REPORT FOR THE COMPARATIVE *IN VITRO* ADHESIVES STUDY: MICROINFILTRATION

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INTRODUCTION:

Bacterial microinfiltration into the tooth cavity is one of the leading causes of pulp inflammation and necrosis and secondary caries in restored teeth. The symptoms of this process include post-operative sensitivity, enamel fractures and marginal staining.

The main factors that influence microinfiltration include cavity design and contraction of the restorative material upon polymerisation, which can lead to maladaptation at the bonding interface between the composite and the tooth, and therefore loss of retention (2,5). Dental adhesives, which are solutions of resin monomers that bind the restorative material to the dental substrate (dentine or enamel) after polymerisation, were therefore developed to minimise these problems.

As such, current adhesive agents are used to obtain restorations that provide an appropriate seal at the interface between the tooth structure and the restorative materials. However, it is very difficult to achieve this objective as adhesion to enamel and dentine differs, mainly due to the organic composition of the latter and its higher water and fluid content with respect to enamel.

Thus, to obtain appropriate adhesion to dentine it is necessary to take into account the smear layer formed when creating the cavity, as these tissue remnants adhere to the surface and the two adhesion strategies currently available, namely etch and wash adhesives, which eliminate the smear layer and can be either two- or three-step, and self-etching adhesives, which preserve and modify this layer and can be either one- or two-step, depend on how the adhesives interact with it. The current literature still considers the etch and wash technique to be more reliable as self-etching systems do not provide an optimal adhesion surface.

According to Van Meerbeek et al., the main problem of adhesives is that no system, not even the most recent ones, is yet able to guarantee a hermetic seal of the restorative material. Furthermore, due to the difficulty in objectively evaluating microinfiltration in clinical studies, laboratory studies are considered to be an acceptable alternative for evaluating adhesive restorative materials.

AIM:
To evaluate the degree of microinfiltration *in vitro* in class V restorations performed using one three-step and three two-step etch and wash adhesives and to determine the effect of thermal cycling on microinfiltration.

MATERIAL AND METHODS:
MATERIAL:
Adhesives:

Adhesive	**Manufacturer**
Optibond FL	Kerr, Orange, CA, USA
Ventura Unibond2	Madespa, S.A, Toledo, Spain
One Coat Bond	Coltène/Whaledent, Altstätten, Switzerland
Single Bonding	DMP Dental LDA, Greece

METHODS
Microinfiltration into class V cavities in permanent molars was studied *in vitro* at the Universitat Internacional de Catalunya. A non-probabilistic sampling procedure, which involved selecting 48 caries-free molars with no prior restorative treatment, was employed to construct the sample. These teeth were stored in water until treatment. The molar collection period was completed in 30 days.
Any molar with caries, fillings, root canal treatment or drilling was excluded. Teeth with remnants of tartar were cleaned in a universal point 1 ultrasound descaling apparatus with the help of a Gracey number 9/10 periodontal curette for molar scaling.
The apices of all samples were then sealed with wax and embedded in acrylic resin to facilitate handling and to be able to make the appropriate sections.
A class V cavity was then created in the cement/enamel boundary of each molar using a flame-shaped diamond bur with a large-grained elliptical point and the perimeter bevelled using a fine-grained diamond finishing bur. A high-speed turbine with abundant irrigation was used for this purpose. The cavities were measured prior to treatment using a periodontal probe and calliper to ensure they were as homogeneous as possible (Figure 1).

Figure 1. Measurement with a calliper

These dimensions are equivalent to (Figure 2):

- Mesiodistal length: 3-4 mm.
- Apico-coronal length: 2.5-3 mm.
- Depth: 1.5-2 mm.

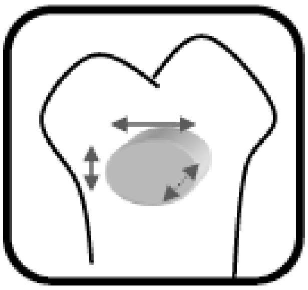


Figura 2. Esquema de la cavidad

Once prepared, the samples were divided at random into four groups (A-D) containing 12 molars each. A different type of adhesive was applied to each group according to the manufacturer's instructions. All were filled using the Ventura Nanolux composite (MADESPA, S.A. – Toledo, Spain).
The filling procedure was performed by a single operator and comprised the following steps:

1. Drying of the cavity to be filled with air.

2. Preparation of the cavity using Ventura Gel Conditioner containing 37% phosphoric acid according to the manufacturer's instructions.

