Pit and fissure nanocomposite sealants reinforced with organically modified montmorillonite: A study of their mechanical properties, surface roughness and color stability

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The aim of the present work was to investigate the effect of the different organically modified nanoclays on clinically significant properties of new synthesized dental pit and fissure nanocomposite sealants. Their morphological characteristics were examined by means of X-ray diffraction (XRD) analysis and scanning electron microscopy (SEM). A universal testing machine was used to conduct the flexural and compression tests. Surface roughness measurements were taken by using a 3D-optical profilometer. Color changes after aging in black tea were determined by recording UV-visible spectra. XRD plots depicted possible structures governed by intercalated regions along with some “tactoids” nanoparticles. SEM images revealed a better dispersion for the methacrylated clay nanofiller. Flexural modulus and microhardness were found to be higher for sealants reinforced with such polymerizable clay nanoclays. These specific nanocomposites yielded smoother surfaces, as well as clinically accepted color changes even after 1 week aging in black tea.

Keywords: Pit and fissure sealants, Dental nanocomposite materials, Nano-clay

INTRODUCTION

Dental caries constitutes one of the most widespread chronic diseases affecting children and adolescents1-2. Occlusal pit and fissures may be often susceptible to cariogenic bacteria attacks due to their specific morphological characteristics3,4. A physical barrier against food remnants6, invasion of bacteria and plaque accumulation7 is usually achieved by applying pit and fissure sealants8 in clinical practice. In this way, incipient carious lesions and their consequent progression can be avoided9. As resin-based materials, pit and fissure sealants are capable of satisfactory retention rates, but they are often challenged with polymerization shrinkage that could lead to microleakage6,9,10. Hence, bacteria can easily find pathways initiating secondary caries11. If these phenomena persist, then carries lesions, and finally pain and pulp exposure cannot be excluded12.

The utilization of fillers in nanometer scale could promote their antibacterial and mechanical properties of pit and fissure sealants. Towards this direction several aspects have been reported in the literature. Pit and fissure sealants with bioactive glass nanoparticles showed higher fluoride release and recharge capabilities and similar or better retention than the commercial counterparts12. Addition of tricalcium phosphate nanoparticles to the fissure sealants significantly increased the remineralization potential without affecting the mechanical properties13. Flexural strength and hardness was found to be improved by incorporating chitosan nanofibers but no antibacterial activity was observed14. Chitosan whiskers had a comparable antimicrobial activity to commercial resin sealants without reducing the curing depth or degree of double bond conversion, but a minimal reduction in hardness was not avoided15.

Montmorillonite (MMT) is a 2:1 smectic type of clay containing metal cations such as Na+, K+, Ca2+ or Mg2+ between the galleries of its lamellar structure15. Due to its hydrophilic nature, metal ions are usually exchanged with quaternary ammonium intercalates, in order to render nanoclay hydrophobic and capable to be well dispersed into polymer matrix16-17. It is well known that polymer-clay nanocomposites might present remarkable performance regarding their mechanical, physicochemical, thermal and antibacterial properties18-20. Recently, commercially available clay nanoparticles were utilized in composite resins exhibiting encouraging results concerning their polymerization shrinkage reduction and mechanical properties improvement21-23. Furthermore, commercially available organomodified montmorillonites (OMMTs)23 with variant alkylammonium cations were tested against representative pathogenic bacteria proving significant antibacterial activity.

In the present work, novel pit and fissure sealant nanocomposites were synthesized and characterized by inserting clay nanofillers into the polymer matrix. To the best of our knowledge, there is no literature data associated with the usage of clay nanofillers in pit and fissure sealants, and hence the potential of incorporation of such nanoparticles into sealants’ formulations could be considered as a quite promising approach to meet the performance requirements needed in clinical practice. For
this purpose, different organomodified clays (OMMTs), in terms of their intercalating agent, were tested: a) one having polar groups, b) a hydrophobic clay containing only alkyl groups and, c) a methacrylate functionalized OMMT. Subsequently, an effort was made to investigate whether the clay organomodifier have any effect on structural characteristics, mechanical properties, surface roughness and color stability of the obtained pit and fissure sealants. The significance of the obtained results could provide valuable information towards the designing of modern dental restorative materials.

