.01	1.71	3.70
			Deformation_Energy_DIFF	-3.22	1.35	-4.25	-2.18	-4.73	-1.16	-3.72	-4.32	-2.31
			Resilience_BEFORE	91.24	3.59	88.48	94.00	85.64	95.74	90.05	89.28	94.92
			Resilience_AFTER	92.26	6.73	87.08	97.43	83.59	101.86	94.34	84.83	97.65
			Resilience_DIFF	1.02	5.69	-3.36	5.39	-5.69	11.85	-0.33	-2.79	2.69





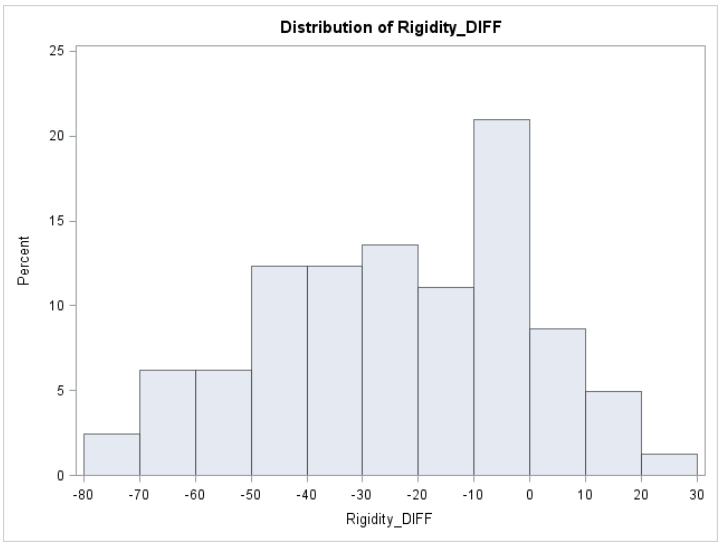


Fig. 4.1 Histogram plots of rigidity distribution before and after solvent exposure, and the differences between the two measurements.

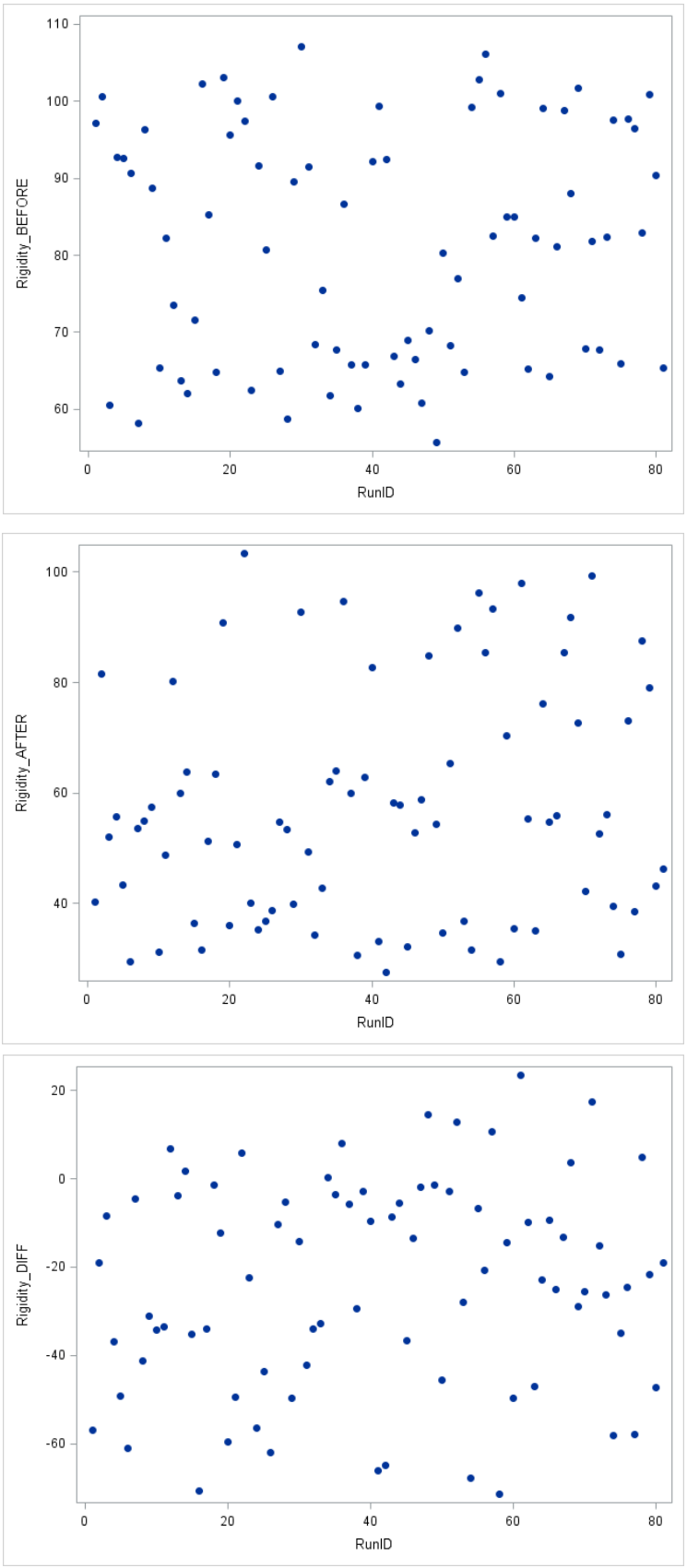


Fig. 4.2 Run order plotting for rigidity (BEFORE, AFTER and DIFF).

4.3. Comparison with pilot data

The results of the treatments which corresponded between the pilot and main studies (i.e. for Xylene and the three GP types) were compared using the Wilcoxon rank sum test (this is the non-parametric equivalent of the independent samples t-test and was used here due to the small sample size of the pilot study group). The results are shown in Table 4.4. To ensure that the pilot and main studies were comparable, there should be no significant differences noted between the two studies.

Observations deduced from Table 4.4 are as follows:

- The rigidity results (BEFORE, AFTER, and DIFF) were not significantly different between the pilot and main studies – this is as expected.
- The deformation energy results were completely different between the pilot and main studies:
    - Across the three GP types, the BEFORE values were approximately 4-7 times higher in the main study than in the pilot study. The AFTER values were not significantly different (the difference for Thermafil was marginal). Thus the DIFF values were significantly different: in the pilot study, deformation energy increased from BEFORE to AFTER, while it decreased in the main study.
- The Resilience results were completely different between the pilot and main studies:
    - Across the three GP types, the BEFORE values were approximately 3 times higher in the main study than in the pilot study, while the AFTER values were 4-5 times higher in the main study than in the pilot study The DIFF values were not significantly different between the two studies.

Table 4.4 Univariate statistics comparing the pilot data to the main data set.

Solvent	Variable	Pilot study					Main study					p-value for H0: no significant difference between means	Ratio of means: Main/Pilot
		n	Mean	Standard Deviation	95% Confidence Limits for Mean		n	Mean	Standard Deviation	95% Confidence Limits for Mean			
Xylene – Conventional GP	Rigidity_BEFORE	4	64.02	2.62	59.86	68.18	9	65.02	2.43	63.15	66.88	0.88	1.0
	Rigidity_AFTER		32.40	3.00	27.63	37.17		40.56	10.41	32.56	48.57	0.24	1.3
	Rigidity_DIFF		-31.62	3.50	-37.19	-26.05		-24.45	10.88	-32.81	-16.09	0.27	0.8
	Deformation_Energy_BEFORE		0.95	0.01	0.94	0.96		3.92	0.13	3.82	4.02	0.019	4.1
	Deformation_Energy_AFTER		1.31	0.14	1.08	1.53		2.02	0.82	1.38	2.65	0.13	1.5
	Deformation_Energy_DIFF		0.36	0.14	0.13	0.58		-1.90	0.92	-2.61	-1.20	0.019	-5.3
	Resilience_BEFORE		22.65	0.55	21.77	23.52		84.73	6.10	80.04	89.42	0.019	3.7
	Resilience_AFTER		18.57	2.48	14.62	22.51		96.28	6.37	91.38	101.17	0.019	5.2
	Resilience_DIFF		-4.08	1.93	-7.16	-1.00		11.55	11.61	2.62	20.47	0.059	-2.8
Xylene - GuttaCore	Rigidity_BEFORE	4	96.59	15.75	71.54	121.65	9	85.83	12.70	76.07	95.60	0.50	0.9
	Rigidity_AFTER		37.00	3.57	31.32	42.68		34.73	4.64	31.17	38.30	0.37	0.9
	Rigidity_DIFF		-59.60	14.57	-82.79	-36.40		-51.10	14.33	-62.11	-40.09	0.45	0.9
	Deformation_Energy_BEFORE		0.76	0.02	0.72	0.79		4.53	0.82	3.90	5.17	0.019	6.0
	Deformation_Energy_AFTER		1.15	0.18	0.86	1.43		1.58	0.43	1.25	1.91	0.21	1.4
	Deformation_Energy_DIFF		0.39	0.17	0.12	0.66		-2.95	0.92	-3.66	-2.24	0.019	-7.6
	Resilience_BEFORE		27.44	0.68	26.35	28.52		93.87	3.87	90.89	96.84	0.019	3.4
	Resilience_AFTER		19.98	2.61	15.83	24.13		83.84	6.44	78.89	88.79	0.019	4.2
	Resilience_DIFF		-7.46	2.29	-11.10	-3.82		-10.02	6.40	-14.94	-5.10	0.88	1.3
Xylene - Thermafil	Rigidity_BEFORE	4	84.74	4.13	78.18	91.31	9	91.42	8.33	85.02	97.83	0.21	1.1
	Rigidity_AFTER		49.76	5.34	41.26	58.26		59.94	15.50	48.03	71.85	0.30	1.2
	Rigidity_DIFF		-34.98	9.11	-49.49	-20.48		-31.48	13.81	-42.09	-20.86	0.50	0.9
	Deformation_Energy_BEFORE		0.78	0.03	0.72	0.83		5.82	0.42	5.50	6.14	0.019	7.5
	Deformation_Energy_AFTER		1.01	0.07	0.90	1.12		2.60	1.31	1.60	3.61	0.045	2.6
	Deformation_Energy_DIFF		0.24	0.08	0.10	0.37		-3.22	1.35	-4.25	-2.18	0.019	-13.7
	Resilience_BEFORE		27.07	1.73	24.31	29.83		91.24	3.59	88.48	94.00	0.019	3.4
	Resilience_AFTER		23.49	1.38	21.30	25.68		92.26	6.73	87.08	97.43	0.019	3.9
	Resilience_DIFF		-3.58	1.74	-6.34	-0.81		1.02	5.69	-3.36	5.39	0.12	-0.3

It was subsequently established that the test probe speed in the pilot data had been set at $0.5mm.s^{-1}$, whereas in the main study it was set as $5mm.s^{-1}$. Changes in probe speed affect the contact time of the probe on material constituents, and thus change the resultant graph plot. Hence, any increase or decrease in probe speed would ultimately alter the resultant values for any parameter (Klatzky et al, 2003). However, of importance is the DIFF data which indicate solvent efficiency. For that reason, even with differing BEFORE and AFTER data, the DIFF values were not significantly different and therefore valid.

4.4. Comparison of within-GP types solvent groups before treatment

In order to validate the use of the DIFF variables to evaluate the effect of solvent treatment, randomisation was employed to ensure that there were no significant differences in the BEFORE measurements between the three groups, within each GP type, assigned to each of the three solvents.

A general linear model with main effect for GP type, solvent (nested in GP type), and experiment day as a blocking variable, was used to model each of the BEFORE dependent variables in turn. The variable transformations described in the previous section were retained; the solvent effect should be non-significant. The source table is shown in Table 4.5, with the box-and-whisker plots illustrating the data shown in Fig. 4.3 and 4.4.

Table 4.5 Source table for the dependent variables.