3. The sample was then divided into the four groups according to the adhesive to be applied:

- Group A (n=12): Optibond FL (Kerr, Orange, CA, USA). Optibond FL Primer was applied by passing the tip of a disposable applicator brush over the entire surface of the cavity and bevelled perimeter for 15 seconds. It was then gently dried for 5 seconds and Optibond FL Adhesive applied to the entire prepared surface using the tip of a new disposable applicator brush for 15 seconds. Air was applied gently to distribute the adhesive, and it was then photo-polymerised for 20 seconds.
- Group B (n=12): Ventura Unibond2 (MADESPA, S.A, Toledo, Spain). A thick layer of adhesive was applied to the previously prepared surface. This was impregnated for 20 seconds using a disposable applicator brush, then photo-polymerised for 30 seconds.
- Group C (n=12): One Coat Bond (Coltène/Whaledent, Alstätten, Switzerland). The adhesive was applied directly using a syringe and rubbed on with a disposable application brush for 20 seconds. It was then photo-polymerised for 30 seconds.
- Group D (n=12): Single Bonding (DMP Dental LDA, Greece). The adhesive was rubbed on with a disposable application brush for 15 seconds. It was then air-dried for 5 seconds and photo-polymerised for 20 seconds.

4. The Ventura Nanolux composite (colour A2) was inserted.

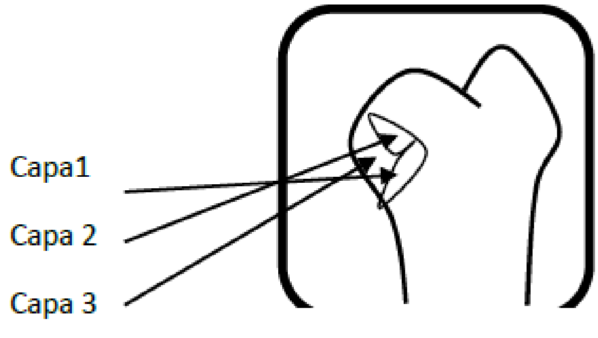


Figure 3. Incremental reconstruction

As can be seen in Figure 6, this was performed by applying three different layers and polymerising each layer for 20 seconds.

5. Once filled, all samples were stored in water at 37ºC ± 1ºC for 24 hours. After this period half the samples form each group (n=6) were thermally cycled for 1200 cycles in the temperature range 5ºC to 55ºC, spending 20 seconds at each temperature. The remaining samples (n=6) were prepared and stained immediately after the period at 37ºC.

The thermal cycling process took 6 days, after which time the samples were prepared and stained.

Preparation of samples for staining:

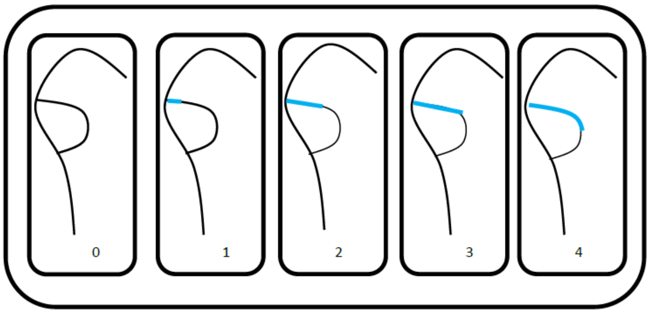
The perimeter of the filled cavity was marked with a permanent marker at 1 mm form its edge. The rest of the molar was painted with two layers of nail varnish (a clear layer first and then a darker layer), as shown in Figure 9. Once the varnish had dried, the samples were immersed in a 1% methylene blue solution for 24 hours.

Figure 4. Samples prepared for staining.

6. They were then removed from the staining solution, cleaned with water and dried before being sectioned in a vestibuloingual direction, along the longitudinal axis of the tooth, passing through the midpoint of the filling, using an ISOMET 1000 instrument.

The samples obtained were observed through a stereomicroscope, at a magnification of 15x, and analysed using a software program.