MATERIALS AND METHODS

Materials

The monomers triethyleneglycol dimethacrylate (TEGDMA), 95%, and 2,2-bis[4-(2-hydroxy-3-methacrylyloxy-propoxy)-phenyl] propane (Bis-GMA) were both provided by Aldrich (Sigma-Aldrich Chemie, Taufkirchen, Germany). Co-initiator 2-(Dimethylamino) ethylmethacrylate (DMAEMA), 99%, and initiator camphorquinone, 98%, were purchased from J&K (J&K Scientific, Lommel, Belgium). Commercially available OMMTs with the trade name Nanomer® I.34MN (Nanocor, Hoffman Estates, IL, USA) and Cloisite® 20A were supplied by Aldrich (Sigma-Aldrich Chemie) and BYK Additives & Instruments (BYK-Chemie, Wesel, Germany) respectively. They are -onium ion modified clays containing methyl dihydroxyethyl hydrogenated tallow and dimethyl dehydrogenated tallow quaternary ammonium ions respectively. Another OMMT with dimethylaminooctadecyl methacrylate as intercalating organomodifier (MMT-DMAODM) prepared in our previous work was also used. The specific chemical structures of all MMT organomodifiers are represented in Table 1. Phosphate buffered saline (PBS) 10× was provided by Aldrich (Sigma-Aldrich Chemie) and diluted to a 1× working solution. All other chemicals used were of reagent grade.

Methods

1. Sample preparation

Three groups of experimental composites were prepared by initially mixing a Bis-GMA/TEGDMA base (50:50 wt/wt) which contained camphorquinone (0.2%) and DMAEMA (0.8%) as photo-initiating system. Afterwards, the different organomodified clays were mixed with the resin by manual mixing until the powder was completely wetted with organic matrix, and the obtained mixture was ultrasonicated for 10 min. The nanofiller loading was 40% to ensure paste handling properties almost similar to commercial pit and fissure sealants. Bis-GMA/TEGDMA pure matrix was also prepared to the same composition to be used as control material.

2. Measurements

X-ray Diffraction (XRD) analysis of cured materials and nanopowder Cloisite® 20A were performed over the 2θ range of 2–10°, at steps of 0.05°, and counting time of 5 s per step, using a XRD system (Miniflex II, Rigaku, Tokyo, Japan) with Cu Kα radiation (λ=0.154 nm).

Scanning electron microscopy (SEM) was carried out using a scanning microscope (JEOL, JSM-6390LV, JEOL USA, Peabody, MA, USA) equipped with an energy-dispersive X-ray (EDX) microanalytical system (INCA PentaFET×3, Oxford Instruments, Abingdon, England). All the studied surfaces were coated with carbon black to avoid charging under the electron beam.

For flexural testing, bar-specimens were prepared by filling a Teflon mold (2×2×25 mm) with unpolymerized paste in accordance to ISO 4049. The mold surfaces were overlaid with glass slides covered with a Mylar sheet to avoid air entrapping and adhesion of the final set material. The assembly was held together with

| Nanoclay type (40%) | Chemical structure of organomodifier | d<sub>001</sub> (nm) | Δd<sub>001</sub> (nm) |
|---------------------|-------------------------------------|---------------------|---------------------|
| Nanomer® I.34MN     | CH<sub>2</sub>CH<sub>2</sub>OH         | 3.90                | 2.04                |
|                     | CH<sub>3</sub>–N–HT                  |                     |                     |
|                     | CH<sub>2</sub>CH<sub>2</sub>OH       |                     |                     |
| MMT-DMAODM          | CH<sub>3</sub>–COOCH<sub>2</sub>CH<sub>2</sub>–N–C<sub>18</sub>H<sub>17</sub> | 4.12                | 2.35                |
|                     | CH<sub>3</sub>                      |                     |                     |
| Cloisite® 20A       | CH<sub>3</sub>–N–HT                  | 4.05                | 2.08                |
|                     | HT                                  |                     |                     |