Rigidity_BEFORE (inverse square root transformation)					
Source	**Degrees of freedom**	**Type 3 Sum of Squares**	**Mean Square**	**F**	**p**
GP_type	2	0.00602	0.00301	97.98	<0.0001
Solvent(GP_type)	6	0.000072	0.000012	0.39	0.88
Date	2	0.000053	0.000027	0.87	0.42
Deformation Energy_BEFORE (inverse square root transformation)					
GP_type	2	0.0752	0.0376	34.25	<0.0001
Solvent(GP_type)	6	0.0081	0.0013	1.22	0.31
Date	2	0.0008	0.0004	0.37	0.69
Resilience_BEFORE					
GP_type	2	234.94	117.47	8.24	0.0006
Solvent(GP_type)	6	822.49	137.08	9.61	<0.0001
Date	2	19.06	9.53	0.67	0.52

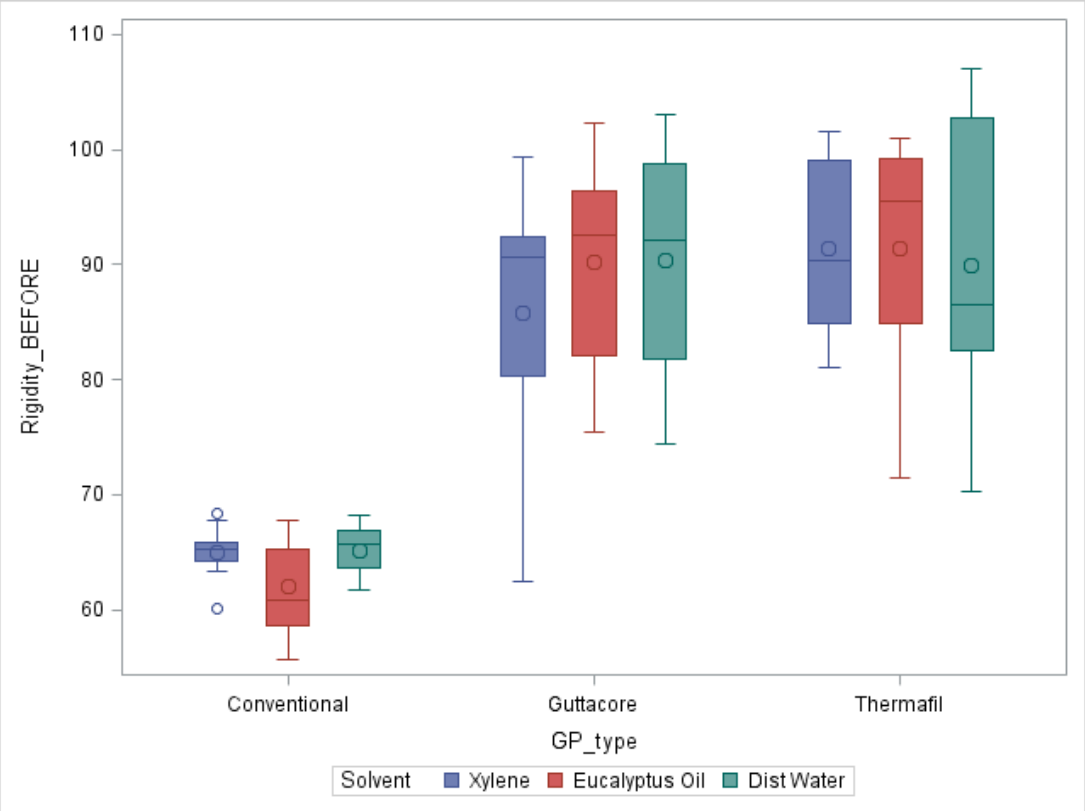


Fig. 4.3 Box-and-whiskers plot for rigidity (BEFORE).

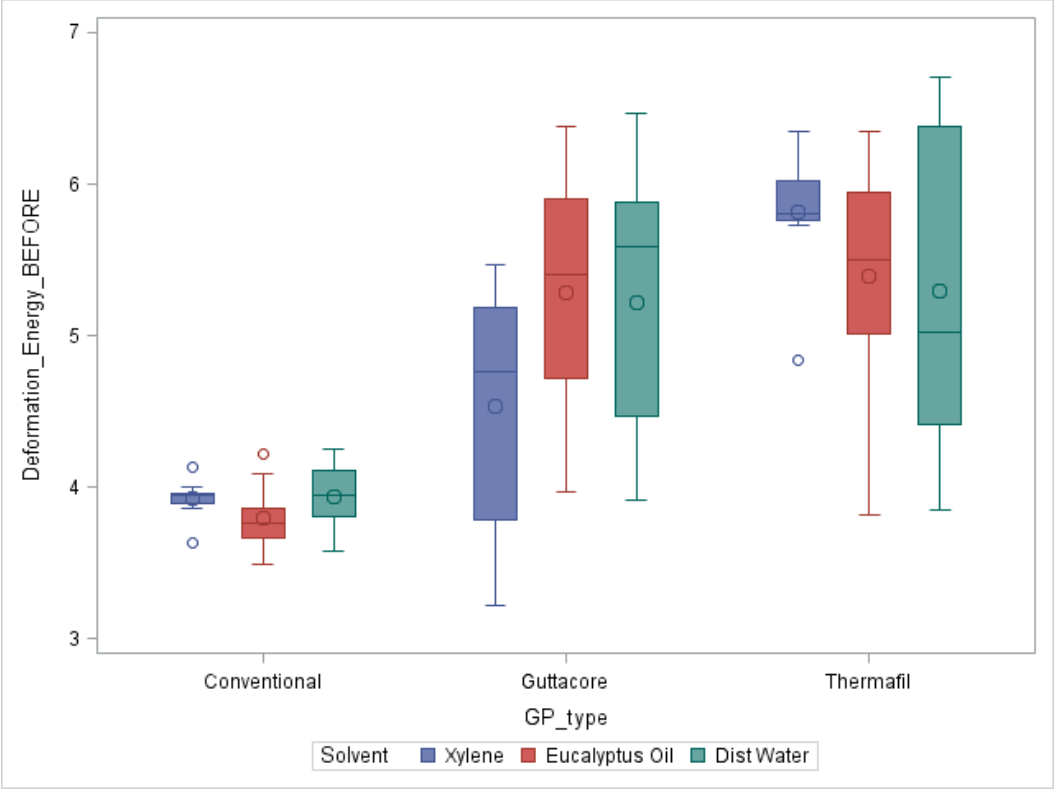


Fig. 4.4 Box-and-whiskers plot for deformation energy (BEFORE).

For rigidity and deformation energy, only the GP type main effect was significant ($p<0.0001$), and the solvent effect was confirmed as non-significant. For resilience however, the main effect of solvent ($p = 0.0006$) as well as the 'Solvent x GP type' interaction ($p<0.0001$), were highly significant. Post-hoc tests showed that the Conventional/Xylene (C/X) group had significantly lower resilience than the Conventional/Eucalyptus Oil (C/EO) and Conventional/Distilled water (C/DW) groups. This is illustrated in the box-and-whisker plot in Fig. 4.5.

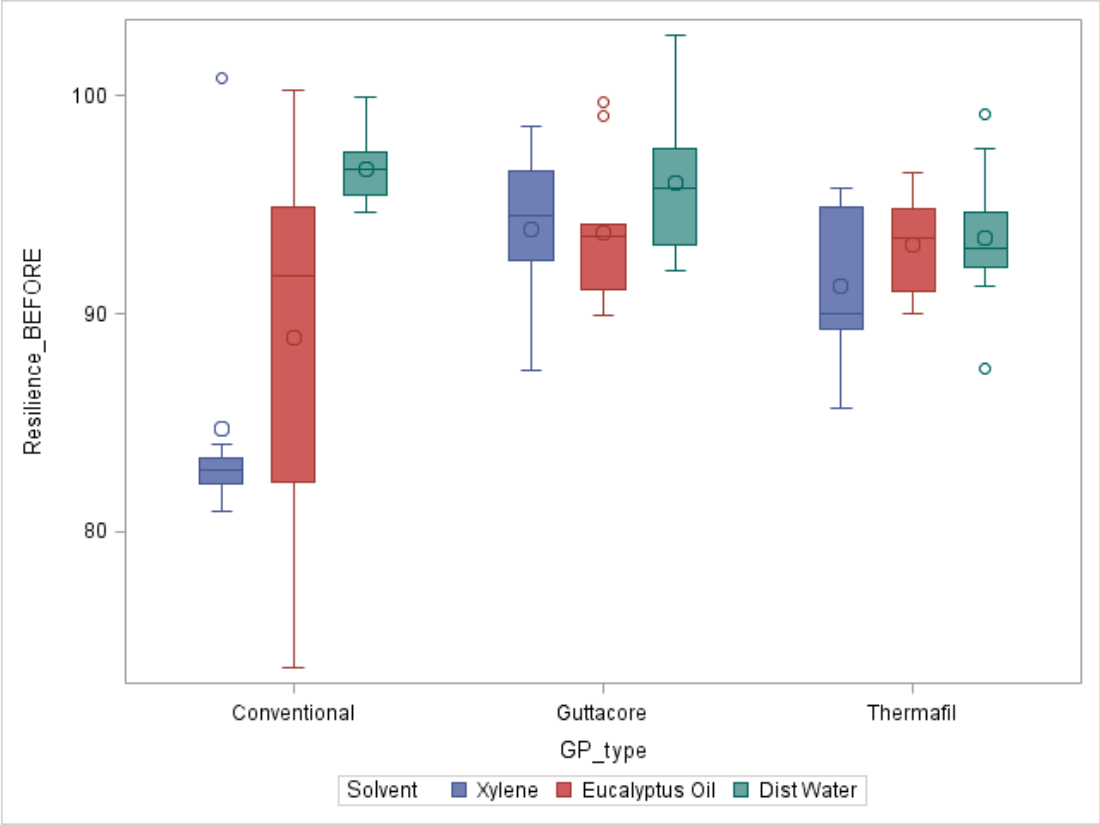
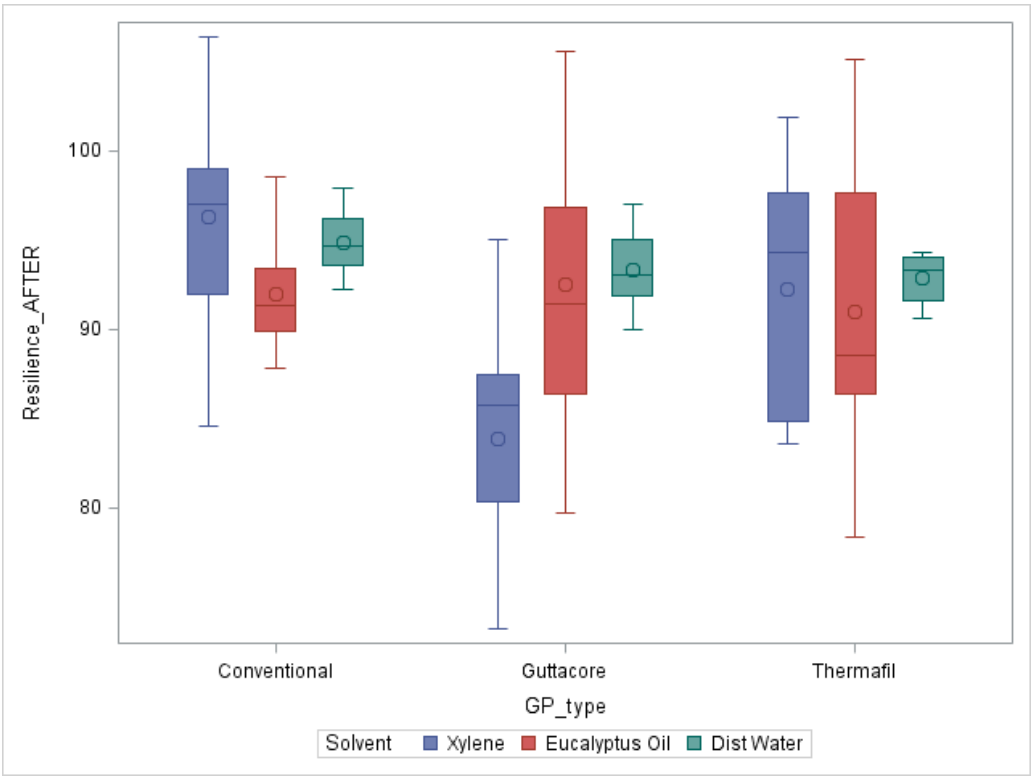


Fig. 4.5 Box-and-whiskers plot for resilience (BEFORE).

The AFTER results for C/X are roughly in line with those for the other solvents for the conventional groups. However when DIFF was calculated, C/X was the only one of the 9 treatment groups to display a significant increase in resilience after solvent treatment, against expectations (Fig. 4.6). The reason for the discrepancy remains unclear. Furthermore, with a lack of comparative data in the literature, it is difficult to establish whether this finding is related to experimental error, or is in fact valid. Hence, the resilience data for C/X were treated with caution.



