Influences of Polymeric Magnetic Encapsulated Nanoparticles on the Adhesive Layer for Composite Materials Used for Class I Dental Fillings

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The main properties of dental adhesives should be good marginal adaptation, high retention strength and the possibility of not negatively influencing clinical sustainability. Dental adhesives are continuously improving to increase their retention to dental structures by increasing penetration in these structures, as it was shown in the in-vitro tests requiring imaging and qualitative analysis to allow the evaluation of experimental samples as well as the development of new materials. The main objective of this study is the qualitative and quantitative analysis of the layer of modified dental adhesives with ferrous nanoparticles encapsulated in a SiO$_2$ membrane located between the surface of the dental preparation and the surface of the photopolymerizable composite filling. For qualitative and quantitative analysis of the samples, technologies such as SEM, optical microscopy and EDX were used.

Keywords: dental adhesives, nanoparticles, SEM, EDX, optical microscopy

The development of adhesive techniques in dental medicine has increased the requirements of aesthetic dentistry as well as increasing the number of minimally invasive restorations made on dental surfaces [1].

Contemporary dentistry has been revolutionized by the appearance of dental adhesives, through it, other research directions have emerged for their improvement: the development of methacrylate-based composites, the development of hydrophilic resins and the modification of acid-etched dental surfaces. Surfaces of dental adhesives are susceptible to biodegradation. This biodegradation includes the interaction of bacterial enzymes, endogenous enzymes and dental biofilm. Acidity and hydrophilicity of resins increase the degree of degradation of the adhesive at its interface, and modified forms of dentin and enamel can affect the adhesion of the adhesive to enamel or dentin [2].

Composite resins have surpassed amalgam and have become the most used materials for dental restorations made by direct technique. Adhesive composite restorations are threatened by secondary caries, the degradation of the adhesive layer present at the interface between the tooth and by the various defective restoration materials, which will then be infested by fluids, bacteria and secondary bacterial products, leading ultimately to the failure of the composite filling. Therefore the durability over time of the connections between dentin and adhesives become an issue [3].

Thus, considering the fact that the main reason for the loss of a composite restoration by bacterial colonization and the appearance of secondary caries was generated by the microfissures at the edge of the restoration, the researchers developed the first dental adhesive material that has self-healing properties with antibacterial and remineralizing action. It has increased dental adhesion qualities, but its most important property is the self-healing of fractures at the adhesive interface layer [4].

Dental adhesives have been modified over time to alleviate the deficiencies they have acquired since the first generations, with methacrylamides being added to their component, the main purpose of which is to increase the resistance to hydrolytic and enzymatic degradations occurring at the interface of the adhesive [5].

In the formula of the adhesive components, lysine has been incorporated, which has resulted in good pH modulation outcomes, the effect of this modification can improve the durability of composite dental restorations [6].

Previous studies show that the thickness of the adhesive layer at the interface between the tooth and the restoration material is between 0.02 mm and 0.3 mm [7-9].

The presence of resin tags and the thickness of the hybrid layer do not greatly influence adhesion. [10, 11] The strength of adhesion may vary depending on the action time of the acid on the dental surface. At the same time, the surface of the hybrid layer changes, and the long time action of the acid on the surface of the tooth can influence the adhesion in a negative way [12].

Stress at the dentine-adhesive interface may be influenced by the type of adhesive used, with increased stress levels for self-etch adhesives compared to etch-and-rinse and self-etch-primer, both adhesive systems presenting partial demineralized dentin [13].

Dental adhesives have been evaluated over the years using imaging technologies such as optical coherence tomography (OCT), scanning electron microscopy, Raman spectroscopy and electron paramagnetic resonance spectroscopy [14-20].

Experimental part

In this study, 15 teeth were used, that had Class I Black cavities. The materials used in this study were: phosphoric acid for tooth demineralisation, Evertic Bond (Ivoclar) dental adhesive, Brilliant Flow (Coltene) photopolymerizable composite flow and multicore-shell Fe$_3$O$_4$ SiO$_2$ magnetic nanoparticles (fig.1).
After preparing the cavities on the surface of the teeth, they were restored using the adhesive technique. Demineralisation of dental surfaces was done with phosphoric acid for 15 s for dentin and 30 s for the enamel. The acid was washed with water for 20 s and the surface was dried with air. After demineralization, the dental adhesive doped with nanoparticles was applied by brushing onto the prepared surface of the cavity (fig.2).