7. The scale described by Perdigão et al (12) was used to determine the degree of microinfiltration (Figure 5):



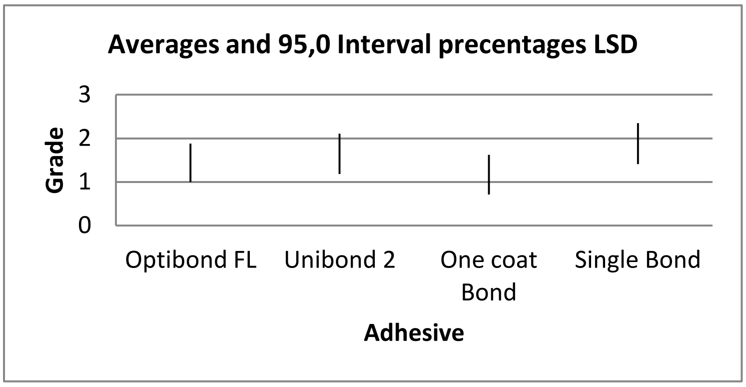
0: Absence of microinfiltration
1: Filtration to the mid-point of the apical or coronal wall.
2: Filtration throughout the apical or coronal wall.
3: Filtration to the cavity angle.
4: Filtration surpasses the cavity angle and reaches the axial wall.

Figure 5. Microinfiltration scale.

9. The degree of microinfiltration was assessed by a single trained operator in order to eliminate observer variability.

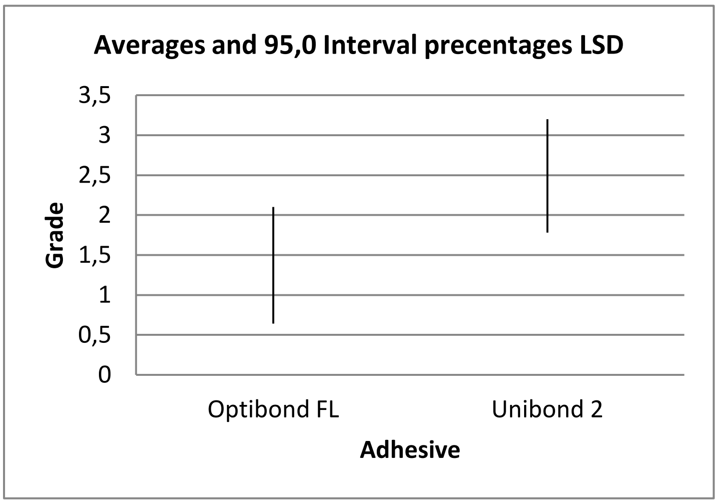
RESULTS:

Evaluation of the microinfiltration (Scheme 1) and degree of microinfiltration (scheme 2) for the adhesives studied gave very similar values, thus meaning that there were no significant differences between them.

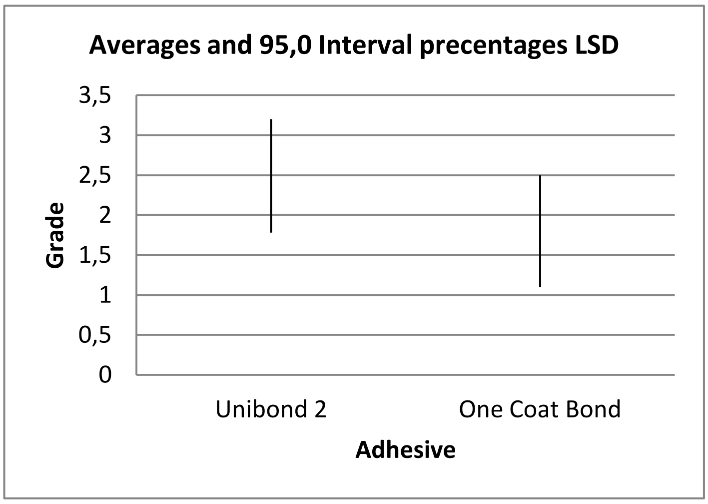


Scheme 1: Microfiltration grade

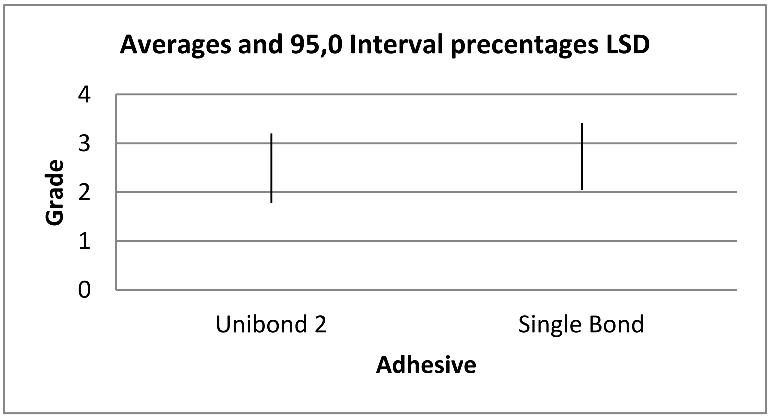
No statistically significant differences were found when comparing Unibond 2 (Adhesive B) with the other adhesives.