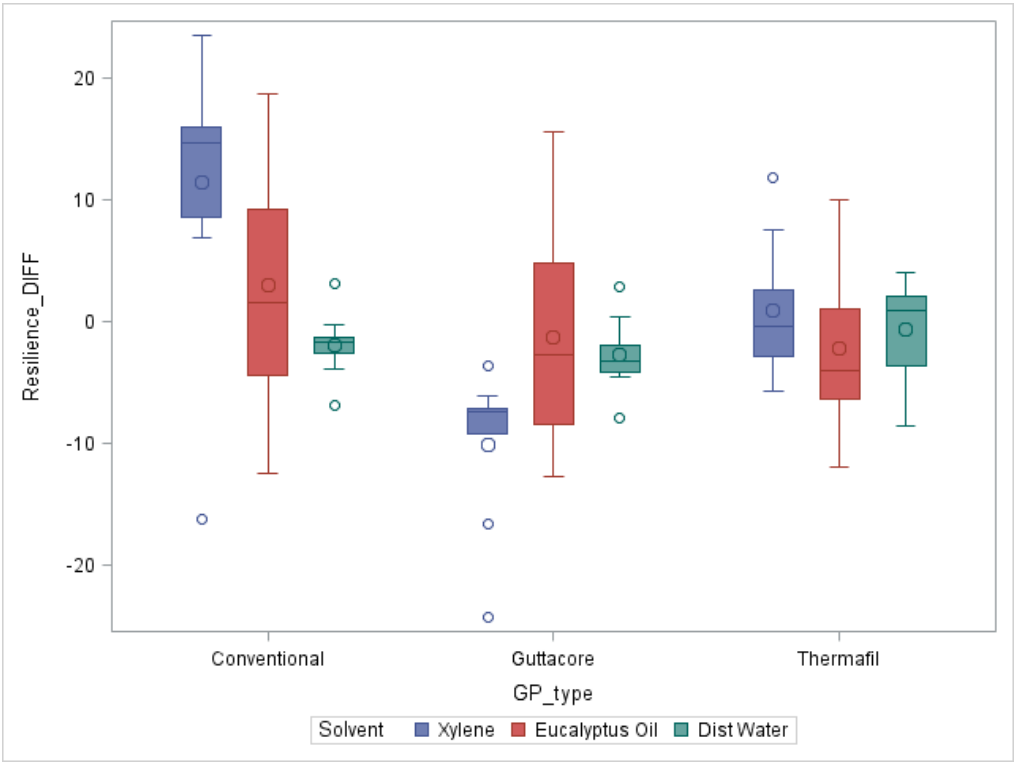


Fig. 4.6 Box-and-whisker plots for resilience (AFTER and DIFF).

4.5. Comparison of physical properties of GP types before solvent treatment

Differences in the three physical properties of the three GP types before solvent treatment were assessed by combining the BEFORE results for the 27 experiments conducted for each GP type. A general linear model with GP type as main effect and experiment day as blocking variable was used to model each of the BEFORE dependent variables in turn. (The use of room, solvent, and desiccator temperatures as covariates was also considered, however kept constant throughout the study.) Post-hoc tests were conducted using the Tukey-Kramer test. Effect sizes were calculated using Cohen's d, which were interpreted as follows:

0.80 and above	large effect
0.50 to 0.79	moderate effect
0.20 to 0.39	small effect
below 0.20	near zero effect

4.5.1. Rigidity

A box-and-whisker plot of the raw data, by GP type, is shown in Fig. 4.7. The variance of the Rigidity_BEFORE increased with its mean; a variance-stabilising transformation was required for the general linear model, although the conclusions from the raw and transformed data were the same. An inverse square root transformation of the Rigidity_BEFORE data was used.

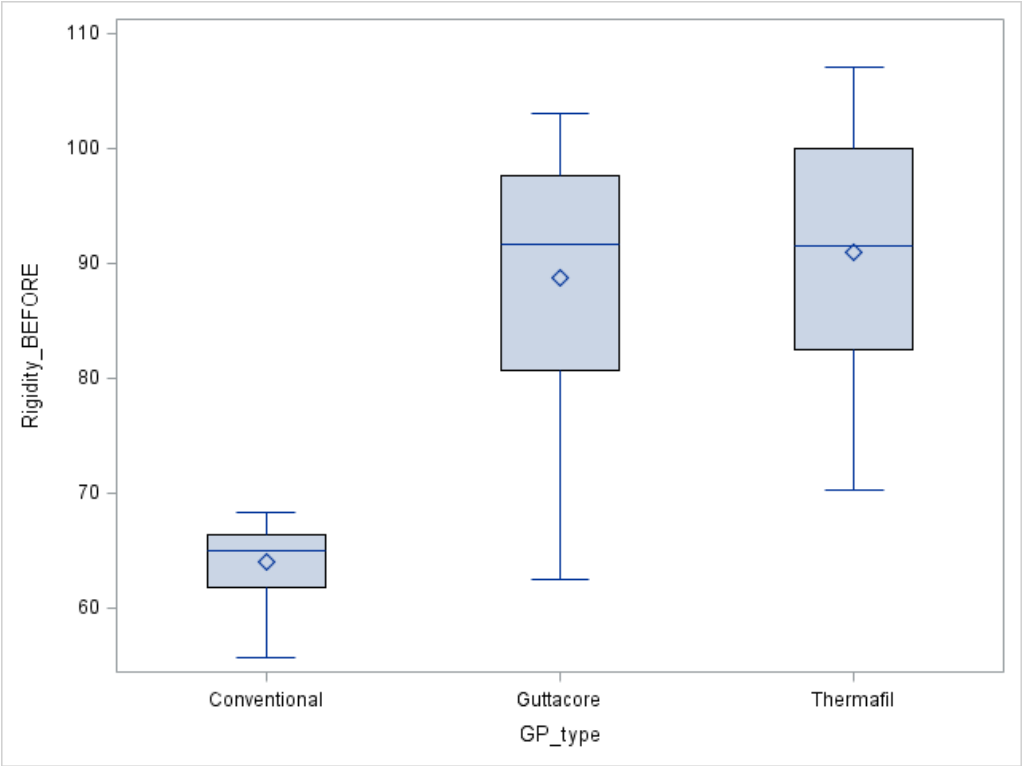


Fig. 4.7 Box-and-whisker plot of the raw data for rigidity prior to solvent exposure.

One of the tests (experiment 11) was excluded as an outlier, based on model diagnostics. The overall model was significant: $F(4,75)=57.9$; $p<0.0001$. The source table in Table 4.6 shows the main effect of GP type to be significant ($p<0.0001$) and the blocking effect of date not significant, as was expected.

Table 4.6 Source table for rigidity prior to solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	0.006069	0.003035	103.87	<.0001
Date	2	0.000059	0.000030	1.01	0.37

Post-hoc tests indicated that the mean rigidity (before solvent treatment) of the conventional GP was significantly lower than that of the other two materials ($p<0.001$). The Least-Squares (LS) means plot is shown in Fig. 4.8. The error bars denote the 95% confidence limits for the

mean. Least Squares means are the means estimated from the model, over balanced values of the other covariates in the model.

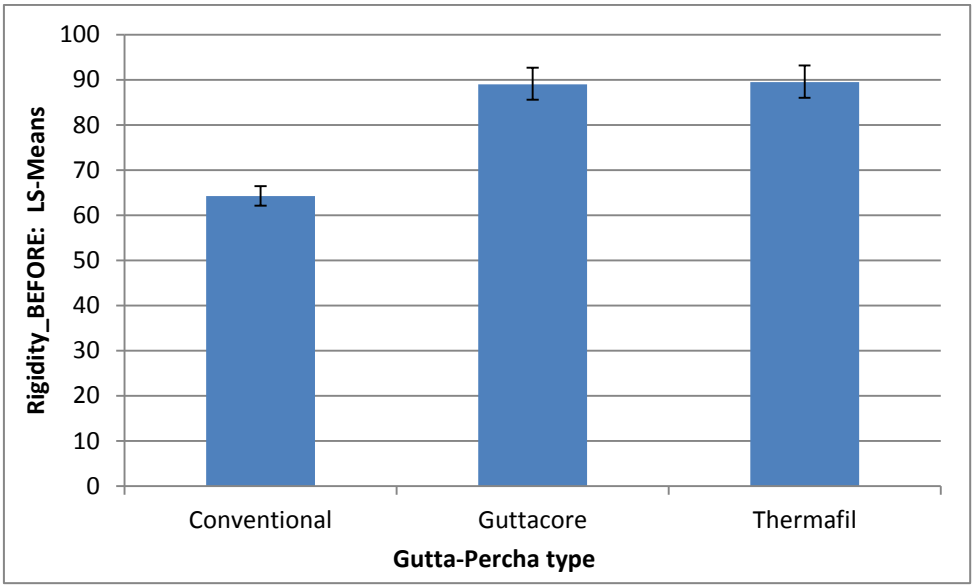


Fig. 4.8 Least-Squares means plot for rigidity prior to solvent exposure.

Conventional GP had a mean (standard deviation) rigidity of 64.1 (3.3), compared to the rigidity for Guttacore of 89.8 (9.5) and that for Thermafil of 90.9 (10.6). The effect sizes were both large (Cohen's d=3.7 and 3.6 for Conventional vs. Guttacore and Thermafil, respectively).

4.5.2. Deformation energy

A box-and-whisker plot of the raw data, by GP type, is shown in Fig. 4.9. As with Rigidity, the variance of the Deformation Energy_BEFORE increased with its mean, and once again a variance-stabilising inverse square root transformation was required.

The overall model was significant: $F(4,76)=20.1$; $p<0.0001$. The source table is shown in Table 4.7 below and, as with rigidity, the blocking effect of date was not significant.

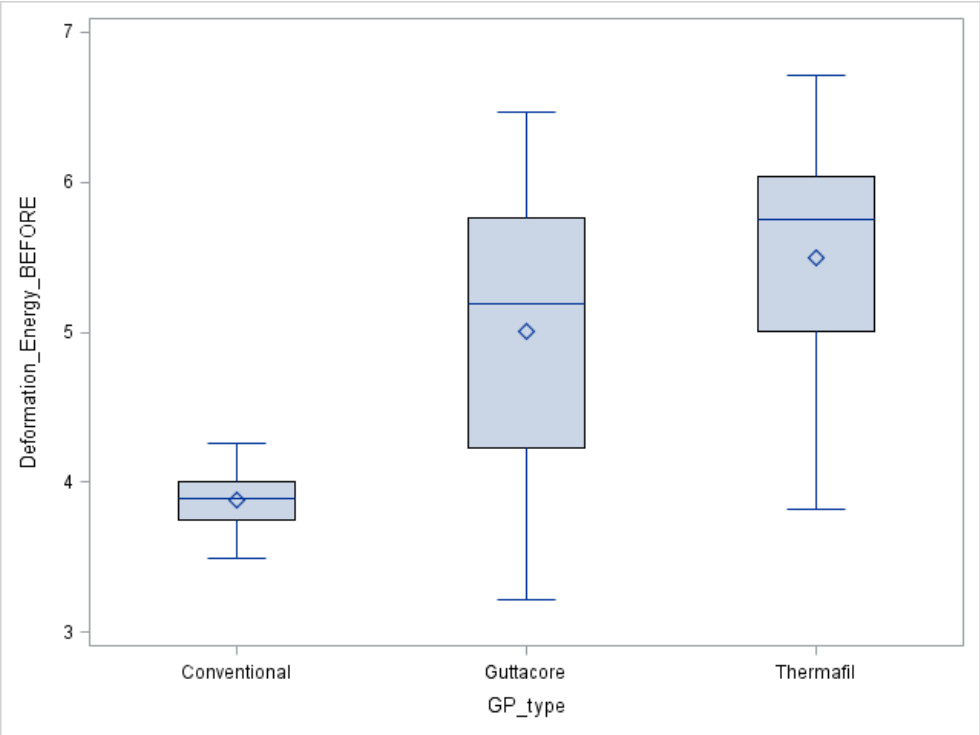


Fig. 4.9 Box-and-whisker plot of the raw data for deformation energy prior to solvent exposure.

Table 4.7 Source table for deformation energy prior to solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	0.072	0.036	32.20	<.0001
Date	2	0.0038	0.0019	1.68	0.19

Post-hoc tests indicated that the mean deformation energy (before solvent treatment) of the Conventional GP was significantly lower than that of the other two materials ($p<0.001$). The Least-Squares means plot is shown in Fig. 4.10.

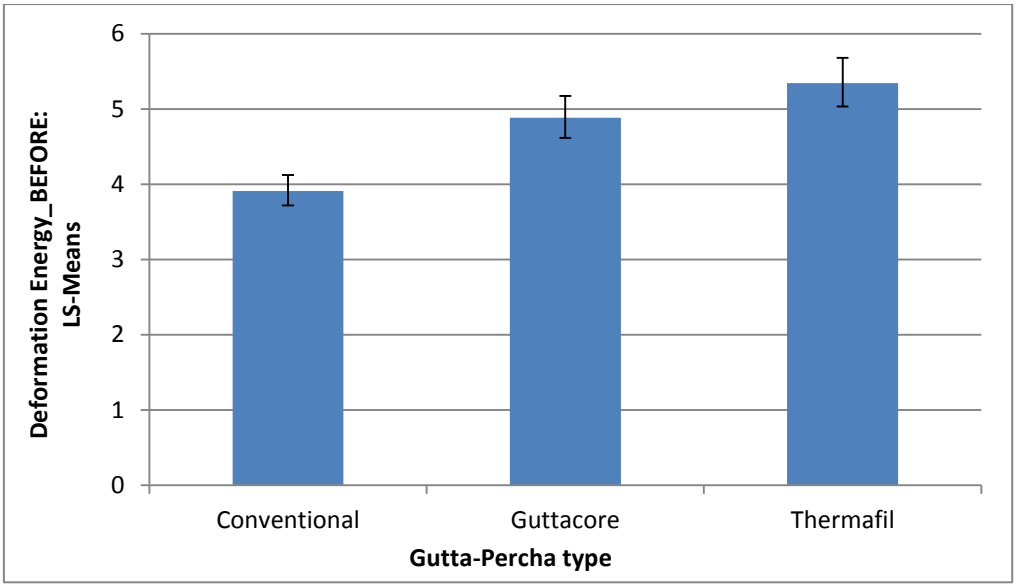


Fig. 4.10 Least-Squares means plot for deformation energy prior to solvent exposure.