After applying the adhesive, it was photopolymerized with the blue light lamp for 40 s, resulting in anaesthetic filling with slightly colored edges due to the nanomagnetic particles (fig.4).

Using a Vibrating Sample Magnetometer ADE Technologies VSM 880 we measured at room temperature the magnetization properties in the field range 0 - 1000 kA/m. The magnetic particles have superparamagnetic behaviour with 7.1 emu/g saturation magnetization, 6.4emu/g remnant magnetization and 4.4 kA/m coercive field.

Further, all 15 samples were sectioned and analysed with the help of FEI Inspect S scanning electron microscope (SEM), optical microscope and Energy Dispersive X-ray analysis (EDX). The scanning electron microscope has the following characteristics: tungsten filament mounted in the tetrode cannery assembly with a resolution of 3.0 nm on standard specimen with gold particles separated on a carbon substrate. Focus domain is between 3 and 99 mm with a magnification from 6x to >1,000,000x.

SEM analyzes generated images at 200x magnification in which the dental adhesive layer was observed between the two interfaces of the composite resin and the surface of the tooth structure.

Samples showing the adhesive loaded with magnetic nanoparticles that was applied to the tooth surface by conventional technique were analyzed using SEM, resulting in images at a 200x magnification. EDX quantitative analysis is showing internal components (fig.5).

The interfaces in which the dental adhesive loaded with magnetic nanoparticles was applied in the magnetic field for 2 and 5 min were also analyzed, generating high resolution images at the same magnification. The internal components diagram was also created (figs. 6, 7).
All the samples were analyzed with A377 optical microscope. The microscope has a magnification range between 20X and 800X and an CMOS acquisition sensor of 2MPX. The focus is between 0 mm and 40 mm and the connection with the computer is made with USB 2.0 port. Luminosity on sample probes is adjusted manually with 10 LED lights (figs. 8-10).

Results and discussions

After recording images using SEM and optical microscopy, they were imported and analyzed using ImageJ software (Wayne Rasband, National Institutes of Health, USA). As a result of the measurements made on the samples where the dental adhesive was loaded with nanoparticles and applied to the surface of the teeth without magnetic field, have resulted thicknesses of the adhesive layer ranging from 10 to 25 microns (table 1).

For adhesives loaded with magnetic nanoparticles and applied in magnetic field for 2 min, the measurements generated adhesive layer sizes between 14 - 36 microns (table 2).

For adhesives loaded with magnetic nanoparticles and applied in the magnetic field for 5 min, the measurements generated adhesive layer sizes between 2-12 microns.

Based on the analysis performed with optical microscopy, the thickness of adhesive layer for samples loaded with magnetic nanoparticles and applied to non-magnetic dental surfaces were between 16-29 microns (table 4).

Table 1

SEM ANALYSIS FOR DENTAL ADHESIVE LAYER THICKNESS OF THE SAMPLES REINFORCED WITH NANOPARTICLES APPLIED WITHOUT MAGNETIC FIELD

| Nr.Crt. | Area | Mean | Min | Max | Angle | Length (microns) |
|--------|------|------|-----|-----|-------|-----------------|
| 1      | 5.463| 182.128 | 77.204 | 235.000 | 21.077 | 18.774 |
| 2      | 5.763| 179.148 | 95.330 | 235.166 | 19.393 | 20.377 |
| 3      | 3.929| 167.167 | 74.141 | 235.000 | 15.811 | 21.431 |
| 4      | 4.129| 145.830 | 73.935 | 235.820 | 18.435 | 22.504 |
| 5      | 4.329| 121.034 | 95.348 | 255.000 | 18.015 | 23.602 |
| 6      | 4.329| 142.062 | 86.020 | 224.110 | 17.832 | 24.701 |
| 7      | 4.362| 135.541 | 79.549 | 231.666 | 18.834 | 24.872 |
| 8      | 3.330| 152.561 | 97.337 | 225.965 | 16.975 | 18.124 |
| 9      | 4.729| 130.432 | 0.000 | 193.336 | 15.219 | 25.720 |
| 10     | 3.963| 177.888 | 111.552 | 255.000 | 17.210 | 21.587 |
| Nr. Crt | Area (mm²) | Mean  | Min   | Max   | Angle (°) | Length (microns) |
|---------|------------|-------|-------|-------|-----------|------------------|
| 1       | 23.445     | 92.648| 83.187| 113.333| -35.150   | 14.595          |
| 2       | 29.836     | 98.373| 85.590| 112.333| -41.987   | 19.313          |
| 3       | 38.361     | 93.172| 85.398| 113.333| -39.806   | 21.175          |
| 4       | 23.443     | 95.261| 83.556| 119.090| -48.814   | 14.468          |
| 5       | 31.907     | 116.133| 100.251| 139.090| -31.340   | 20.064          |
| 6       | 27.705     | 126.778| 101.444| 151.171| -30.026   | 17.545          |
| 7       | 27.705     | 101.435| 88.914| 113.444| -45.000   | 17.935          |
| 8       | 31.907     | 118.346| 98.787| 133.789| -30.711   | 20.825          |
| 9       | 49.015     | 129.149| 101.000| 156.500| -61.189   | 32.234          |
| 10      | 55.410     | 99.278| 83.490| 124.000| -41.820   | 36.532          |