where HT is hydrogenated tallow (~65% C18; ~30% C16; ~5% C14).
spring clips and irradiated by overlapping on both sides using a LED light-curing unit (SATELEC, mini LED, ACTEON Merigniac, France) at 1,250 mW/cm². Each overlap irradiation lasted for 40 s. A radiometer (Hilux, Benioglu Dental, Ankara, Turkey) was used to verify the output irradiance of the LED device. Ten specimen bars (n=10) were prepared for each nanocomposite. The specimens were stored at 37±1°C in dark conditions for 24 h immediately after curing. Then they were immersed in PBS solution. After conditioning at 37±1°C for 10 days, the specimens were bent in a three-point transverse testing rig with 20 mm between the two supports (3-point bending). The rig was fitted to a universal testing machine (Testometric AX, M350-10kN, Testometric, Rochdale, England). All bend tests were carried out at a cross-head speed of 0.5 mm/min until fracture occurred. The load and the corresponding deflection were recorded. The flexural modulus (E) in GPa and flexural strength (σ) in MPa were calculated according to the following equations:

\[
E = \frac{F_1 l^3}{4bh^3} 10^{-9} \quad \text{and} \quad \sigma = \frac{3F_{max}l}{2bh^2}
\]

(1)

where: \(F_1\) is the load recorded in N, \(F_{max}\) is the maximum load recorded before fracture in N, \(l\) is the span between the supports (20 mm), \(b\) is the width of the specimen in mm, \(h\) is the height of the specimen in mm, and \(d_1\) is the deflection (in mm) corresponding to the load \(F_1\).

Cylindrical specimens (4×6 mm) for compressive tests were prepared (n=10), cured and stored in PBS solution as above. Test specimens were fractured on a universal testing machine (Testometric AX, M350-10kN, Testometric). All measurements were carried out at a cross-head speed of 1 mm/min until fracture occurred. The maximum load was recorded and the compressive strength (\(\sigma\)) in MPa was calculated using the standard formula:

\[
\sigma = \frac{4F}{\pi D^2}
\]

(2)

where: \(F\) is the maximum load (N), and \(D\) is the specimen diameter (mm).

Vickers microhardness evaluations were conducted on the top surfaces of circular specimens (5×4 mm), following the previous curing and storage procedures. These surfaces were ground and wet-polished using a sequence of SiC abrasive papers (320–2000 grit) in a grinding/polishing machine (TG250 Automata GP, Jean Wirtz, Essen, Germany), rinsed with water for 30 s, acid-etched for 15 s with a phosphoric acid gel (Scotchbond Etchand Gel, 3M ESPE, St. Paul, MN, USA), rinsed with water for 10 s and finally air dried for 10 s. After conditioning at 37±1°C for 10 days, the measurements were taken by means of a microhardness tester (HMV-2000, Shimadzu, Kyoto, Japan), at a load of 200 g with an indentation time of 10 s (Vickers pyramid: diamond right pyramid with a square base and an angle of α=136° between the opposite faces at the vertex). Ten (n=10) specimens for each sample were prepared, and five readings for each specimen surface were carried out and independently averaged and reported in Vickers hardness number (VHN).

For surface roughness measurements, circular specimens (10×2 mm) were prepared and cured as described above. All specimens were contoured, finished and wet polished with abrasive disks by means of a single operator at low speed (10,000–12,000 rpm) under 0.3–0.6 N pressure, and subsequently rinsed with tap water. The polishing procedure was performed sequentially with a complete series of abrasive disks (coarse, medium, fine and ultra fine). The average surface roughness was evaluated by vertical scanning interferometry (VSI) using a 3D optical profilometer (Contour GT, BRUKER, Tucson, AZ, USA) and magnification ×10. Vision64™ software was used to acquire the data and compute the mean surface roughness in Sa units (nm) of each image. Five images were taken from each specimen and ten specimens (n=10) from each group were tested.