Conventional GP had a mean (SD) deformation energy of 3.88 (0.20), compared to the deformation energy for Guttacore of 5.01 (0.90) and that for Thermafil of 5.50 (0.84). The effect sizes were both large (Cohen's d=1.8 and 2.7 for Conventional vs. Guttacore and Thermafil, respectively).

4.5.3. Resilience

A box-and-whisker plot of the raw data, by GP type, is shown in Fig. 4.11. In contrast to rigidity and deformation energy, the variance did not increase with its mean, and thus a variance-stabilising inverse square root transformation was not required.

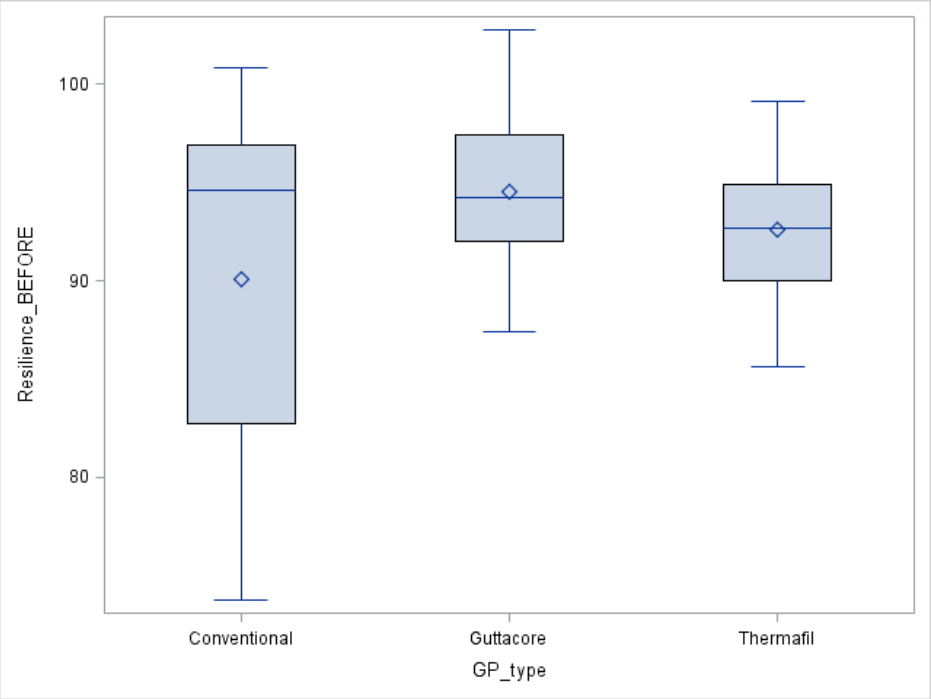


Fig. 4.11 Box-and-whisker plot of the raw data for resilience prior to solvent exposure.

Experiment 30 was excluded as an outlier, based on model diagnostics. The overall model was significant: $F(4,75) = 2.69$; $p = 0.038$. The source table is shown in Table 4.8 below:

Table 4.8 Source table for resilience prior to solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	190.5	95.2	3.77	0.028
Date	2	80.6	40.3	1.60	0.21

Post-hoc tests indicated that the mean resilience (before solvent treatment) of the Conventional GP was significantly lower than that of the Guttacore (only) ($p=0.021$). The Least-Squares means plot is shown in Fig. 4.12.

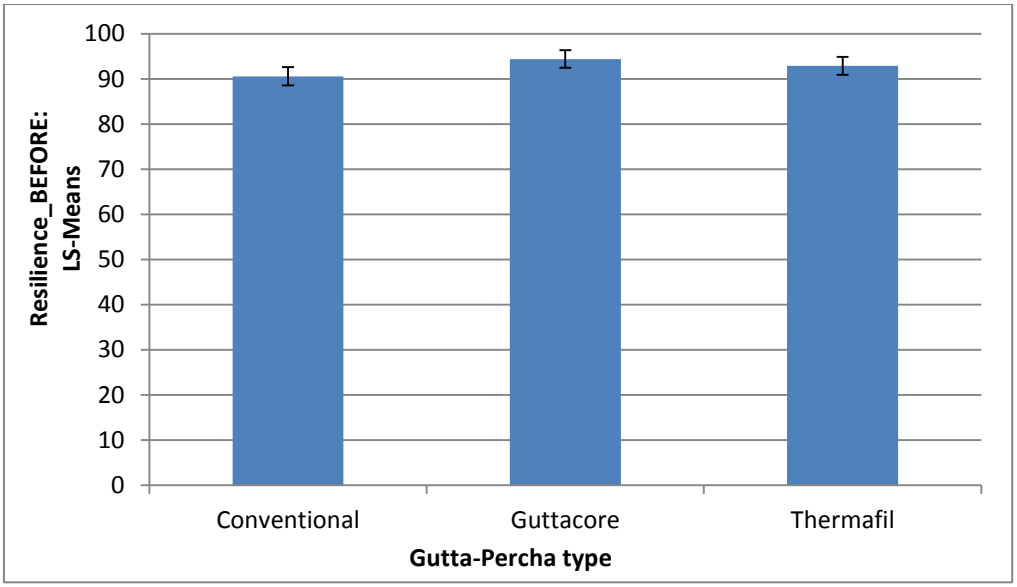


Fig. 4.12 Least-Squares means plot for resilience prior to solvent exposure.

Conventional GP had a mean (SD) resilience of 90.7 (7.4), compared to the resilience for Guttacore of 94.5 (3.7) and that for Thermafil of 92.6 (3.2). The effect size for the significant difference (Conventional vs. Guttacore) was moderate (Cohen's d=0.67).

Exclusion of C/X data :

Repeating the above analysis for resilience, but excluding the C/X data (n=9), and again excluding experiment 30 as an outlier, the overall model was not significant: $F(4,66)=0.92$; $p=0.46$. The source table is shown in Table 4.9.

Table 4.9 Source table for resilience prior to solvent exposure (excluding C/X data).

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	41.31	20.65	1.17	0.32
Date	2	14.67	7.33	0.41	0.66

Thus, there was no significant difference in the resilience of the difference GP types before solvent treatment. The Least-Squares means plot is shown in Fig. 4.13 below.

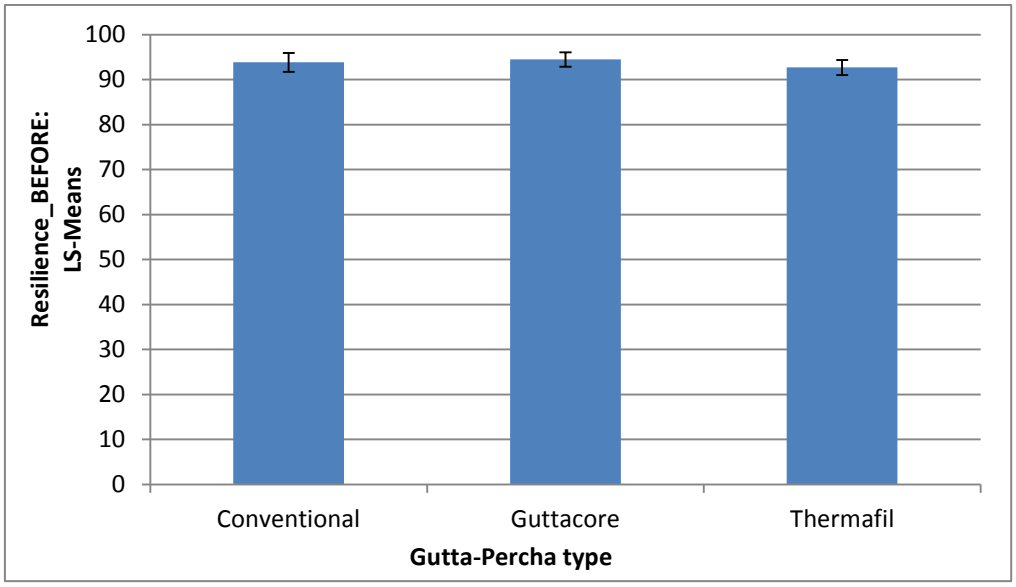


Fig. 4.13 Least-Squares means plot for resilience prior to solvent exposure (excluding C/X data).

Thus conclusions drawn across the three types of GP, prior to solvent exposure, were that:

- Conventional GP had significantly lower rigidity and deformation energy than both Guttacore and Thermafil.
- Conventional GP had significantly lower resilience than Guttacore when all the data were used. However, excluding the C/X group data there was no significant difference in the resilience of the different GP types before solvent treatment.

4.6. Comparison of physical properties of GP types after solvent exposure

The effects of the treatments on the DIFF variables for each of the three dependent variables was assessed using a general liner model with main effects for solvent and GP type, a two-factor interaction between solvent and GP type, and experiment day as the blocking variable.

As mentioned previously, the use of room and solvent temperatures as covariates was also considered, however these remained constant throughout the study. Post-hoc tests were conducted using the Tukey-Kramer test. Effect sizes were calculated using Cohen's d (interpreted as above).

4.6.1. Rigidity DIFF

A box-and-whisker plot of the raw data, by GP type and solvent, is shown in Fig. 4.14.

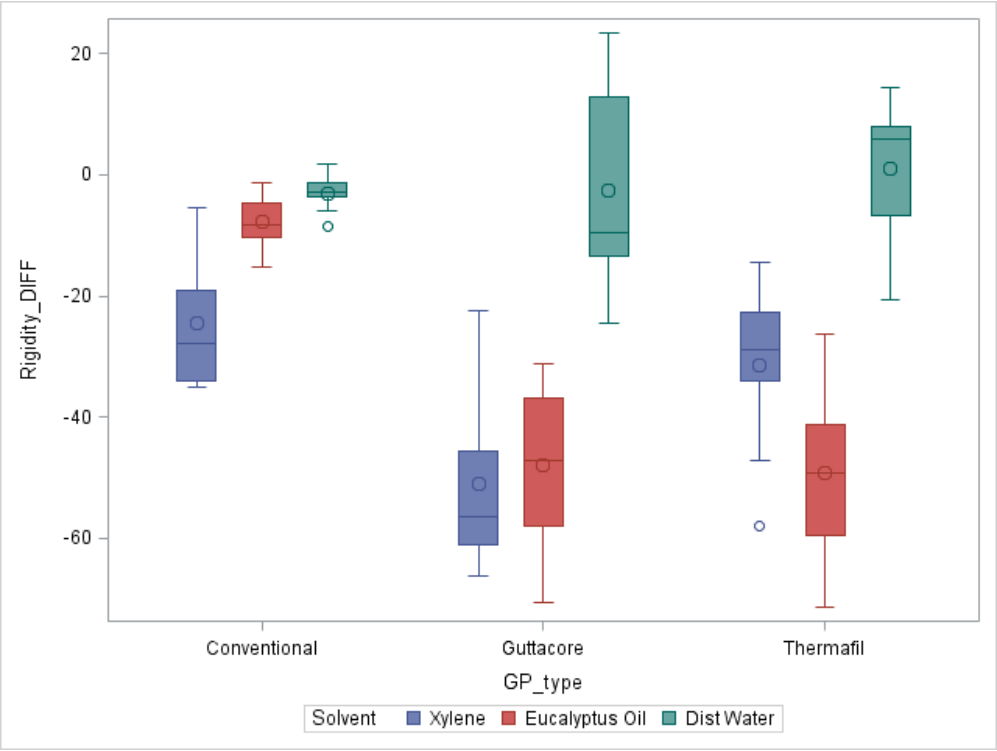


Fig. 4.14 Box-and-whisker plot of the raw data for rigidity DIFF.

The mean DIFFs were zero or negative indicating that the rigidity remained unchanged or decreased after solvent treatment. The overall model was significant: $F(10,70)=21.3$; $p<0.0001$, as were the main effects of GP type. The source table is shown in Table 4.10.

Table 4.10 Source table for rigidity DIFF following solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	6397.08	3198.54	20.03	<.0001
Solvent	2	20006.91	10003.46	62.65	<.0001
GP_type*Solvent	4	6413.38	1603.34	10.04	<.0001
Date	2	78.22	39.11	0.24	0.78

The two-factor interaction for Least-Squares means plot is shown in Fig. 4.15.