**Table 2**
SEM ANALYSIS FOR DENTAL ADHESIVE LAYER THICKNESS OF THE SAMPLES REINFORCED WITH NANOPARTICLES APPLIED WITH MAGNETIC FIELD FOR 2 min

| Nr. Crt | Area (mm²) | Mean  | Min   | Max   | Angle (°) | Length (microns) |
|---------|------------|-------|-------|-------|-----------|------------------|
| 1       | 0.619      | 122.830| 112  | 139   | -31.340   | 6.247           |
| 2       | 1.095      | 119.220| 100.447| 142.939| -51.926   | 11.104          |
| 3       | 0.466      | 115.242| 100.667| 146.000| -49.268   | 4.635           |
| 4       | 0.428      | 116.631| 105.748| 152.000| -45.000   | 4.777           |
| 5       | 0.343      | 118.397| 109.016| 141.000| -43.831   | 3.381           |
| 6       | 0.771      | 119.431| 101.214| 138.398| -45.307   | 7.796           |
| 7       | 1.100      | 142.553| 122.947| 186.000| -49.248   | 12.106          |
| 8       | 1.161      | 154.936| 123.563| 245.098| -47.344   | 11.806          |
| 9       | 0.752      | 170.840| 141.629| 238.320| -52.356   | 7.590           |
| 10      | 0.276      | 151.699| 126.921| 203.439| -52.306   | 2.712           |

**Table 3**
SEM ANALYSIS FOR DENTAL ADHESIVE LAYER THICKNESS OF THE SAMPLES REINFORCED WITH NANOPARTICLES APPLIED WITH MAGNETIC FIELD FOR 5 min

| Nr. Crt | Area (mm²) | Mean  | Min   | Max   | Angle (°) | Length (mm) |
|---------|------------|-------|-------|-------|-----------|-------------|
| 1       | 1.90E-4    | 134.630| 99.000| 186.333| 90.000    | 0.025       |
| 2       | 1.64E-4    | 139.804| 124.333| 162.550| 26.500    | 0.015       |
| 3       | 1.64E-4    | 142.389| 114.667| 172.222| 45.000    | 0.017       |
| 4       | 1.90E-4    | 170.414| 145.222| 195.333| 45.000    | 0.021       |
| 5       | 1.90E-4    | 162.145| 149.444| 190.556| 45.000    | 0.023       |
| 6       | 1.90E-4    | 162.987| 141.704| 191.370| 90.000    | 0.023       |
| 7       | 1.90E-4    | 165.417| 143.481| 197.667| 108.435   | 0.023       |
| 8       | 2.70E-4    | 145.456| 123.312| 179.667| 63.425    | 0.029       |
| 9       | 1.90E-4    | 174.593| 123.667| 209.000| 90.000    | 0.022       |
| 10      | 1.90E-4    | 140.630| 126.889| 164.741| 56.310    | 0.025       |