Scheme 2. Optibond FL vs. Unibond 2

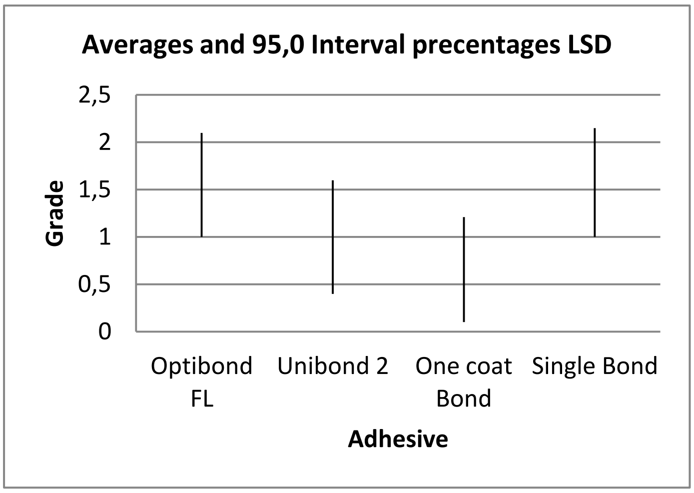


Scheme 3. Unibond 2 vs. One Coat Bond

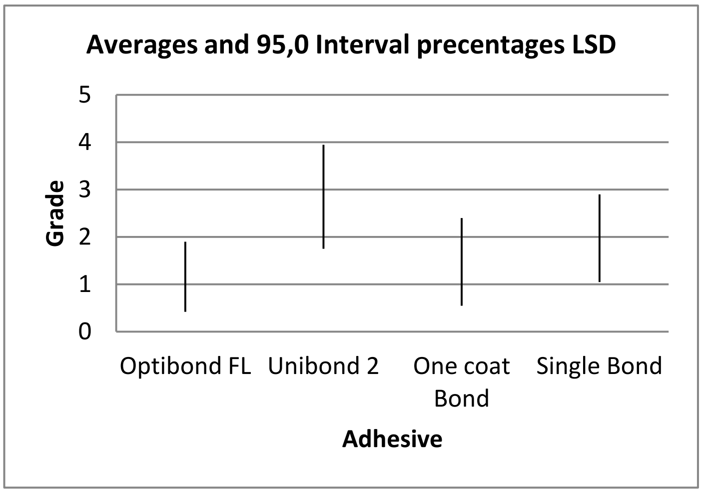


Scheme 4. Unibond vs. Single Bond

A comparative analysis of the various adhesives in the non-thermally and thermally cycled fillings is shown in Schemes 5 and 6, respectively. No statistically significant differences were found between them.



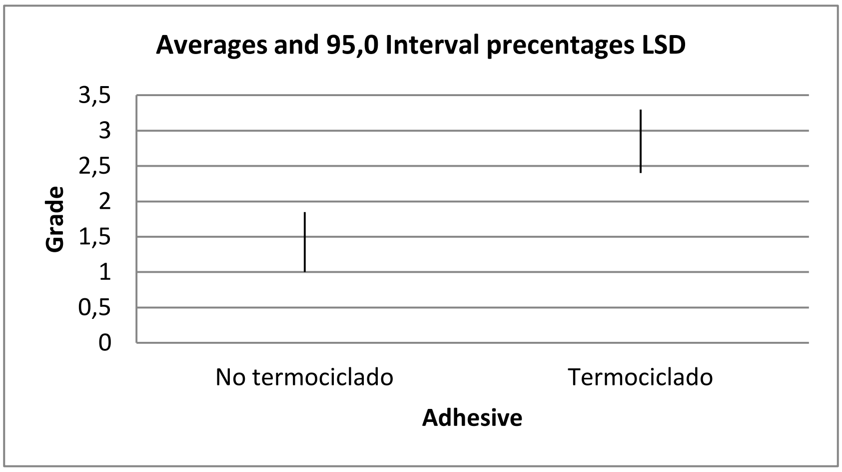
Scheme 5. Non Thermocycled samples



Scheme6. Thermocycled samples

Evaluation of adhesive microinfiltration for thermally and non-thermally cycled samples gave a p value of 0.0485, thus indicating significant differences between the two procedures.