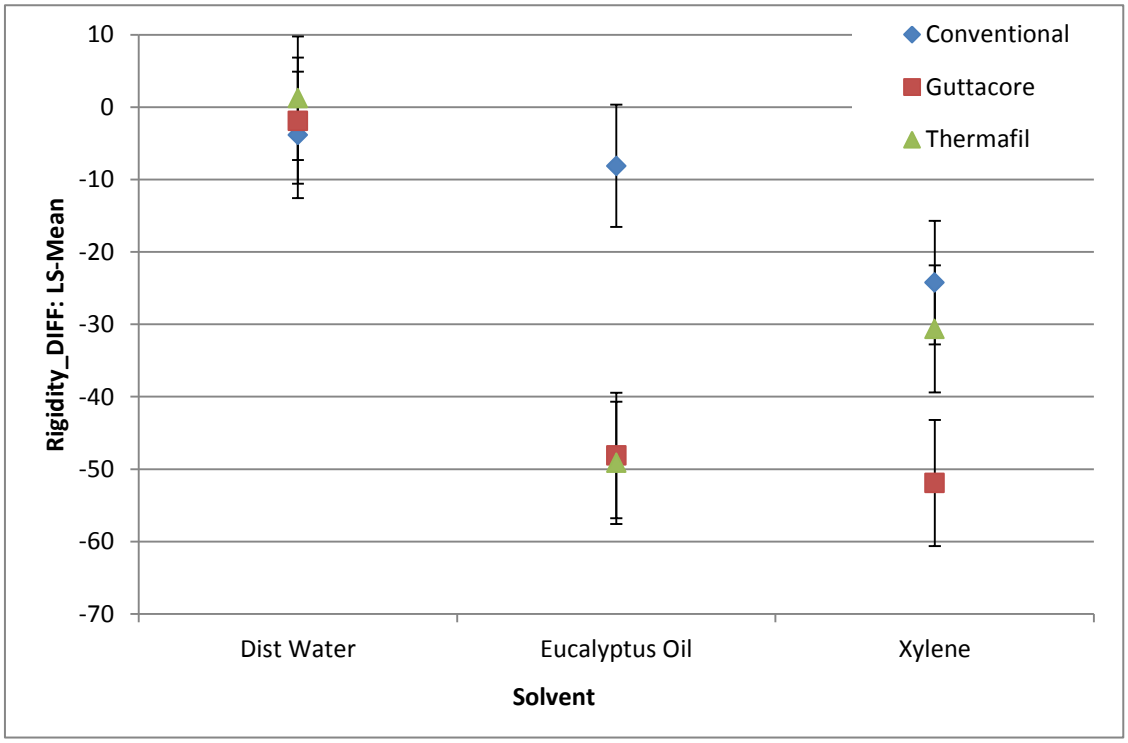


Fig. 4.15 Least-Squares means plot for rigidity DIFF following solvent exposure.

Post-hoc tests indicated significant differences between interactions as per Table 4.11.

Table 4.11 Post hoc tests for Rigidity_DIFF indicating the significant differences in red.

Least Squares Means for effect GP_type * Solvent									
Pr > |t| for H0: LSMean(i)=LSMean(j)									
Dependent Variable: Rigidity_DIFF									
i/j	**C / DW**	**C / EO**	**C / Xyl**	**G / DW**	**G / EO**	**G / Xyl**	**T / DW**	**T / EO**	**T / Xyl**
C / DW		0.998	0.037	1.000	<.0001	<.0001	0.996	<.0001	0.003
C / EO	0.998		0.175	0.983	<.0001	<.0001	0.826	<.0001	0.015
C / Xyl	0.037	0.175		0.012	0.008	0.001	0.002	0.003	0.980
G / DW	1.000	0.983	0.012		<.0001	<.0001	1.000	<.0001	0.000
G / EO	<.0001	<.0001	0.008	<.0001		0.999	<.0001	1.000	0.123
G / Xyl	<.0001	<.0001	0.001	<.0001	0.999		<.0001	1.000	0.038
T / DW	0.996	0.826	0.002	1.000	<.0001	<.0001		<.0001	<.0001
T / EO	<.0001	<.0001	0.003	<.0001	1.000	1.000	<.0001		0.075
T / Xyl	0.003	0.015	0.980	0.000	0.123	0.038	<.0001	0.075	

C:Conventional; G: Guttacore; T: Thermafil; Xyl: Xylene; EO: Eucalyptus Oil; DW: Distilled Water

4.6.2. Deformation Energy DIFF

A box-and-whisker plot of the raw data, by GP type and solvent, is shown in Fig. 4.16. As with Rigidity, the mean DIFFs were zero or negative, i.e. the deformation energy remained unchanged or decreased after solvent treatment.

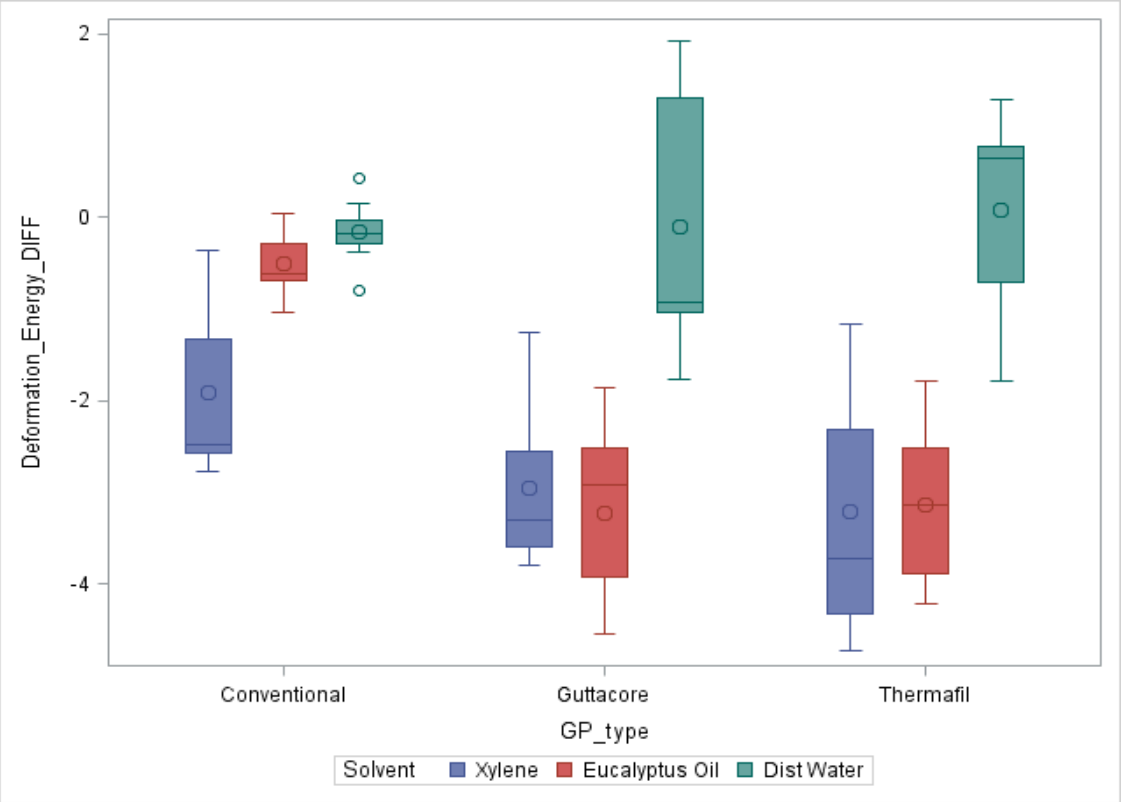


Fig. 4.16 Box-and-whisker plot of the raw data for deformation energy DIFF following solvent exposure.

The overall model was significant: $F(10,70)=16.4$; $p<0.0001$, as were the main effects of GP type and solvent, as well as their interaction. The source table is shown in Table 4.12.

Table 4.12 Source table for deformation energy DIFF following solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	25.20	12.60	12.83	<.0001
Solvent	2	104.22	52.11	53.05	<.0001
GP_type*Solvent	4	21.88	5.47	5.57	0.0006
Date	2	0.36	0.18	0.18	0.83

The two-factor interaction for the Least-Squares means plot is shown in Fig. 4.17.

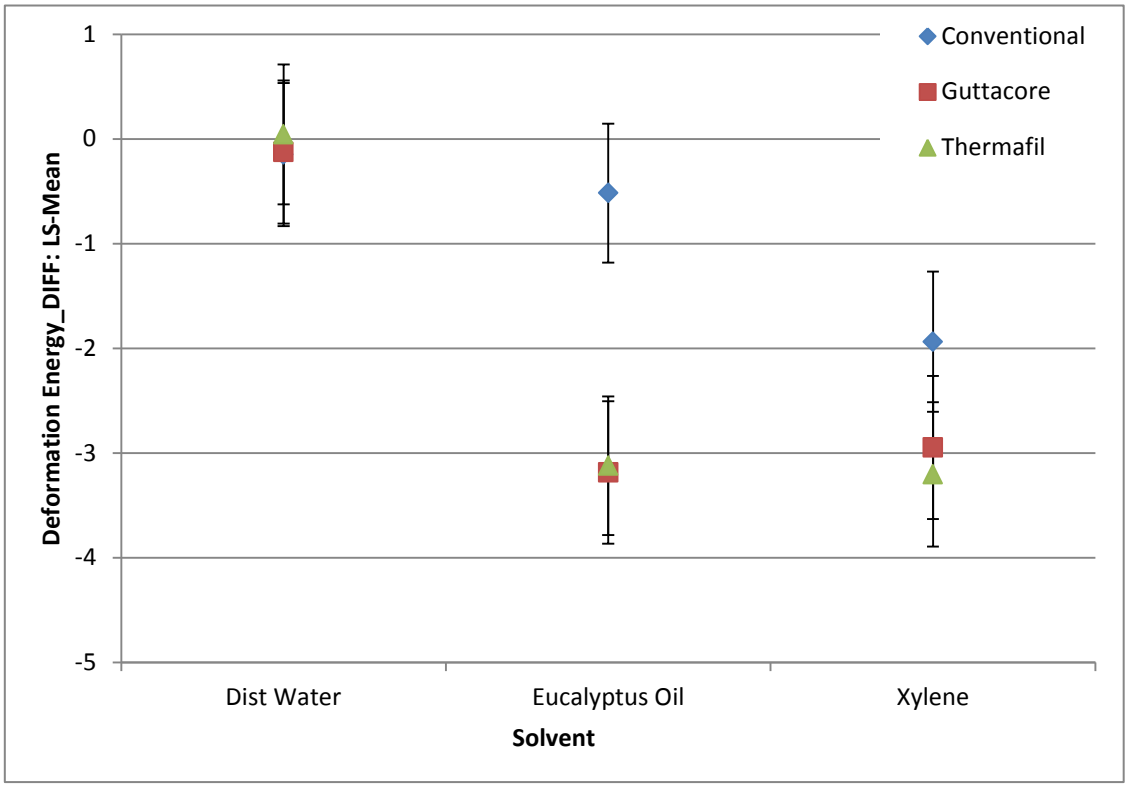


Fig. 4.17 Least-Squares means plot for deformation energy DIFF following solvent exposure.

Post-hoc tests indicated significant differences between interactions as per Table 4.13.

Table 4.13 Post hoc tests for Deformation Energy_DIFF indicating the significant differences in red.

Least Squares Means for effect GP_type*Solvent									
Pr > |t| for H0: LSMean(i)=LSMean(j)									
Dependent Variable: Deformation Energy_DIFF									
i/j	**C / DW**	**C / EO**	**C / Xyl**	**G / DW**	**G / EO**	**G / Xyl**	**T / DW**	**T / EO**	**T / Xyl**
C / DW		0.997	0.012	1.000	<.0001	<.0001	1.000	<.0001	<.0001
C / EO	0.997		0.082	0.996	<.0001	<.0001	0.957	<.0001	<.0001
C / Xyl	0.012	0.082		0.008	0.224	0.490	0.002	0.254	0.192
G / DW	1.000	0.996	0.008		<.0001	<.0001	1.000	<.0001	<.0001
G / EO	<.0001	<.0001	0.224	<.0001		1.000	<.0001	1.000	1.000
G / Xyl	<.0001	<.0001	0.490	<.0001	1.000		<.0001	1.000	1.000
T / DW	1.000	0.957	0.002	1.000	<.0001	<.0001		<.0001	<.0001
T / EO	<.0001	<.0001	0.254	<.0001	1.000	1.000	<.0001		1.000
T / Xyl	<.0001	<.0001	0.192	<.0001	1.000	1.000	<.0001	1.000	

C:Conventional; G: Guttacore; T: Thermafil; Xyl: Xylene; EO: Eucalyptus Oil; DW: Distilled Water

The following were observed focusing on the DIFF per material type:

C/DW > C/X (Cohen's d=2.7)

G/DW > G/X, G/EO (Cohen's d=2.5 and 2.6 respectively)

T/DW > T/X, T/EO (Cohen's d = 2.9 and 3.4 respectively).

All the effect sizes are classified as 'large'.

4.6.3. Resilience DIFF

The data obtained for resilience were interpreted with caution, given the unexpected results obtained for Conventional GP/Xylene (C/X) group BEFORE solvent treatment. A box-and-whisker plot of the raw data, by GP type and solvent, is shown in Fig. 4.18.