**Table 4**
OPTICAL MICROSCOPY ANALYSIS FOR DENTAL SAMPLES WITH ADHESIVE REINFORCED WITH NANOPARTICLES APPLIED ON TEETH SURFACE WITHOUT MAGNETIC FIELD

| Nr. Crt | Area (mm²) | Mean  | Min   | Max   | Angle (°) | Length (mm) |
|---------|------------|-------|-------|-------|-----------|-------------|
| 1       | 2.37E-4    | 95.037| 69.000| 121.000| 45.000    | 0.022       |
| 2       | 1.78E-4    | 127.444| 102.256| 149.370| 26.765    | 0.015       |
| 3       | 1.78E-4    | 165.333| 152.333| 177.000| 90.000    | 0.015       |
| 4       | 1.78E-4    | 131.888| 111.056| 151.259| 45.000    | 0.018       |
| 5       | 1.78E-4    | 131.130| 126.444| 137.778| 45.000    | 0.018       |
| 6       | 2.37E-4    | 119.483| 114.813| 125.889| 0.000     | 0.021       |
| 7       | 1.78E-4    | 133.307| 127.607| 139.333| 0.000     | 0.014       |
| 8       | 2.97E-4    | 101.519| 91.880| 110.667| 56.310    | 0.029       |
| 9       | 2.97E-4    | 90.217| 68.056| 133.778| 63.435    | 0.023       |
| 10      | 2.37E-4    | 114.065| 104.087| 133.444| 18.435    | 0.023       |

**Table 5**
OPTICAL MICROSCOPY ANALYSIS FOR DENTAL SAMPLES WITH ADHESIVE REINFORCED WITH NANOPARTICLES APPLIED ON TEETH SURFACE WITH MAGNETIC FIELD FOR 2 min
For samples using a magnetic field for 2 min, the analyzes generated thicknesses of adhesive layer between 13-33 microns (table 5). For samples using a magnetic field for 5 min, the analyzes generated adhesive layer thicknesses between 3 - 10 microns (table 6).

EDX semi quantitative analysis for samples with adhesive reinforced with nanoparticles applied on teeth without magnetic field has highlighted the presence of C (49.74 %), O (23.43 %), Na (0.46 %), Mg (0.4 %), Al (0.39 %), Si (3.07 %), P (6.61 %), Ca (12.7 %), Ba (2.23 %) and Zn (7.58 %).

EDX semi quantitative analysis for samples using a magnetic field for 2 min has highlighted the presence of C (59.46 %), O (23.21 %), Al (0.15 %), Si (3.42 %), P (2.01 %), Ca (2.57 %), Ba (1.24 %), Fe (0.36 %) and Zn (0.97 %).

EDX semi quantitative analysis for probes where we used magnetic field for 2 min has highlighted the presence of C (59.46 %), O (23.21 %), Al (0.15 %), Si (3.42 %), P (2.01 %), Ca (2.57 %), Ba (1.24 %), Fe (0.36 %) and Zn (0.97 %).

EDX semi quantitative analysis for samples where we used magnetic field for 5 min has highlighted the presence of C (59.46 %), O (23.21 %), Al (0.15 %), Si (3.42 %), P (2.01 %), Ca (2.57 %), Ba (1.24 %), Fe (0.36 %) and Zn (7.58 %).

Measurements made using scanning electron microscopeand optical microscopy generated results that fall within the same intervals.

Conclusions

The use of magnetic nanoparticles after incorporation into dental adhesives, can reduce the thickness of the adhesive layer by 30% by applying a magnetic field on the tooth surface for 2 min and by 85% for applying the same magnetic field for 5 min compared to the application of dental adhesives by conventional techniques.

Further studies are needed for adhesion strength evaluations.

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Table 6

| Nr. Crt. | Area  | Mean  | Min   | Max    | Angle (°) | Length (mm) |
|---------|-------|-------|-------|--------|-----------|-------------|
| 1       | 1.095E-4 | 103.861 | 99.278 | 108.444 | -45       | 0.010       |
| 2       | 1.095E-4 | 89.389  | 78.333 | 100.444 | -90       | 0.007       |
| 3       | 1.095E-4 | 115.528 | 113.667 | 117.389 | 0         | 0.007       |
| 4       | 1.095E-4 | 114.833 | 107.333 | 122.333 | -45       | 0.008       |
| 5       | 5.475E-5 | 142.111 | 142.111 | 142.111 | 0         | 0.003       |
| 6       | 1.095E-4 | 143.315 | 151.983 | 156.667 | -45       | 0.008       |
| 7       | 1.095E-4 | 143.444 | 140.778 | 146.111 | 0         | 0.005       |
| 8       | 1.095E-4 | 153.690 | 143.824 | 163.355 | 0         | 0.006       |
| 9       | 1.095E-4 | 119.583 | 118.944 | 120.722 | 0         | 0.004       |
| 10      | 1.095E-4 | 119.534 | 108.319 | 131.389 | -90       | 0.008       |
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