In order to estimate the color stability, circular specimens (10×2 mm) were formed (n=10) and cured as previously described. The specimens were stored at 37±1°C in dark conditions for 24 h immediately after curing. Then they were polished according to the procedure that was followed for the VHN measurements. Afterwards, they were immersed in black tea and stored at 37°C for time periods of 72 h and 1 week. Color data were recorded using an ultraviolet-visible spectroscopy recording spectrophotometer (UV-2401 PC, Shimadzu, Tokyo, Japan) supported by software (UVPROBE v2.21, Shimadzu). Data were retrieved relative to the illuminant D65 standard, corresponding to average daylight with 10 degrees observation in the reflectance mode on a black background, by means of color measurement software (UVPC Color Analysis Ver. 3.02, Shimadzu). The spectrophotometer was equipped with an integrating sphere (ISR-240 A, Shimadzu) with a 7-mm opening directing the light at the specimen at an angle of 0 degrees. The color change values of specimens were examined before and after each aging test using the CIEDE2000 color system and determined based on the following formula:

\[
\Delta E_0 = \sqrt{\left(\frac{\Delta L}{K_L S_L}\right)^2 + \left(\frac{\Delta C}{K_C S_C}\right)^2 + \left(\Delta H/\alpha\right)^2 + \left(R_T (\frac{\Delta C}{K_C S_C}) + \Delta H/\alpha\right)^2 + \left(K_H \Delta H/\alpha\right)^2}
\]

(3)

where: \(\Delta E_0\) is color differences, \(\Delta L'\), \(\Delta C'\), and \(\Delta H'\) are the differences in lightness (L), chroma (C), and hue (H) for a pair of specimens in CIEDE2000, \(R_T\) is the rotation factor that accounted for interactions between chroma and hue differences in the blue region. Weighting functions, \(S_L, S_C, S_H\) adjusted the total color difference for variation in the location of the color difference pair in the \(L^*, a^*, b^*\) coordinates, and the parametric factors \(K_L, K_C, K_H\), where the terms for experimental conditions. The parametric factors of the equation (3) were set to 1.10.
3. Statistical analysis

Since the experimental data of flexural modulus, compression strength and microhardness were not normally distributed, Kruskal-Wallis statistic test (nonparametric ANOVA), followed by a Dunn's multiple comparisons test were performed, for multiple comparisons between means to determine significant differences. One-way ANOVA test followed by Tukey-Kramer multiple comparisons test was applied for the obtained flexural strength, surface roughness and color changes values. The values of the measured properties represent mean values±standard deviation (SD) and median values±interquartile range (IQR) of replicates (Tables 2 and 3).

RESULTS

XRD diffractograms for both neat OMMTs and corresponding nanocomposites with 40% nanofiller are represented in Fig. 1. It is obvious that the diffraction peaks of the three OMMTs were shifted to lower angles in the obtained nanocomposites, implying a possible intercalation of macromolecular chains between the interlayer galleries of clay in each case. Concerning the nanocomposites' spectra, the residual diffraction peaks of the pristine OMMTs are still observed in the initial 2θ angles. Nanocomposites containing MMT-DMAODM and Cloisite® 20A exhibited a higher intensity of the first peak compared to the secondary peak. On the other hand, the composite with Nanomer® I.43MN showed higher intensity of the secondary peak.

The d001-basal spacing values were calculated for all nanocomposites according to Bragg’s law (nλ=2dsinθ) and are given in Table 1. The d001 changes (Δd001) caused by clay intercalation in nanocomposite structures were also determined and listed in Table 1. The d001-value of pure Cloisite® 20A was found 1.97 nm, whereas the corresponding values were measured in our previous work32 for pure Nanomer® I.34MN, MMT-DMAODM and found 1.86 and 1.77 nm respectively. It can be seen that nanocomposite containing MMT-DMAODM exhibited the highest Δd001-value (2.35 nm) when compared to Nanomer® I.34MN and Cloisite® 20A (2.04 and 2.08).