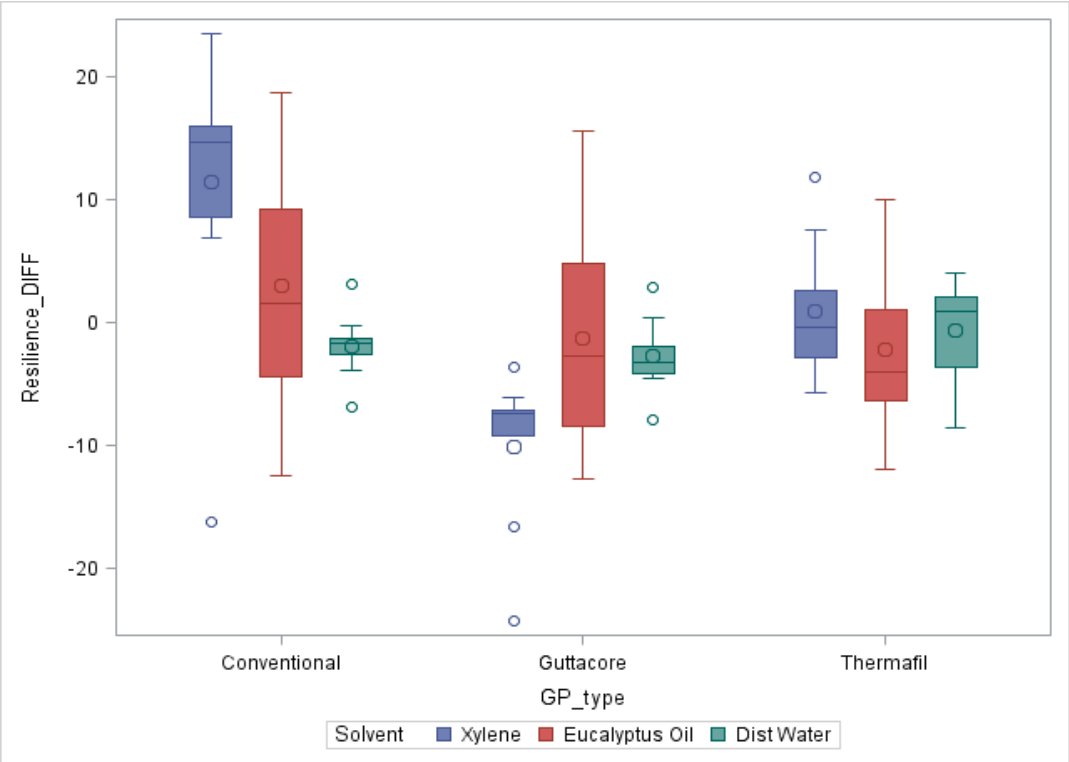


Fig. 4.18 Box-and-whisker plot of the raw data for resilience DIFF following solvent exposure.

The mean DIFFs were zero or negative, i.e. the resilience remained unchanged or decreased after solvent treatment except for C/X, where the resilience increased after solvent treatment due to the values for Resilience_BEFORE for this group. Experiment 2 was excluded as an outlier based on model diagnostics. The overall model was significant: $F(10,69)=7.1$; $p<0.0001$, as were the main effect of GP type, as well as the 'GP type x Solvent' interaction. The source table is shown in Table 4.14:

Table 4.14 Source table for resilience DIFF following solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	1320.92	660.46	15.76	<.0001
Solvent	2	181.59	90.80	2.17	0.12
GP_type*Solvent	4	1475.92	368.98	8.80	<.0001
Date	2	37.96	18.98	0.45	0.64

The two-factor interaction for the Least-Squares means plot is shown in Fig 4.19.

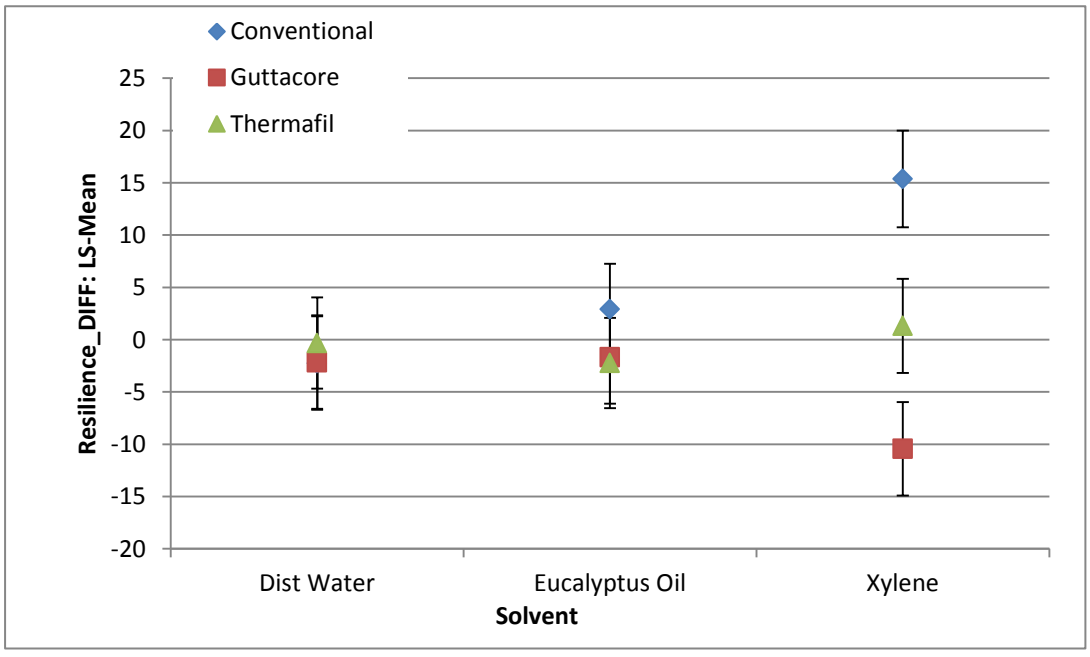


Fig. 4.19 Least-Squares means plot for resilience DIFF following solvent exposure.

Post-hoc tests indicated significant differences between interactions as per Table 4.15.

Table 4.15 Post hoc tests for Resilience_DIFF indicating the significant differences in red.

Least Squares Means for effect GP_type*Solvent									
Pr > |t| for H0: LSMean(i)=LSMean(j)									
Dependent Variable: Resilience_DIFF									
i/j	**C / DW**	**C / EO**	**C / Xyl**	**G / DW**	**G / EO**	**G / Xyl**	**T / DW**	**T / EO**	**T / Xyl**
C / DW		0.757	<.0001	1.000	1.000	0.174	1.000	1.000	0.975
C / EO	0.757		0.006	0.797	0.864	0.002	0.979	0.759	1.000
C / Xyl	<.0001	0.006		<.0001	<.0001	<.0001	0.000	<.0001	0.001
G / DW	1.000	0.797	<.0001		1.000	0.236	1.000	1.000	0.968
G / EO	1.000	0.864	<.0001	1.000		0.138	1.000	1.000	0.989
G / Xyl	0.174	0.002	<.0001	0.236	0.138		0.050	0.196	0.018
T / DW	1.000	0.979	0.000	1.000	1.000	0.0498		1.000	1.000
T / EO	1.000	0.759	<.0001	1.000	1.000	0.196	1.000		0.965
T / Xyl	0.975	1.000	0.001	0.968	0.989	0.018	1.000	0.965	

C:Conventional; G: Guttacore; T: Thermafil; Xyl: Xylene; EO: Eucalyptus Oil; DW: Distilled Water

4.7. Comparison of the DIFF results for Xylene and Eucalyptus oil

A one-sample t-test of the DIFF value with respect to 0 was performed to establish whether a significant reduction for a specified parameter was achieved following exposure to Xylene and to Eucalyptus oil. A two-sample t-test was performed to determine if there was a significant difference between the DIFFs of Xylene and Eucalyptus oil. Where the assumptions of these tests were not met, non-parametric alternatives were used, namely the Wilcoxon Signed Rank test and the Wilcoxon Rank sum test, respectively. The results of these tests are depicted in Table 4.16.

Table 4.16 Comparison of the DIFF results for Xylene and Eucalyptus oil, indicating the significant differences in red.

GP type	Parameter	Xylene						Eucalyptus Oil						p-value for H_0*	Direction of difference	Effect size (Cohen's d)**
		Mean	95% Confidence Limits for Mean		Interquartile Range		p-value for H_0: mean DIFF = 0	Mean	95% Confidence Limits for Mean		Interquartile Range		p-value for H_0: mean DIFF = 0			
Conventional	Rigidity_DIFF	-24.45	-32.81	-16.09	-33.96	-19.09	0.0001	-7.84	-11.60	-4.08	-10.32	-4.66	0.0013	0.0007	X < EO	2.09 (large)
	Def Energy_DIFF	-1.90	-2.61	-1.20	-2.57	-1.33	0.0002	-0.51	-0.78	-0.24	-0.69	-0.28	0.0023	0.0006	X < EO	2.13 (large)
	Resilience_DIFF	11.55	2.62	20.47	8.60	16.03	0.0175	3.02	-4.36	10.40	-4.33	9.27	0.37	0.041	X > EO	0.43 (small)
Guttacore	Rigidity_DIFF	-51.10	-62.11	-40.09	-61.13	-45.55	<0.0001	-47.92	-58.31	-37.53	-57.91	-36.92	<0.0001	0.63	-	-
	Def Energy_DIFF	-2.95	-3.66	-2.24	-3.59	-2.55	<0.0001	-3.24	-3.96	-2.51	-3.92	-2.52	<0.0001	0.53	-	-
	Resilience_DIFF	-10.02	-14.94	-5.10	-9.15	-7.07	0.0015	-1.25	-8.50	6.01	-8.34	4.91	0.70	0.11	-	-
Thermafil	Rigidity_DIFF	-31.48	-42.09	-20.86	-34.02	-22.82	0.0001	-49.15	-60.63	-37.68	-59.54	-41.29	<0.0001	0.019	X > EO	1.30 (large)
	Def Energy_DIFF	-3.22	-4.25	-2.18	-4.32	-2.31	<0.0001	-3.14	-3.83	-2.44	-3.89	-2.51	<0.0001	0.89	-	-
	Resilience_DIFF	1.02	-3.36	5.39	-2.79	2.69	0.61	-2.12	-7.68	3.43	-6.26	1.08	0.40	0.32	-	-

* p-value for H_0: no significant difference between mean DIFF for X and EO.

** Effect size (Cohen's d) if difference is significant.

CHAPTER 5
DISCUSSION

Endodontic therapy is completed when the root canal system is obturated to provide a liquid tight seal that will facilitate periapical repair. However, at times, even meticulously performed endodontic therapies fail which then necessitate endodontic retreatment (Whitworth and Boursin, 2000; Martos et al, 2006). The primary objective of retreatment is the removal of the filling material from the canal/s and to regain access to the apical foramen (Tasdemir et al, 2008). Whilst mechanical instrumentation serves as the primary method for removing the GP, chemical solvents assist by softening and partially dissolving the GP in the canal

Several authors have investigated the dissolving ability of solvents by measuring the weight of GP before and after exposure to them. However, none of these studies tested the effects of the solvents on the physical properties of the GP (Tanomaru-Filho et al, 2010; Faria-Júnior et al, 2011; Mushtaq et al, 2012). A change in the physical properties following solvent exposure can render the material more easily removable by mechanical instrumentation or conversely make retreatment more difficult. Properties such as hardness and penetration of the filling material are particularly significant. The hardness of a material refers to its ability to resist indentation, which affects the mechanical file's ability to engage the GP in the root canal. Penetration is difficult if not impossible to measure directly, but deformation energy and resilience can serve as its proxies. Deformation energy is the energy required to deform a material during penetration, whilst resilience refers to the ability of a material to deform

while absorbing the energy of the applied load, with subsequent recovery. Thus, a decrease in deformation energy and resilience would ease file penetration into the GP.

This study employed textural analysis to assess the changes in the physical properties of three types of GP following exposure to two endodontic solvents. Whilst textural analysis features very scarcely, if at all, in the dental literature, it is frequently employed in pharmaceutical research laboratories and the food industry. It plays an invaluable role in determining properties of materials including rigidity, resilience, cohesiveness and adhesiveness amongst others. Rigidity, deformation energy and resilience were the parameters applicable to this study, representing hardness and penetration.

5.1. Hardness

The property of hardness (rigidity) was calculated as the gradient on a Force-Distance graph obtained from textural analysis (see Fig. 3.5). The results obtained prior to solvent exposure displayed that Thermafil and Guttacore had comparably higher rigidities than Conventional GP which had a lower rigidity overall. The increased rigidity of the former types of GP can probably be attributed to their strengthened central cores which provide additional support to the different type of gutta-percha wrapped around them. The increased rigidity may be indicative of the differing hardness found between the different phases of gutta-percha. Distilled water, employed as a control, was incapable of causing any significant reduction in rigidity across all groups.