An ANOVA was performed to assess the degree of microinfiltration between thermally and non-thermally cycled samples (Scheme 7). A multiple range test was applied to detect differences between the means of the differences, which were found to differ significantly.



Scheme 7. ANOVA for degree of microinfiltration. p = 0.0028

CONCLUSIONS:

- No statistically significant differences were found between the Unibond 2 adhesive and the other adhesives studied in terms of microinfiltration.
- However, statistically significant differences in microinfiltration and degree of microinfiltration were found between the thermally and non-thermally cycled samples.

REPORT FOR THE STUDY COMPARING VARIOUS ADHESIVES SUBJECTED TO SHEAR OR TANGENTIAL FORCES

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Introduction:

Adhesive agents are currently used to obtain restorations that provide an appropriate seal at the interface between the tooth structure and the restorative materials.

In this respect, Buonacore has shown that adhesion between these restorative materials and the enamel can be achieved by treating the latter with orthophosphoric acid. However, due to the fact that adhesion to dentine is a much more complex and less predictable process than adhesion to the enamel as a result of the presence of moisture in the tubules and its organic composition,

Etching dentine does not significantly increase the adhesive strength of restorative materials. As a result, an understanding of the smear layer is required to obtain good adhesion as it affects both adhesion and the substrate and could therefore condition the restoration process itself. Indeed, the two adhesion strategies currently available, namely etch and wash adhesives, which remove this layer, and self-etch adhesives, which make it more permeable but do not remove it, depend precisely on how the adhesives interact with this smear layer.

Application of the adhesive results in transformation of the dentine as its organic matrix combines with residual hydroxyapatite crystals, monomers from the resin and solvents to form a hybrid layer.

As the adhesion strength to enamel and dentine is often used as an indicator of the efficacy of adhesive systems, various tests have been developed to measure it. One of these is the shear bond strength (SBS) test, a simple technique that allows us to perform an efficient analysis of adhesive systems and evaluate the adhesion to different types of substrate and the type of adhesion failure (adhesive or cohesive) that occurs.

The aim of this study was to determine and compare adhesion strength by applying the shear bond strength test to four etch and wash adhesives, one of the three-step type and the remaining three of the two-step type.

Materials and methods
Four etch and wash adhesives, three of the two-step type and of the three-step type, which acted as control, were selected (Table1 and Figure 1).

ADHESIVE	MANUFACTURER
VENTURA Unibond 2	MADESPA S.A., Toledo, Spain
One Coat Bond	Coltène/Whaledent, Altstätten, Switzerland
Single Bonding	DMP Dental LDA, Greece
Optibond FL	Kerr, Orange, CA, USA

The teeth sample consisted of 32 caries-free third molars with no prior restorative treatment of any sort.

Sample preparation: the molars were cut at the crown level using an ISOMET 1000 cutting machine to expose the dentine surface. They were then embedded in acrylic resin. Once set, the crown surface was polished using 600-grain, water-cooled silicon carbide polishing discs until a smooth dentine surface had been achieved.

All samples were etched with 37% orthophosphoric acid (Ventura conditioning gel, MADESPA, S.A, Toledo, Spain) according to the manufacturer's instructions. The sample was then divided into four experimental groups:

- Group I (n=8): control group, Optibond FL (Kerr). Primer was rubbed into the dentine surface for 15 seconds and then air-dried for 5 seconds. A thin bonding layer was subsequently applied for 15 seconds, followed by air for 5 seconds, and this layer polymerised for 20 seconds using an LED lamp.
- Group II (n=8): Unibond2 (MADESPA, S.A) was rubbed into the dentine surface for 15 seconds, then air was applied from a distance for 10 seconds and the adhesive polymerised for 30 seconds using an LED lamp.
- Group III (n=8): One Coat Bond (Coltène/Whaledent) was rubbed into the dentine surface for 20 seconds, then air was applied from a distance for 10 seconds and the adhesive polymerised for 30 seconds using an LED lamp.
- Group IV (n=8): Single Bonding (DMP Dental LDA) was rubbed into the dentine surface for 15 seconds, then air was applied from a distance for 10 seconds and the adhesive polymerised for 15 seconds using an LED lamp.

Figure 1. Adhesion methodology for the control group. It is the same for the other groups.

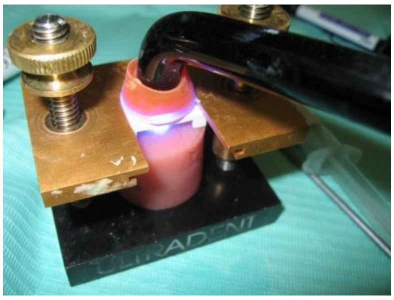
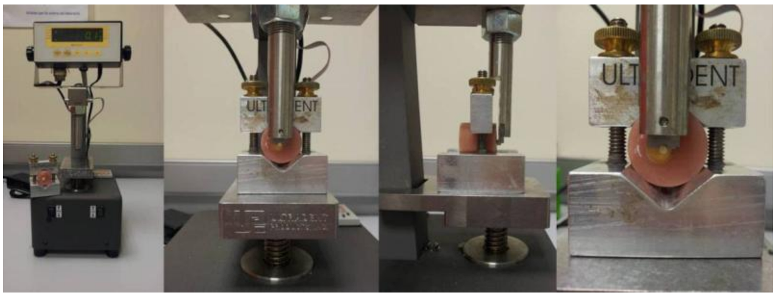


Figure 2. Jig in which the composite is applied to conduct the test.

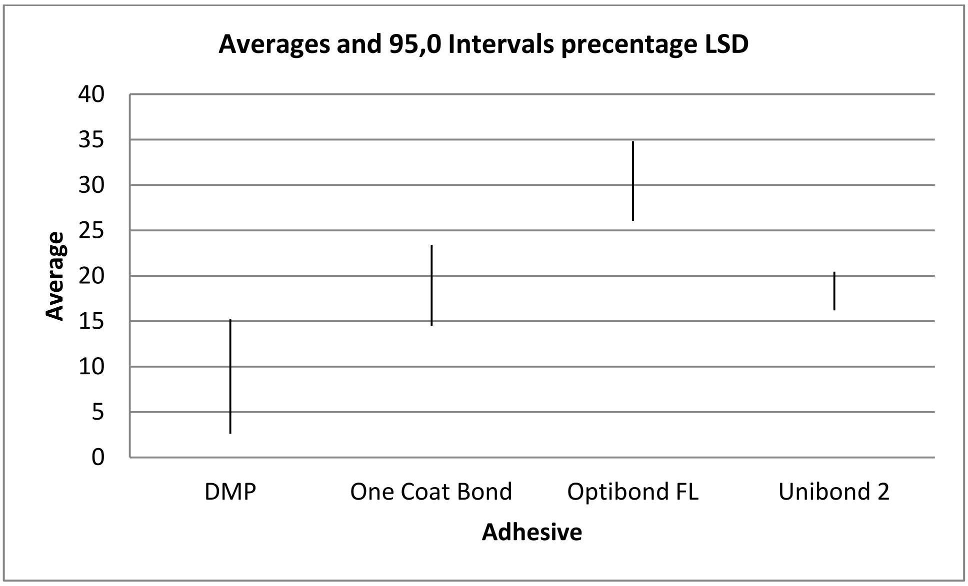
Once the samples had been prepared, they were subjected to a shear bond strength test using an Ultratester instrument. This test involved applying a cross velocity of 1 mm/min until failure or fracture. The shear bond strength, in MPa, was calculated by dividing the fracture load by the transversal section of the adhesion sample.



Results:

A comparison of the shear bond strength for the four groups studied showed statistically significant differences.

Thus, the Optibond FL three-step adhesive presented thee highest adhesion strength in this test (30.45±4.39 MPa) and the Single Bonding adhesive the lowest (8.91±6.31 MPa). Unibond 2 (18.33±2.14 MPa) behaved similarly to One Coat Bond (19.025±4.18 MPa) (Scheme 1).



Scheme 1:

Conclusions:

At this stage of the research we can conclude that

- the Unibond" adhesive has a similar standard behaviour to the One Coat Bond adhesive in terms of adhesive strength.
- Similarly, the Optibond FL three-step adhesive, which was used as a control, exhibits the highest adhesive strength.