Figure 2 represents the SEM microphotos captured for each sealant nanocomposite prepared. The narrower white dots correspond to clay particles that sometimes

### Table 2

| Nanocomposite resin | Flexural modulus (GPa) | Flexural strength (MPa) | Compressive strength (MPa) | Microhardness (VHN) |
|---------------------|------------------------|-------------------------|---------------------------|---------------------|
| BisGMA/TEGDMA       | 0.77 (0.19)            | 41.71 (7.14)            | 133.47 (56.43)            | 25.79 (3.14)        |
| Nanomer® I.34MN     | 1.53 (0.09)            | 24.77 (3.97)            | 35.20 (6.46)              | 10.28 (2.56)        |
| MMT-DMAODM          | 1.78 (0.51)            | 13.90 (3.83)            | 33.13 (10.78)             | 12.82 (10.23)       |
| Cloisite® 20A       | 1.09 (0.16)            | 18.71 (1.88)            | 34.25 (6.27)              | 12.15 (3.70)        |
| Guardian Seal 3M-ESPE (St. Paul, MN, USA) | 1.20 | 39.00 | — | 13.50 |
| Clinpro Kerr (Orange, CA, USA) | 1.70 | 50.00 | 123.54 | 9.50 |

In same column, superscript capital and lower letters indicate no statistical significant differences between mean values, as compared by nonparametric and one-way ANOVA tests (p<0.01).

### Table 3

| Nanocomposite resin | Surface roughness, Sa (nm) | Color change (ΔE) |
|---------------------|-----------------------------|-------------------|
|                     | 72 h                        | 1 week            |
| Nanomer® I.34MN     | 370.87 (185.33)             | 1.66 (0.94)       | 3.30 (0.84) |
| MMT-DMAODM          | 225.22 (91.96)              | 1.15 (0.60)       | 1.80 (0.59) |
| Cloisite® 20A       | 313.49 (68.83)              | 5.60 (2.62)       | 8.27 (2.06) |

In same column, superscript capital and lower letters indicate statistical significant differences between mean values, as compared by one-way ANOVA test (p<0.05).
may appear close to nanometer scale, while the larger and frequently light gray dots to the formed agglomerates. Particularly, the nanocomposite having 40% MMT-DMAODM (Fig. 2b) exhibited rather a favorable dispersion of clay particles concurrently limiting the appearance of agglomerates compared to other types of OMMT. On the contrary, the incorporation of Nanomer® I.34MN at the same amount level (Fig. 2a) may lead to bulky aggregates, sometimes even around 50 μm at a relatively high abundance. In case of Cloisite® 20A (Fig. 2c), the smaller clay particles seem to be in a population balance with the formed clusters, while the size of the latter is much lower than that of Nanomer® I.34MN agglomerates.

The mechanical behavior of the current sealant nanocomposites in terms of their flexural modulus, flexural and compression strength, as well as microhardness is illustrated in Figs 3, 4 and 5. The corresponding values of the specific mechanical parameters are summarized in Table 2. Typical data are also included for some commercially available pit and fissure sealants based on previous studies. Neat resin (Bis-GMA/TEGDMA) was also measured in each case as control material. It can be seen that the flexural modulus can be significantly affected by the presence of clay at a concentration of 40%, regardless the type of clay organomodifier (Fig. 3a). In particular, the insertion of MMT-DMAODM into the resin gave a 131.2% rise to the final flexural modulus, while a 98.7% increment was observed for Nanomer® I.34MN followed by a 41.6% modulus increase due to the presence of Cloisite® 20A.

Figure 3b reveals that flexural strength deteriorates when 40% clay nanofiller is added. The flexural resistance decrease for nanocomposite can be reached to 67% for nanocomposite with MMT-DMAODM compared to pure resin. In other respects, Nanomer® I.34MN yielded the highest flexural strength (24.77 MPa) followed by Cloisite® 20A (18.71 Mpa).

Compressive strength tendencies depending on the different OMMT are presented in Fig. 4. It can be seen that a significant decrease (up to 75.18%) of strength might take place when clay nanoparticles are inserted. However, strength values (Table 2) were very close for
Fig. 3 Effect of the clay nanofiller type on a) flexural modulus (box plots including median and interquartile range), and b) flexural strength of the experimental pit and fissure sealant nanocomposites.

Nanomer® I.34MN (35.20 MPa), Cloisite® 20A (34.25 MPa) and MMT-DMAODM (33.13 MPa).

Microhardness tendencies are represented in Fig. 5. All nanocomposites exhibited lower microhardness values in comparison to neat resin. The highest VHN value was calculated for MMT-DMAODM nanofiller (18.33), followed by Cloisite® 20A (14.38) and Nanomer® I.34MN (10.72) respectively.

The mean surface roughness values ($S_a$, in nm) and standard deviations for each sealant nanocomposite are listed in Table 3. Representative topographic surface maps of nanocomposite specimens are illustrated in Fig. 6, accompanied with a color scale indicating the discrimination between peaks (red areas) and valleys (blue areas). According to Table 3 data, the utilization of different OMMT clay might have a remarkable effect on the polishing characteristics of each nanocomposite surface. Particularly, nanocomposites containing MMT-DMAODM exhibited the smoothest surface ($S_a=225.22$ nm) after polishing treatment (Fig. 6b), while the Cloisite® 20A analogue revealed somehow irregular regions (Fig. 6c) raising the average surface roughness ($S_a=313.49$ nm). Nanocomposites having Nanomer® I.34MN seem to be more susceptible to biofilm growing or stain retention, showing a relatively abrasive surface (Fig. 6a) characterized by the highest mean roughness value ($S_a=370.87$ nm).

In terms of the color stability estimations of new nanocomposites, UV-Vis spectra were taken by recording the reflection changes of incident light in different wavelengths (Fig. 7a). The solid lines correspond to scans gained after nanocomposite aging in black tea for 72 h, whereas 1 week measurements are represented by dot lines. The mean values of the calculated color...
changes ($\Delta E$) are given in Table 3, whereas the relative overall $\Delta E$ tendencies in function to clay type are displayed in Fig. 7b. Comparing $\Delta E$ values it is obvious that MMT-DMAODM and Nanomer® I.34MN imparted better color stability to nanocomposites ($\Delta E=1.15$ and 1.66) than Cloisite® 20A ($\Delta E=5.60$) after 72 h aging in black tea. Furthermore, discoloration increment after 1 week aging was lower for MMT-DMAODM ($\Delta E=1.80$), followed by Nanomer® I.34MN ($\Delta E=3.30$) and Cloisite® 20A ($\Delta E=8.27$).

**DISCUSSION**

In the present study, XRD and SEM techniques were chosen to provide information about the clay lamellae conformation and dispersion in the polymer matrix of each nanocomposite. The claim of obtained nanocomposite sealants with intercalated structures as supported by XRD results is in accordance with previous observations reported by other researchers when filler concentration was varied from 20 up to 50%\(^{29,30}\). The secondary peaks of pure OMMTs remained in the starting $2\theta$ angles denote structures mostly characterized by a potential co-existence of clay agglomerates well known as “tactoids”\(^{36,37}\) together with polymer intercalated regions. A higher ratio of intercalated clay to “tactoids” could be ascribed to nanocomposites containing MMT-DMAODM and Cloisite® 20A, due to the higher intensity of the first diffraction peak, while the incorporation of Nanomer® I.34MN leads rather to an established presence of agglomerates as it was indicated by the respective intensity peak ratio. The maximum $\Delta d_{001}$-value found for MMT-DMAODM nanocomposite reveals the highest degree of intercalation among the other composites. This trend could be explained by the presence of vinyl groups in the chemical structure of MMT-DMAODM (Table 1).
which render it compatible enough to be well dispersed into the polymer matrix and potentially polymerizable with the resin monomers. Although someone could expect that hydroxy-groups of Nanomer® I.34MN would impart a better intercalation than Cloisite® 20A —because of their capability of hydrogen bonding with monomers— however the excess of hydrogenated tallow ligands in Cloisite® 20A structure could ensure a sufficient hydrophobicity which may allow an almost equal intercalation of macromolecules.

According to SEM images, the distribution of clay could be characterized quite efficient for the total of nanocomposites, always taking into account that at filler loadings as high as 40% the formation of some “tactoids” is almost inevitable. It is generally considered that the extensive dispersion of nanofillers into the polymer matrix can assure the homogeneity of the nanocomposite, and thus it is believed to have a strong efficacy on the ultimate properties of the nanocomposite, and thus it is believed to have a strong efficacy on the ultimate properties of the nanocomposite, and thus it is believed to have a strong efficacy on the ultimate properties of the nanocomposite, and thus it is believed to have a strong efficacy on the ultimate properties of the nanocomposite, and thus it is believed to have a strong efficacy on the ultimate properties of the nanocomposite. Based on a correlation between the emerged tendencies of flexural modulus and the differences in the chemical structures of the clay organomodifiers (Table 1), it is apparent that chemical interactions could play a key role towards the improvement of flexural modulus. Specifically, methacrylated groups located in the quaternary ammonium molecule of MMT-DMAODM are capable of forming covalent bonds with Bis-GMA/TEGDMA system through a polymerization reaction, contributing to the highest stiffness of the corresponding nanocomposite. Furthermore, the significant augmentation of modulus due to Nanomer® I.34MN nanoparticles could be attributed to their hydroxy-groups which might be involved in hydrogen bonding with ester groups of monomers.

In contrast to flexural modulus improvement, the flexural strength considerably lowered when 40% nanoclay was added in each case. Similar flexural resistance attitude has been also reported in other studies when clay filler concentration varied from 20–50%. According to XRD and SEM results the occurrence of some clay agglomerates was detected for all nanocomposites. It could be stated that the existence of tactoids at high clay loadings signals a probable structural defect as the interfacial interactions between nanofillers and organic matrix become lower. As a result, the balanced distribution of the exerted stresses throughout the bulk of the nanocomposite is disrupted and an unfavorable impact is reflected on the ultimate strength. Possible hydrogen bonds formed between Nanomer® I.34MN clay and resin monomers maybe sustained better resistance against flexural stresses. Discacciati and Orié have suggested the frequent occurrence of larger tactoids in composites with nanoclay modified with vinyl groups, probably favored by an adhesion between particles. Although SEM pictures did not indicate relatively larger agglomerates for MMT-DMAODM, however a little possibility of forming such structural irregularities cannot be excluded, accounting for the lowest flexural strength value observed.

An experimental model of both flexural along with compression tests was selected in order to simulate occlusal forces growing on resin restorations in clinical practice. A combination of the high clay concentration together with a long (6 mm height) compression test specimen may comprise a limiting factor in the penetration of visible light radiation. In this manner, it was proven that the size of brittle materials constitutes an additional factor which could strongly affect the obtained values of compressive strength. The experimental data showed that the variant of clay organomodifiers have no significant effect on the strength values, even if Nanomer® I.34MN clay slightly increase the strength. A possible explanation is that the agglomerates of clay detected in three nanocomposites could similarly influence the light transmittance. A potential hydrogen bonding due to Nanomer® I.34MN could affect the ultimate strength.

Surface microhardness provides an indication of the nanocomposite resistance to scratching or abrasion. In addition, VHN is associated with the yield strength and finally with the capability of the tested region to absorb mechanical stresses. A softening effect appears when organic matrix is enriched with any type of clay. These results are almost in accordance with microhardness literature data when organomodified nanoclays were applied as new fillers for mechanical reinforcement of dental composites at a filler loading of 25%. Cross-linked networks involving MMT-DMAODM nanoparticles bonded through vinyl groups on the nanocomposite surface may be responsible for the better performance of the nanocomposite against abrasive forces. These phenomena rather dig up a correlation especially between flexural modulus and microhardness concerning nanocomposites with MMT-DMAODM. On the other hand, the lower VHN values observed for nanocomposites with Cloisite® 20A and Nanomer® I.34MN denote softer materials.

The surface roughness of dental restorative materials is an important factor for their esthetic appearance and longevity. Increased surface roughness is often associated with plaque formation, discoloration, gingival inflammation and solubility of the organic matrix due to the formation of acids by plaque. Measurements for organic matrix were also taken but, in the absence of clay
nanofillers, the polishing treatment led to non-uniform areas yielding random distribution of experimental values, and thus complicating the proper estimation of 

\[ \Delta E \]

values even at a large number of scanned regions (5×10 specimens). Polishing treatment of nanocomposites containing MMT-DMAODM led to smoother surfaces while those reinforced with Nanomer\textsuperscript{®} I.34MN seem to be more susceptible to biofilm growing or stain retention. The surface roughness measurements also verified the improving effect of MMT-DMAODM regarding the surface microhardness behavior, highlighting the significance of covalently bonded clay nanoparticles on the surface layers rendering them resistant to abrasive stresses. The estimation of the overall tendencies regarding the microhardness and surface roughness measurements denote a constant relation between both parameters for the total of the examined nanocomposites.

The importance of color stability in dental restorative materials due to esthetic reasons is reflected by many studies dedicated to the evaluation of color changes caused by discoloration factors, polishing and bleaching treatments etc\textsuperscript{49-52}. Several reports regarding restorative materials claim that color alteration is perceptible when \( \Delta E>1 \), clinical acceptable values are limited to \( \Delta E<3.3 \textsuperscript{53-55} \). According to \( \Delta E \) values reported in Table 3, it is shown that all nanocomposites exhibited perceptible color changes in each case. However, it is worthy to point out that composite sealant containing MMT-DMAODM maintained a \( \Delta E \) value (1.80) fairly lower than clinically accepted limit, even after a period of 1 week immersion in black tea. It is well known that clay aggregates might favor new pathways for water diffusion at the clay/polymer interfacial zones and hence increase water permeability\textsuperscript{56}. As it was ensured from SEM results dealing with nanocomposite having MMT-DMAODM, the relatively lower portion of agglomerates could better promote the resistance to water and its dissolved colorants’ diffusion on the surface of composite resin. On the other hand, the most hydrophobic Cloisite\textsuperscript{®} 20A is possibly more capable of hydrophobic interactions with polyphenolic compounds\textsuperscript{57} existed in black tea\textsuperscript{58} rather than MMT-DMAODM and Nanomer\textsuperscript{®} I.34MN, leading to a critical staining retention in the earlier stage of 72 h.

CONCLUSIONS

Pit and fissure sealant nanocomposites were successfully synthesized by adding 40% nanoclay, and subsequently characterized in terms of their morphology and ultimate properties. According to XRD and SEM observations, the structures of the obtained nanocomposites were mostly governed by intercalated regions along with some OMMT agglomerates. Sealants containing MMT-DMAODM with methacrylated groups exhibited higher mechanical properties such as remarkable flexural modulus, and microhardness in comparison to the other studied nanocomposites, as they were characterized by a better dispersion of clay into the polymer matrix. A possible formation of hydrogen bonds between Nanomer\textsuperscript{®} I.34MN and monomers was capable of sustaining mainly flexural parameters and compressive strength. Furthermore, the sealant nanocomposites enhanced with MMT-DMAODM seemed to be less susceptible to plaque formation and discoloration yielding smoother surfaces after polishing treatment, as well as clinically accepted color changes even after 1 week aging in black tea. It can be postulated that vinyl functionality of MMT-DMAODM clay nanofillers might have an efficient impact on the morphology and final properties of sealant nanocomposites, due to the potential of covalent bonding of clay with monomers via a co-polymerization process. In this way, a probable further reinforcement of the polymer network not only into the bulk but also on the surface of the corresponding nanocomposite can be easier achieved, rather than by incorporating either the hydroxylated Nanomer\textsuperscript{®} I.34MN or the most hydrophobic Cloisite\textsuperscript{®} 20A.

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