The rigidities of Conventional GP, Thermafil and Guttacore were significantly reduced following exposure to Eucalyptus oil ($p<0.05$) and Xylene ($p<0.05$). The extent to which the rigidity decreased varied between the type of GP and solvent. There was no significant

difference in the extent of rigidity reduction for Guttacore between the two solvents. However, the decrease in rigidity for Conventional GP was significantly greater following exposure to Xylene than to Eucalyptus Oil ($p=0.0007$; Cohen's $d=2.09$). In contrast, Eucalyptus oil elicited a significantly greater reduction in rigidity with Thermafil as opposed to Xylene ($p=0.019$; Cohen's $d=1.30$).

Conventional GP was less susceptible to a reduction in rigidity across all solvents. This resistance could be attributed to the quantity of β-phase gutta-percha possessed by Conventional GP, as opposed to the thermoplastic α-phase gutta-percha found in the circumferential layer of Guttacore and Thermafil. The comparably thicker gutta-percha in Conventional GP may hinder complete penetration of the solvent, thus leaving the properties of the deeper gutta-percha intact. Xylene has been shown to weaken the polysulfone core of Thermafil, contributing to a reduction in its rigidity (Ibarrola et al, 1993). The internal cross-linked gutta-percha core of Guttacore has been shown to resist softening when exposed to solvents and is less amenable to a change in its physical properties (Beasley et al, 2013). These results further support the findings of Mushtaq et al (2012) and Rubino et al (2012) who reported Xylene as a solvent with "major capacity of dissolution of gutta-percha". Magalhães et al (2007) also verified Eucalyptus oil as an acceptable solvent, dissimilar to previous studies that observed significantly less dissolution efficiency with Eucalyptus oil (Görduysus et al, 1997).

5.2. Penetration

As discussed previously, deformation energy and resilience serve as representative parameters for penetration. Deformation energy is calculated as a measure of area in a Force-

Distance graph whereas resilience is calculated as a measure of area in a Force-Time graph (see Fig. 3.6).

Any increase in *deformation energy* following solvent exposure infers that a greater force is required by the retreatment file when penetrating the GP. The deformation energies of Conventional GP, Thermafil and Guttacore were significantly reduced with Eucalyptus oil ($p<0.05$) and Xylene ($p<0.05$). The decrease in deformation energy for Conventional GP was significantly greater for Xylene than for Eucalyptus Oil ($p=0.0006$; Cohen's $d=2.13$). There was no significant difference between the two solvents for Thermafil and Guttacore. Upon comparison of the changes in deformation energies, a significantly greater reduction was observed with Thermafil and Guttacore than with Conventional GP ($p<0.05$). This is in accordance with Tanomaru-Filho et al (2010) who demonstrated that Xylene and Eucalyptus oil presented a more favoured solvent effect on Thermoplastic GP than on Conventional GP.

An increase in *resilience* denotes that the material will absorb a greater energy of the applied force before yielding to penetration or fracture. A decrease in file penetration reduces the surface area of the file that engages the GP. This reduction in contact leads to a decline in the amount of GP being removed with each successive file withdrawal This increases clinical procedure time and possibly operator frustration as the retreatment procedure becomes more arduous to perform.

Eucalyptus oil showed no significant reduction in the resilience of Thermafil and Guttacore, with an insignificant increase observed with Conventional GP ($p=0.37$). Following exposure to Xylene, there was a significant increase in resilience with Conventional GP ($p=0.0175$), whilst there was a significant decrease with Guttacore ($p=0.0015$), and an insignificant

reduction with Thermafil (p=0.61). Hence, while the solvents may aid the penetration of retreatment files into Thermafil and Guttacore, they may actually confound the retreatment procedure when Conventional GP is present in the canal Nonetheless, with resilience being closely related to the ability of the polymer chain to rotate freely, additional factors such as the rate and extent of deformation, the applied force, as well as temperature will also affect the resilience value of the material (Huson and Maxwell, 2006). However, increasing the force applied to the retreatment file will amplify the resultant mechanical stresses, which in turn can lead to instrument separation (Bahcall, 2013).

Conventional GP consists of mainly β-phase gutta-percha and zinc-oxide, whereas Thermafil and Guttacore have α-phase gutta-percha circumferentially around their respective cores. The two crystalline phases (α-phase and β-phase) are molecularly both *trans* isomers, differing only in single bond configuration and molecular repeat distance. However, the molecular repeat distance for β-phase gutta-percha is shorter than that of α-phase gutta-percha. This results in the α-phase being more flexible than the β-phase and contributes to the vulnerability of α-phase gutta-percha to solvents, as illustrated by Tanomaru-Filho et al in 2010. Both, Thermafil and Guttacore rely on heating to make their circumferential GP flowable during canal insertion. Heating the material to a temperature range of 46-48^{o}C, causes the α-phase gutta-percha to transform into β-phase and lose flexibility. Should the heating temperature exceed 58^{o}C, the gutta-percha then transforms into an irreversible amorphous phase which then exhibits entirely different mechanical properties (Goodman et al, 1974; Schilder et al, 1985; Maniglia-Ferreira et al, 2007). Thermal treatment was not used in the present study and since thermal exposure causes molecular phase transformations, and changes the bond structure and orientation of the GP, these data may not necessarily reflect

the properties of the material at chairside. However, they provide a base reference for physical changes that occur in GP following solvent exposure.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

The endodontic solvents, Xylene and Eucalyptus oil, have both proven effective in their ability to significantly change the physical properties of all three types of GP.

Conventional GP is more resistant to changes in its physical properties when compared with thermoplastic GP forms. Despite the fact that the hardness and deformation energy of conventional GP does significantly decrease, changes in its resilience may not always prove beneficial Whilst a decrease in GP hardness may aid its removal from the root canal system, an increased resilience may in fact hinder penetration and subsequent engagement of the retreatment file to the GP.

The thermoplastic forms of GP, Thermafil and Guttacore, are vulnerable to endodontic solvents which alter their physical properties more readily. However, whilst the circumferential gutta-percha may be amenable to removal, the central core may portray entirely different properties which will either assist or hinder its removal It is therefore recommended that this study be repeated using only the core GP of these thermoplastic materials.

Thermal exposure causes molecular phase transformations, and changes the bond structure and orientation of the GP. Thus further study is required to assess the changes in the physical properties that occur following thermal exposure.

A greater reduction in the hardness of thermafil GP was observed with Eucalyptus oil, while no significant difference was found in the interactions between thermafil GP and either solvent. Conventional GP was susceptible to a significant reduction in hardness with both solvents, however its penetrability may be compromised by the use of Xylene. No significant differences were found in the interactions between Guttacore and either solvent. Thus, considering the toxicity profile of Xylene, and the biocompatibility and antimicrobial effects of Eucalyptol, Eucalyptus oil is recommended for use during endodontic retreatment.

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APPENDIX A

COMPARISON OF THE PHYSICAL PROPERTIES OF THE THREE GP TYPES FOLLOWING SOLVENT EXPOSURE

The effects of the treatments on the AFTER variables for each of the three dependent variables was assessed using a general liner model with main effects for solvent and GP type, a two-factor interaction between solvent and GP type, and experiment day as the blocking variable. As mentioned previously, the use of room and solvent temperatures as covariates was also considered, however these remained constant throughout the study. Post-hoc tests were conducted using the Tukey-Kramer test.

A.1 Rigidity AFTER:

A box-and-whisker plot of the raw data, by GP type and solvent, is shown in Fig A.1.

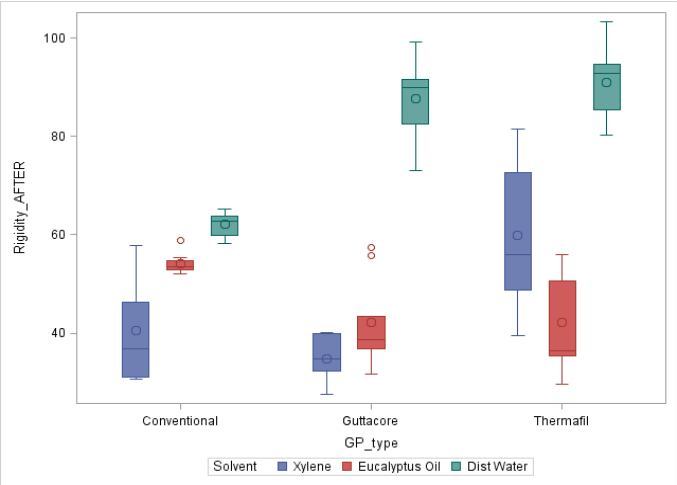

Fig. A.1 Box-and-whisker plot of the raw data for rigidity following solvent exposure.

The overall model was significant: $F(10,70)=38.2$; $p<0.0001$. The source table is shown in Table A.1.

Table A.1: Source table for rigidity following solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	2047.30	1023.65	12.99	<.0001
Solvent	2	21069.02	10534.51	133.63	<.0001
GP_type*Solvent	4	5687.94	1421.98	18.04	<.0001
Date	2	7.03	3.51	0.04	0.96

Since the two-factor interaction is significant, we interpret this (and not the main effects). The Least-Squares means plot is shown in Fig. A.2.

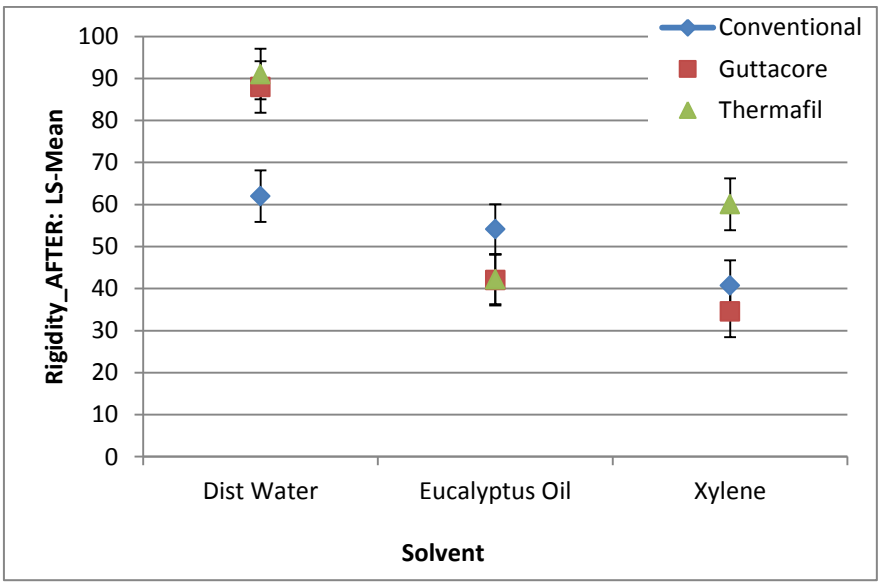

Fig. A.2 Least-Squares means plot for rigidity following solvent exposure.

Table A.2 Post hoc tests for Rigidity_AFTER indicating the significant differences in red.

Least Squares Means for effect GP_type*Solvent									
Pr > |t| for H0: LSMean(i)=LSMean(j)									
Dependent Variable: Rigidity_AFTER									
i/j	**C / DW**	**C / EO**	**C / Xyl**	**G / DW**	**G / EO**	**G / Xyl**	**T / DW**	**T / EO**	**T / Xyl**
C / DW		0.640	0.000	<.0001	0.001	<.0001	<.0001	0.001	1.000
C / EO	0.640		0.053	<.0001	0.127	0.001	<.0001	0.124	0.909
C / Xyl	0.0002	0.053		<.0001	1.000	0.887	<.0001	1.000	0.001
G / DW	<.0001	<.0001	<.0001		<.0001	<.0001	0.998	<.0001	<.0001
G / EO	0.001	0.127	1.000	<.0001		0.719	<.0001	1.000	0.003
G / Xyl	<.0001	0.001	0.887	<.0001	0.719		<.0001	0.697	<.0001
T / DW	<.0001	<.0001	<.0001	0.998	<.0001	<.0001		<.0001	<.0001
T / EO	0.001	0.124	1.000	<.0001	1.000	0.697	<.0001		0.002
T / Xyl	1.000	0.909	0.001	<.0001	0.003	<.0001	<.0001	0.002	

C:Conventional; G: Guttacore; T: Thermafil; Xyl: Xylene; EO: Eucalyptus Oil; DW: Distilled Water

The lowest rigidity after solvent treatment was obtained with G/X, whose mean Rigidity_AFTER was not significantly different from C/X, G/EO, or T/EO.

Focusing on the DIFF per material type, we can conclude the following:

C/DW > C/X (Cohen's d=2.8)

G/DW > G/X, G/EO (Cohen's d=3.2 and 3.1 respectively)

T/DW > T/X, T/EO (Cohen's d = 2.7 and 3.9 respectively).

All the effect sizes are classified as 'large'.

Thus the best combinations of low Rigidity_AFTER solvent treatment, combined with high change in rigidity as a result of solvent treatment, were G/X, G/EO, and T/EO.

A.2 Deformation Energy AFTER

A box-and-whisker plot of the raw data, by GP type and solvent, is shown in Fig. A.3.

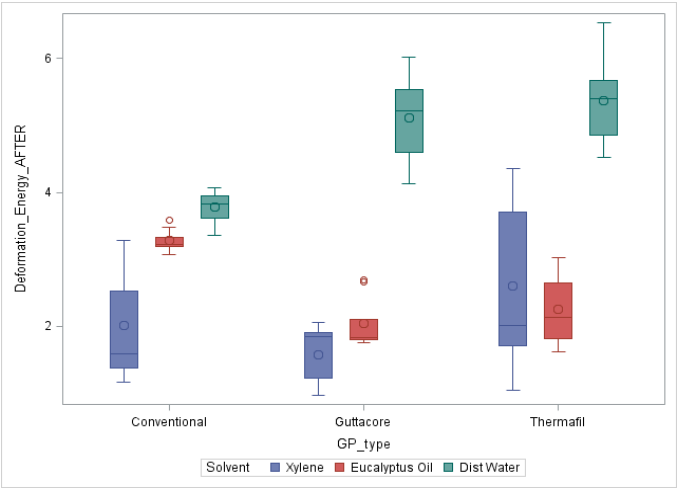

Fig. A.3 Box-and-whisker plot of the raw data for deformation energy following solvent exposure.

The overall model was significant: $F(10,70)=31.2$; $p<0.0001$. The source table is shown in Table A.3 below:

Table A.3 Source table for deformation energy following solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	3.01	1.50	3.41	0.039
Solvent	2	108.01	54.01	122.44	<.0001
GP_type*Solvent	4	18.60	4.65	10.54	<.0001
Date	2	0.32	0.16	0.37	0.69

The main effects of GP type and Solvent, as well as their interaction, were significant. We note that the blocking effect of date was not significant.

Since the two-factor interaction is significant, we interpret this (and not the main effects). The Least-Squares means plot is shown in Fig. A.4.

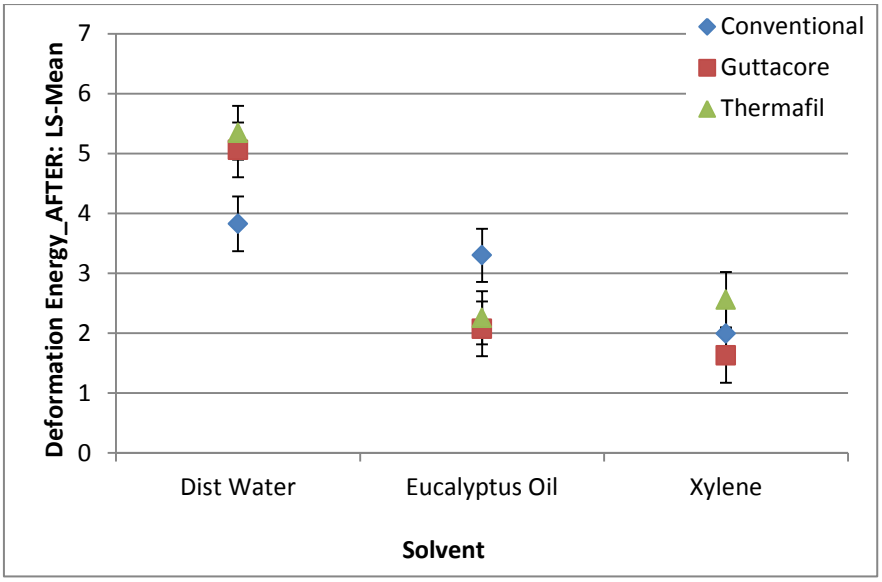

Fig. A.4 Least-Squares means plot for deformation energy following solvent exposure.

Table A.4 Post hoc tests for Deformation Energy_AFTER indicating the significant differences in red.

Least Squares Means for effect GP_type*Solvent									
Pr > |t| for H0: LSMean(i)=LSMean(j)									
Dependent Variable: Deformation Energy_AFTER									
i/j	**C / DW**	**C / EO**	**C / Xyl**	**G / DW**	**G / EO**	**G / Xyl**	**T / DW**	**T / EO**	**T / Xyl**
C / DW		0.766	<.0001	0.013	<.0001	<.0001	0.000	0.000	0.011
C / EO	0.766		0.003	<.0001	0.008	<.0001	<.0001	0.038	0.375
C / Xyl	<.0001	0.003		<.0001	1.000	0.968	<.0001	0.996	0.710
G / DW	0.013	<.0001	<.0001		<.0001	<.0001	0.993	<.0001	<.0001
G / EO	<.0001	0.008	1.000	<.0001		0.900	<.0001	1.000	0.852
G / Xyl	<.0001	<.0001	0.968	<.0001	0.900		<.0001	0.576	0.149
T / DW	0.0004	<.0001	<.0001	0.993	<.0001	<.0001		<.0001	<.0001
T / EO	0.0002	0.038	0.996	<.0001	1.000	0.576	<.0001		0.989
T / Xyl	0.011	0.375	0.710	<.0001	0.852	0.149	<.0001	0.989	

C:Conventional; G: Guttacore; T: Thermafil; Xyl: Xylene; EO: Eucalyptus Oil; DW: Distilled Water

Possible interpretations:

The lowest deformation energy after solvent treatment was obtained with G/X, whose mean Deformation Energy _AFTER was not significantly different from C/X, G/EO, T/X or T/EO.

Thus the best combinations of low Deformation Energy_AFTER solvent treatment, combined with high change in deformation energy as a result of solvent treatment, were G/X, C/X, G/EO, T/X and T/EO.

A.3 Resilience AFTER

A box-and-whisker plot of the raw data, by GP type and solvent, is shown in Fig. A.5.

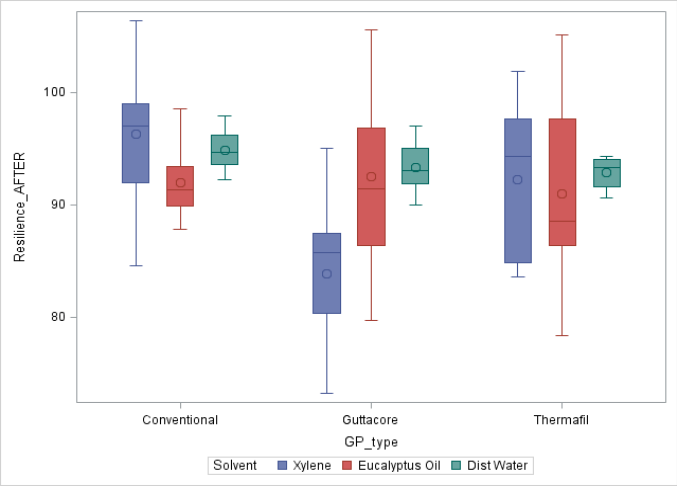

Fig. A.5 Box-and-whisker plot of the raw data for resilience following solvent exposure.

The overall model was significant: $F(10,70)=2.91$; $p=0.0042$. The source table is shown in Table A.5.

Table A.5 Source table for resilience DIFF following solvent exposure.

Source	Degrees of freedom	Type 3 Sum of Squares	Mean Square	F	p
GP_type	2	279.75	139.88	4.42	0.016
Solvent	2	129.63	64.81	2.05	0.14
GP_type*Solvent	4	469.43	117.36	3.71	0.009
Date	2	53.81	26.90	0.85	0.43

The main effect of GP type, as well as the 'GP type x Solvent' interaction, were significant. We note that the blocking effect of date was not significant.

Since the two-factor interaction is significant, we interpret this (and not the main effects). The Least-Squares means plot is shown in Fig. A.6.

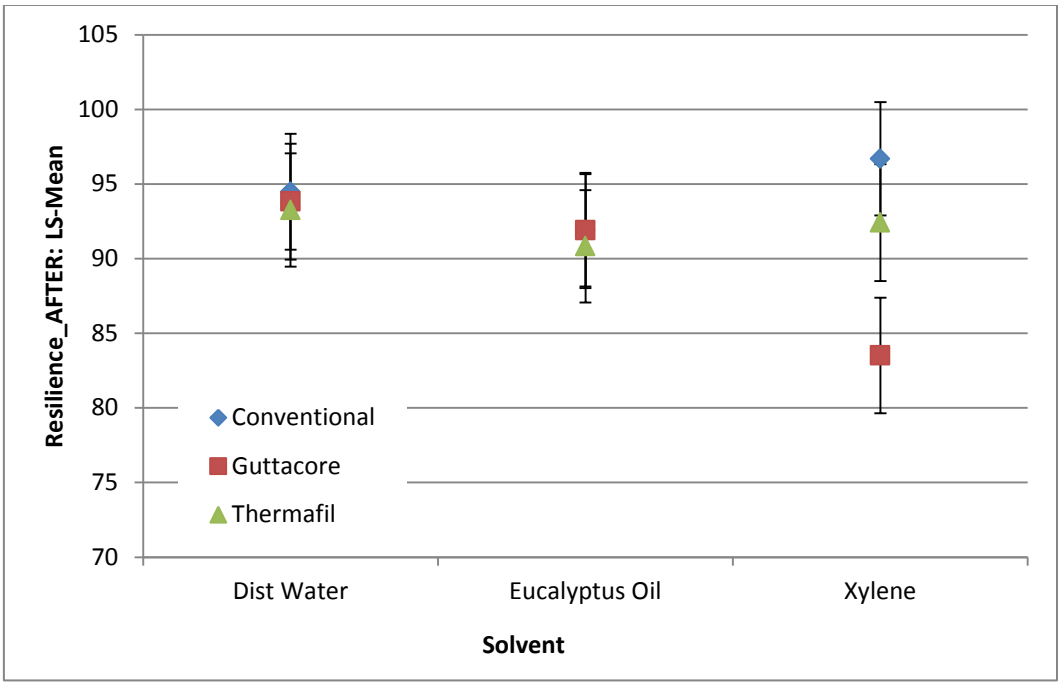

Fig. A.6 Least-Squares means plot for resilience following solvent exposure.

Table A.6 Post hoc tests for Resilience_AFTER indicating the significant differences in red.

Least Squares Means for effect GP_type*Solvent									
Pr > |t| for H0: LSMean(i)=LSMean(j)									
Dependent Variable: Resilience_AFTER									
i/j	**C / DW**	**C / EO**	**C / Xyl**	**G / DW**	**G / EO**	**G / Xyl**	**T / DW**	**T / EO**	**T / Xyl**
C / DW		0.988	0.996	1.000	0.989	0.003	1.000	0.913	0.998
C / EO	0.988		0.688	0.999	1.000	0.059	1.000	1.000	1.000
C / Xyl	0.996	0.688		0.977	0.724	0.000	0.929	0.432	0.819
G / DW	1.000	0.999	0.977		0.999	0.015	1.000	0.972	1.000
G / EO	0.989	1.000	0.724	0.999		0.070	1.000	1.000	1.000
G / Xyl	0.003	0.059	0.0003	0.015	0.070		0.019	0.169	0.064
T / DW	1.000	1.000	0.929	1.000	1.000	0.019		0.992	1.000
T / EO	0.913	1.000	0.432	0.972	1.000	0.169	0.992		1.000
T / Xyl	0.998	1.000	0.819	1.000	1.000	0.064	1.000	1.000	

C:Conventional; G: Guttacore; T: Thermafil; Xyl: Xylene; EO: Eucalyptus Oil; DW: Distilled Water

Possible interpretations:

The lowest resilience after solvent treatment was obtained with G/X, whose mean Resilience_AFTER was not significantly different from C/EO, G/EO, T/X or T/EO.

Thus the best combinations of low Resilience_AFTER solvent treatment, combined with high change in resilience as a result of solvent treatment, were G/X, G/EO, and T/EO.

Finally, combining the conclusions from the three physical properties, the best combinations of low properties AFTER solvent treatment, combined with high change in properties as a result of solvent treatment, were G/X, G/EO, and T/EO.

APPENDIX B

ETHICS WAIVER CERTIFICATE

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

Ref: W-CJ-130904-2

04/09/2013

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: **Dr E Patel.**

Project title: **Hardness, penetration and surface changes of gutta-percha when exposed two endodontic solvents.**

Reason: This is a laboratory study of dental materials. There are no human participants.

UNIVERSITY OF THE WITWATERSRAND
JOHANNESBURG

Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)

copy: Anisa Keshav, Zanele Ndlovu, Wits Research Office

APPENDIX C

FACULTY PROTOCOL APPROVAL

Faculty of Health Sciences
Medical School, 7 York Road, Parktown, 2193
Fax: (011) 717-2119
Tel: (011)717-2075/6

Reference: Ms Mpumi Mngapu
E-mail: Mpumi.Mngapu@wits.ac.za
05 December 2013
Person No: 0401350G
PAG

Dr E Patel
P O Box 1409
Lenasia
1820
Gauteng South Africa

Dear Dr Patel

Master of Science in Dentistry: Approval of Title

We have pleasure in advising that your proposal entitled "*Hardness and penetration of gutta-percha when exposed to two endodontic solvents"* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences