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The effects of adding fluorescent carbon nanoparticles on various mechanical properties of denture liners

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The aim of this *in vitro* study was to evaluate the effects of incorporating fluorescent carbon nanoparticles (FCNs) on the hardness, tear, and tensile bond strength of an acrylic-based tissue conditioner and a silicone-based soft denture liner. FCNs added to an acrylic-based tissue conditioner (Viscogel, Dentsply; Group V) and a silicone-based soft denture liner (Ufigel P, Voco; Group U) were divided into subgroups according to the concentrations (Group 0: no water, Group 1: with only water, Group 2: 0.5% FCNs, Group 3: 1% FCNs and Group 4: 10% FCNs) (n=10/per group). Shore A hardness, tear, and tensile bond strength tests were performed. Significant decreases occurred in Groups U2, U3, and U4 compared to the control groups (Groups U0 and U1) in the tear and tensile bond strength test parameters (p<0.025). However, in both types of the tested materials, there were no statistically significant differences among the shore A hardness test results (p>0.025).

Keywords: Fluorescent carbon nanoparticle, Denture liner, Tensile bond strength, Tear strength, Hardness

INTRODUCTION

Tissue conditioners and soft denture liners are often preferred for the rehabilitation of edentulous patients suffering from the pain that stems from traumatized oral mucosa[1]. These materials can reduce the stresses on denture-bearing areas by absorbing the chewing forces of the prosthesis during function[2-4].

Tissue conditioners and soft denture liners are sometimes categorized according to the duration as short and long-term[3]. However, they are generally categorized according to the chemical type: acrylic resin-based and silicone-based[1,5]. Acrylic based soft denture liners are commonly used as tissue conditioners for a short periods up to seven days to facilitate the healing process of inflammatory, hypertrophic, hyperemic and/or traumatized oral tissues[4,6]. Silicone-based soft denture liners are used in clinical situations, such as inadequate tissue and crest support, bone undercuts, bruxism, the presence of thin and non-resilient mucosa and congenital or acquired defects, for longer periods of 1–3 years[7-9].

The essential properties of tissue conditioners and soft denture liners are good dimensional stability, sufficient tear resistance, permanent softness, viscoelasticity, ease of cleaning, color stability, strong bond to denture base material, low water sorption and solubility, and high biocompatibility[1,3]. However, there is no ideal tissue conditioner or silicone-based soft denture liner, many experience problems such as bond failures to denture base, accumulation of oral microorganism, poor tear strength, and hardness changes[5,10]. To adequately perform their function, these materials should remain bonded to the acrylic denture bases[2]. Protecting against the formation of biofilm is another important issue[5]. The treatment of oral stomatitis requires frequently denture replacement and topical and systemic antifungal drugs[11,12]; however, saliva flow or the lack of patient compliance sometimes render such drug ineffective[13,14]. To extend the clinical longevity of tissue conditioners and soft denture liners and diminish the possibility of biofilm formation, some researchers have incorporated antifungal or antimicrobial agents in these materials, such as silver nanoparticles[2,7,10].

Nanomaterials, as defined by the American Society for Testing and Materials (ASTM), refer to materials 1–100 nm long[15]. These nanoparticles are classified as (1) organic nanomaterials-based on carbon (fullerenes, graphenes, carbon nanotubes *etc.*), (2) metal nanoparticles (gold colloids, nanorods *etc.*), and (3) semiconductor-based nanoparticles (quantum dots *etc.*)[16].

Fluorescent carbon nanoparticles (FCNs) were first discovered in 2004, and they became a new member of the carbon-based nanomaterials class[17]. Recently, FCNs also known as 'carbon nanodots' have been used as an alternatives to inorganic dyes and heavy metal-based quantum dots. This is due to their biocompatibility, low cytotoxicity, photostability, chemical inertness, high water solubility and high photoluminescence behavior, which are essential for their further use in biological application, including biosensors, medical diagnosis, gene transmission, drup delivery and florescence labeling[18-23]. Nanoparticles can function in a therapeutic way without causing drug resistance by following a mechanism different from traditional antibiotics[20]. Carbon based nanomaterilas have demonstrated as a new class of photoactivated antimicrobial agents[20,22].

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The *in vitro*/*in vivo* antioxidant activities of FCNs have many biological applications were previously reported[20,24]. In addition to bacterial identification, developing new strategies to fight against bacterial infection and drug resistance is also important[22]. FCNs contains hydroxyls, aminos and carbony and carboxylate groups on their surfaces for effective surface passivation[25]. These functional groups give rise to synergistic anti-inflammatory effects[23]. Surface charges on FNCs also play an important roles in the growth of bacteria and exhibit antibacterial activity against Gram-positive bacteria and Gram-negative bacteria[26]. Li *et al.*[25] reported that FCNs have unique antioxidant capacites as demostrated by their free-radical scavenging activities. In addition to antioxidant properties, FCNs show antibacterial activities not only in loading antibacterial drugs, but also in immobilizing bacteria on their surfaces[27].

Previous studies have reported that incorporating antioxidants into tissue conditioners and soft denture liners can affect their mechanical and structural properties[4,28]. Today, the addition of plants to biomaterials for medical purposes might be an alternative route of natural origin[6,29], however, only metals and pharmaceutical or herbal ingredients have been added into denture liners so far[11,12,29-31]. No studies on the addition of naturally sourced nanoparticles are available *via* green synthesis. Licorice, however, is a naturally and rich carbon source for the green synthesis of FCN, and it has an antioxidant properties[24]. Also, although previous studies have reported the antibacterial properties of FCNs, an exploration of FCN usage in dental materials is still needed[26,27]. In the light of these gaps in the literature and the advantages of FCNs, we are motivated to explore the mechanical effects of adding FCNs derived from licorice to tissue conditioners and soft denture liners. The aim of this *in vitro* study was to evaluate the effects of incorporating FCNs on the hardness, tear, and tensile bond strength of an acrylic-based tissue conditioner and a silicone-based soft denture liner. The null hypothesis is that the tear strength, tensile bond strength, and hardness of an acrylic-based tissue conditioner and a silicone-based soft denture liner depend on the concentration of added FCNs.

MATERIALS AND METHODS

Preparation and characterization of FCNs

Licorice root extract-based FCNs were synthesized through thermal treatment at 250°C following the method reported by Alas and Genc[24]. Only 1 g of hot water extract of rootstock was collected in an ethanol/water solution (1:2 v/v) and 5 mL of the extract was then mixed with 2 mL of 1:1 water/ethanol solution in a Teflon oven vessel (approximately 20 mL inner volume). The resulting mixture was carbonized for 30 min at 250°C and then dissolved in 6 mL of MilliQ-water (Millipore, Bedford, MA, USA). The obtained suspension was then centrifuged at 6,000 rpm for 20 min, and the supernatant was taken. The final product was vacuum-dried (BINDER, Tuttlingen, Germany) at 90°C for 12 h. The size and surface zeta potentials of FCNs were measured using a Nanosizer/Zetasizer Nano-ZSZEN 3600 (Malvern Instruments, Worcestershire, UK).

Sample fabrication protocols

Table 1 presents the materials tested in this study, and Table 2 presents the percentages of FCN in the subgroups.

Soft denture liner and FCN mixture

The catalyst and base of the soft denture liner (Group U) were mixed in a 1:1 ratio (w/w) for 30 s according to the manufacturer's recommendations. The test specimens in Group U0 and U1 consisted of soft denture liners (Ufi Gel P, VOCO, Cuxhaven, Germany) without added FCNs (Table 2).

To prepare the Group U subgroups, FCNs were incorporated into the catalyst and base during the mixture process at the concentrations of 0.5%, 1% and 10% by weight, respectively. After all the materials were mixed, they were applied to a round, stainless steel mold to ensure uniform size. The mixture was sandwiched between glass slides with surface roughnesses of about 0.009 μm under a constant vertical load of 9.8 N until it solidified. The test specimens were allowed to autopolymerize at room temperature (22±2°C) for

Table 1 The tested materials used in this study

Tested materials	The codes of tested groups	Main composition	Manufacturer	Lot number
Autopolymerized acrylic based tissue conditioner (Visco-gel)	Group V	Powder: polyethyl methacrylate Liquid: ethanol phthalyl butyl glycolate, dibutyl phthalate	Dentsply DeTrey, Konstanz, Germany	1610000172
Autopolymerized silicone based soft denture liner (Ufi Gel P)	Group U	Polydimethylsiloxane and platinum catalyst	Voco, Cuxhaven, Germany	1645226
Conventional heat-polymerized PMMA resin (Meliodent)	—	Powder: polymethyl methacrylate Liquid: methyl methacrylate, ethylene glycol dimethacrylate	Heraeus Kulzer, Hanau, Germany	R010023

Table 2 The classification of the test groups according to the mixture ratios

Test groups	FCN addition ratios (%)	Mixture ratios
Group U0	0	0.1 g base, 0.1 g catalyst
Group U1	0 (water addition without FCN)	40 μL water, 0.1 g base, 0.1 g catalyst
Group U2	0.5	30 μL water, 10 μL FCN, 0.1 g base, 0.1 g catalyst
Group U3	1	20 μL water, 20 μL FCN, 0.1 g base, 0.1 g catalyst
Group U4	10	0 μL water, 200 μL FCN, 0.1 g base, 0.1 g catalyst
Group V0	0	0.2 g powder, 146.6 μL liquid
Group V1	0 (water addition without FCN)	40 μL water, 0.2 g powder, 146.6 μL liquid
Group V2	0.5	30 μL water, 10 μL FCN, 0.2 g powder, 146.6 μL liquid
Group V3	1	20 μL water, 20 μL FCN, 0.2 g powder, 146.6 μL liquid
Group V4	10	0 μL water, 200 μL FCN, 0.2 g powder, 146.6 μL liquid

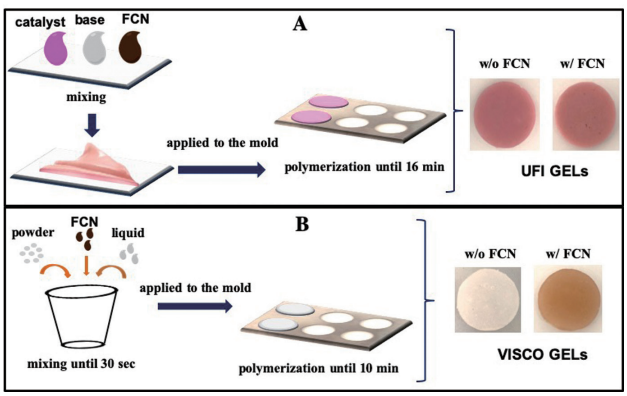


Fig. 1 Schematic representation of preparation procedure for (a) Group U (Soft denture liner and FCN mixture and (b) Group V (Tissue conditioner and FCN mixture).

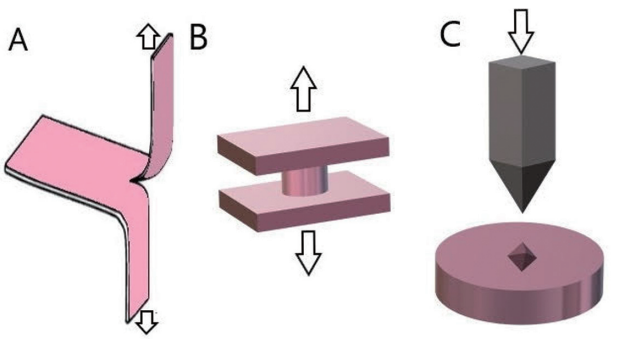


Fig. 2 Schematic illustrations of test methods. A: Tear strength test (denture liners: 50×10×1 mm), B: Tensile bond strength test (denture liners: 10 mm Ø×3 mm; acrylic denture base plates: 25×25×3 mm), C: Shore A hardness test (denture liners: 35 mm Ø×6 mm)

approximately 16 min (Fig. 1).

Tissue conditioner and FCN mixture

Tissue conditioner liquid was added to the powder and mixed for 30 s a powder/liquid ratio designated by the manufacturer's instructions. The test specimens in Groups V0 and V1 consisted of tissue conditioner (Viscogel, Dentsply DeTrey, Konstanz, Germany) without added FCNs (Table 2).

To prepare the subgroups of Group V, FCN was incorporated into the liquid of tissue conditioner at the concentration ranging from 0.5%, 1% and 10% respectively by weight. Then, the mixture was homogenized in a sterile glass beaker. After that, the modified liquid was mixed with powder, and test specimens were processed as described above. The test specimens were allowed to autopolymerize at room temperature (22±2°C) for approximately 10 min (Fig. 1).

Fourier-transformed infrared spectroscopy

The FCNs incorporated into surface functional groups were investigated by a Fourier-transformed infrared spectrometer (Perkin Elmer Frontier, Waltham, MA, USA). FCN-embedded test samples were analyzed in the frequency range of 450–4,000 cm^{-1} with a 4-cm^{-1} resolution.

Tear strength test

Fifty trouser leg–shaped test specimens per tested materials (total 100) were prepared (50×10×1 mm). They were cut (25 mm long) with a blade (#15) to form the trouser leg shapes. The 50 specimens per tested materials were divided into five subgroups (n=10/per group). Their legs of them were placed vertically in opposite directions. Mechanical testing (tear strength tests) was performed on a universal testing machine (Shimadzu AGS-X, Kyoto, Japan) at a crosshead speed of 50 mm/min (Fig. 2A).



Fig. 3 Tensile bond strength test set up.

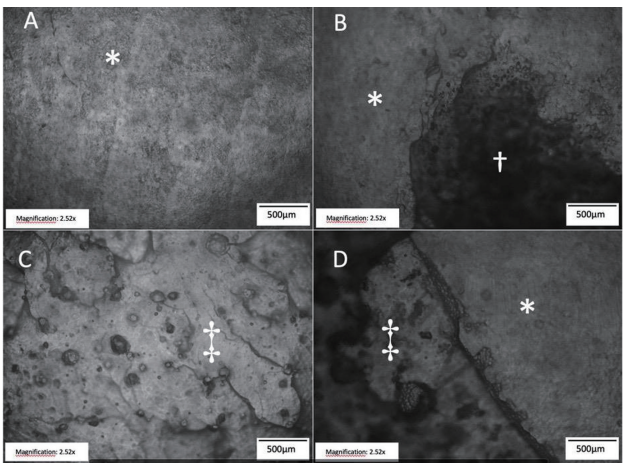


Fig. 4 Stereomicroscopic examination of bond failure, * heat-polymerized PMMA resin, † silicone-based soft denture liner, ‡ acrylic-based tissue conditioner. A: Adhesive failure type of Group U, B: Mixed failure type of Group U, C: Cohesive failure type of Group V, D: Mixed failure type of Group V

Tensile bond strength test

The tensile bond strength of the tested materials to the denture base material was measured by the method presented in ISO 10139-2[32]. Conventional heat-polymerized PMMA resin (Meliodent, Heraeus Kulzer, Hanau, Germany) plates (25×25×3 mm) were prepared by the method recommended by the manufacturer. The plates were ground using abrasive papers (200 and 400 grit) to standardize their surface. They were then rinsed and dried with air for 15 s. In groups U0, U1, U2, U3, and U4, soft denture liner adhesive was applied with a brush on the PMMA resin plates before test specimens were prepared. A polyethylene ring (10 mm internal diameter and 3 mm thickness) was placed in the middle of the plate. The mixed soft denture liner was manually mixed and placed into the ring; then, a second acrylic plate was then placed over the test material. In total, 100 test specimens were made from each test material (n=10/per group). In groups V0, V1, V2, V3 and V4, the test material was applied directly to the surface of the acrylic plates without any adhesive before being mounted on the testing machine (Shimadzu AGS-X). The tensile bond strength tests were performed at a cross-head speed of 10 mm/min (Figs. 2B and 3). The tensile bond strength (MPa) was calculated according to the following formula:

$$\text{Tensile bond strength}=F_{max}/A$$

where F_{max} is the maximum force (N) recorded during debonding, and A is the bonding surface area between the tested materials and PMMA resin (mm^2). Failure types were also evaluated under a stereomicroscope (U-DA, Olympus, Tokyo, Japan) at ×2.52 to determine the failure mode of each test specimen according to following evaluation method (Fig. 4): adhesive (no test material was left on the denture base) (Fig. 4A), mixed (less than 50% of test material left on the denture base) (Figs. 4B and D) or cohesive (more than 50% of test material left on PMMA resin) (Fig. 4C).

Shore A hardness test

The measurements were made according to ISO 10139-2[32] using a shore A durometer (Zwick, Roell, Germany) at 50-gf loading. Three test specimens (35 mm in diameter and 6 mm in thickness) were created for each test group. The hardness was measured immediately after 24 h of aging in distilled water at 37±1°C without any dehydration or drying process. Five measurement points at least 5 mm far from the edges were used to measure the hardness of test specimens (Fig. 2C). No measurements were made closer than 2 mm to a previous one. The mean hardness values of each specimen group were obtained as shore A hardness value.

SEM analyses

Two specimens were randomly selected from the tested groups, the surfaces were air dried and were coated in a gold-palladium coating unit (Quorum Q 150 R ES DC Sputter, Kent, UK). The specimens were evaluated at ×50,000, ×100,000, and ×200,000 magnifications SEM (Zeiss, SUPRA-55, Carl Zeiss NTS, Oberkochen, Germany) (Figs. 5 and 6).

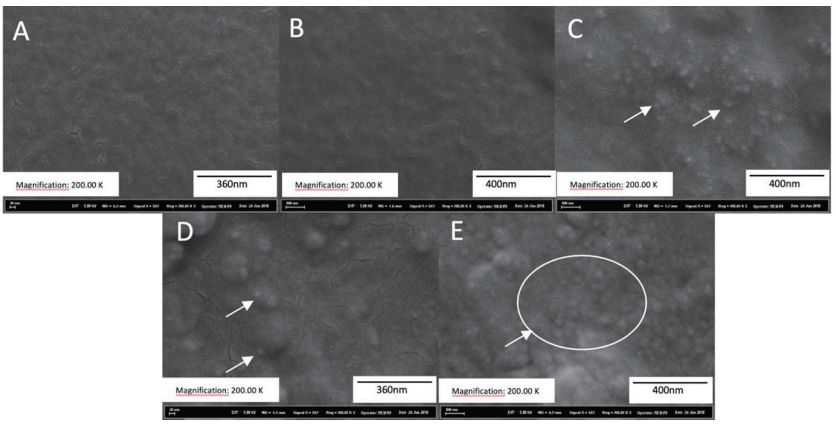


Fig. 5 Representative SEM images of the tested materials.
A: SEM image of Group U0 (control group without FCN), B: SEM image of Group U1 (incorporated with water without FCN), C: SEM image of Group U2 (incorporated with water and 0.5% FCN) (white arrow shows FCN), D: SEM image of Group U3 (incorporated with water and 1% FCN) (white arrow shows FCN), E: SEM image of Group U4 (incorporated with water and 10% FCN) (white arrow and circle show FCN accumulation)

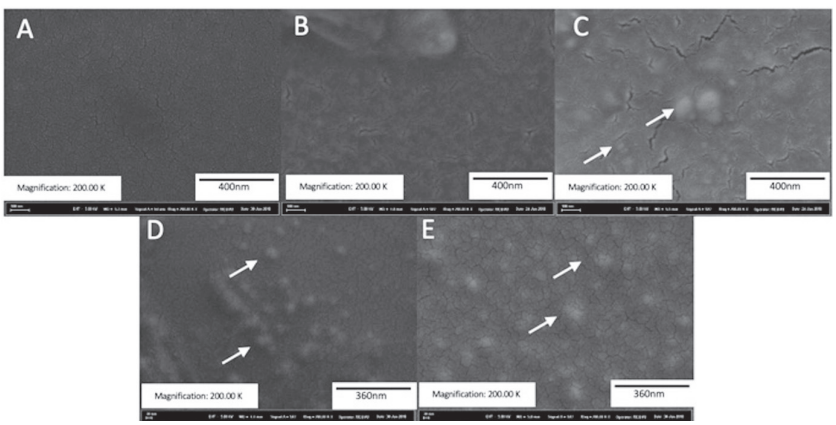


Fig. 6 Representative SEM images of the tested materials.
A: SEM image of Group V0 (control group without FCN), B: SEM image of Group V1 (incorporated with water without FCN), C: SEM image of Group V2 (incorporated with water and 0.5% FCN) (white arrow shows FCN), D: SEM image of Group V3 (incorporated with water and 1% FCN) (white arrow shows FCN), E: SEM image of Group V4 (incorporated with water and 10% FCN) (white arrow shows FCN)

Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 17.0 software (IBM, Armonk, NY, USA). To discover whether the distributions of continuous variables were normal or not a Kolmogorov Smirnov test was applied. A Levene test was used to the evaluate the homogeneity of variances. The data were shown as mean±standard deviation (SD) or median interquartile range (IQR), where applicable. While the mean differences between groups were compared with a Student's t test, a Mann-Whitney U test was applied to compare the non-normally distributed data. When the number of independent groups was more than two, data analyses were performed using one-way analysis of variance (ANOVA) or a Kruskal-Wallis test, where appropriate. Given statistically significant differences, a *post-hoc* Tukey HSD or Conover's multiple comparison test was used to discover which group differed from the others. Values of p less than 0.05 were considered statistically significant. For all possible multiple comparisons, Bonferroni correction was applied to control type I errors.

RESULTS

Characteristics of FCNs and FCN-embedded test materials

Dynamic light scattering (DLS; Zetasizer Nano-ZS, Malvern Instruments, Malvern, UK) measurements of FCNs dispersed in distillated water indicated that hydrodynamic nanoparticle size was in the range of 15–30 nm with a surface charge of −26.3±1.9 mV. Changes in the surface properties of FCN-embedded test specimens were then evaluated at increasing concentrations of nanoparticles using Fourier transform infra-red spectrophotometer (FTIR). As demonstrated in Fig. 7, the presence of FCNs in the test specimens did not affect or change any of the surface functional groups even at FCN concentrations as high as 10% (w/w).

Tear strength test

In all the test groups, the median tear strength values of Group U were statistically higher than Group V (p<0.001) (Table 3).

In Group V, the tear strength values decreased with increasing concentrations of FCN, but the differences between the concentrations were not significant (p=0.028). While Group V0 (control) (0.57±0.18 N) had the highest tear strength value, Group V3 (0.39±0.15 N) had the lowest.

In Group U, the tear strength results of FCN-embedded subgroups indicated a statistically significant decrease, as compared to Group U0 (control) (p<0.025). The addition of FCNs at 0.5%, 1% and 10% concentrations resulted in statistically non-significant differences when compared to each other (p>0.025). Additionally, Group U4 (10%) indicated a statistically significant decrease in tear strength when compared to Group U1 (0%, control) (p=0.023). Group U0 (control) (4.01±1.32 N) had the highest tear strength value, while Group U4 (2.81±0.61 N) had the lowest.

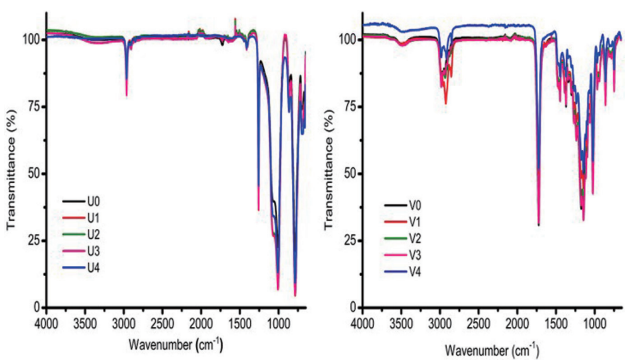


Fig. 7 FTIR spectrum of test specimens.
a) Group U (soft denture liner) and b) Group V (tissue conditioner)

Tensile bond strength test

Of all of the test groups, the median tensile strength values of Group U were higher than Group V (Table 4). In Group 0 (control), Group 1 (0%) and Group 4 (10%), the median tensile bond strengths of Group U were statistically higher than Group V (p<0.001).

In Group V2 (0.5%) and Group V4 (10%), tensile bond strength increased after FCN addition when compared to Group 0 (control), but the difference was not statistically significant (p=0.264). Group V4 (0.63±0.16 MPa) had the highest tensile bond strength value, while Group V1 (0.46±0.19 MPa) had the lowest.

In all of the subgroups of Group U, the addition of FCNs decreased tensile bond strength compared to the control group. The addition of FCN at 0.5%, 1% and 10% concentrations resulted in a statistically significant decreases in tensile bond strength when compared to Group U0 (control). In addition, Group U3 (1%) and Group U4 (10%) resulted in statistically significant decreases in tensile bond strength when compared to Group U1 (0%) (p<0.001). Group U0 (control) (1.84±0.60 MPa) had the highest tensile bond strength value, while Group U4 (1.02±0.27 MPa) had the lowest.

Table 3 Mean values and standard deviations of tear strength of the tested materials

	Group V	Group U	p-value †
Group 0 (control)	0.57 (0.18)	4.01 (1.32)^{a,b,c}	<0.001
Group 1 (0%)	0.55 (0.27)	3.39 (0.82)^{d}	<0.001
Group 2 (0.5%)	0.44 (0.18)	2.93 (0.76)^{a}	<0.001
Group 3 (1%)	0.39 (0.15)	3.25 (0.65)^{b}	<0.001
Group 4 (10%)	0.45 (0.10)	2.81 (0.61)^{c,d}	<0.001
p-value ‡	0.028	0.003	—

The data have been shown in median (interquartile range), † the comparisons among the tested materials according to the FCN concentration added, Mann Whitney U test, the results were accepted as statistically significant for p<0.010 according to the Bonferroni Correction, ‡ the comparisons among the FCN concentrations for each tested material, Kruskal Wallis test, the results were accepted as statistically significant for p<0.025 according to the Bonferroni Correction; The same superscript letters indicate statistically significant differences between the stated groups; a: Group 0 (control) *vs* 2 (p<0.001), b: Group 0 (control) *vs* 3 (p=0.004), c: Group 0 (control) *vs* 4 (p<0.001), d: Group 1 *vs* 4 (p=0.023).

Table 4 Mean values and standard deviations of tensile bond strength of the tested materials

	Group V	Group U	*p*-value †
Group 0 (control)	0.57 (0.17)	1.84 (0.60)[a,b,c]	<0.001
Group 1 (0%)	0.46 (0.19)	1.50 (0.44)[d,e]	<0.001
Group 2 (0.5%)	0.63 (0.16)	1.27 (0.97)[a]	0.075
Group 3 (1%)	0.57 (0.26)	1.03 (0.43)[b,d]	0.015
Group 4 (10%)	0.62 (0.25)	1.02 (0.27)[c,e]	<0.001
p-value ‡	0.264	<0.001	—

The data have been shown in median (interquartile range), † the comparisons among the tested materials according to the FCN concentration added, Mann Whitney *U* test, the results were accepted as statistically significant for $p<0.010$ according to the Bonferroni Correction, ‡ the comparisons among the FCN concentrations for each tested material, Kruskal Wallis test, the results were accepted as statistically significant for $p<0.025$ according to the Bonferroni Correction; The same superscript letters indicate statistically significant differences between the stated groups; a: Group 0 (control) *vs* 2 ($p<0.001$), b: Group 0 (control) *vs* 3 ($p<0.001$), c: Group 0 (control) *vs* 4 ($p<0.001$), d: Group 1 *vs* 3 ($p<0.001$), e: Group 1 vs 4 ($p<0.001$).

Table 5 Mean values and standard deviations of shore A hardness of the tested materials

	Group V	Group U	*p*-value †
Group 0 (control)	14.20 (5.30)	26.40 (1.00)	0.100
Group 1 (0%)	12.10 (3.10)	23.10 (0.90)	0.100
Group 2 (0.5%)	15.00 (4.00)	20.50 (0.90)	0.100
Group 3 (1%)	12.40 (1.30)	21.00 (2.00)	0.100
Group 4 (10%)	10.70 (0.40)	21.20 (1.60)	0.100
p-value ‡	0.276	0.027	—

The data have been shown in median (interquartile range), † the comparisons among the tested materials according to the FCN concentration added, Mann Whitney *U* test, the results were accepted as statistically significant for $p<0.010$ according to the Bonferroni Correction, ‡ the comparisons among the FCN concentrations for each tested material, Kruskal Wallis test, the results were accepted as statistically significant for $p<0.025$ according to the Bonferroni Correction.

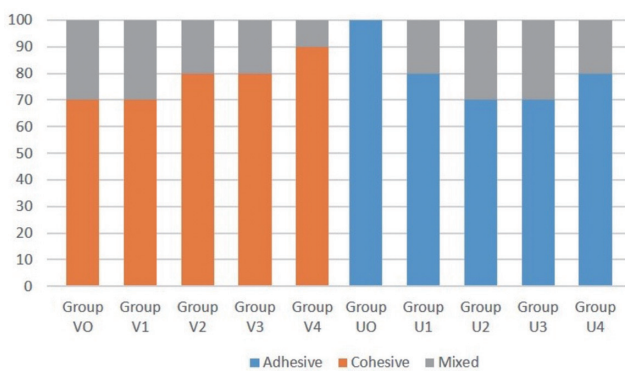


Fig. 8 Failure percentage (%) of test specimens for the tensile bond strength test.

Failure mode analysis

The analysis revealed adhesive failures in Group U0 (control) (100%). Mixed failures were observed in the FCN-embedded subgroups of Group U. Group V0 (control) indicated cohesive type of failures (70%), while the FCN-embedded subgroups of Group V displayed mixed type of failures (Group V1: 30%, Group V2: 20%, Group V3: 20%, Group V4: 10%) (Fig. 8).

In Group U0 (control group), adhesive failure was the dominant failure type observed (100%). Mixed failure was also seen at higher FCN concentrations (30%). For Group V0 (control group), cohesive failure (70%) and mixed failure (30%) dominated.

Shore A hardness

In all test groups, the median shore A hardness values of Group U were higher than Group V, but the difference was not statistically significant ($p=0.100$) (Table 5).

In Group V, the shore A hardness value decreased following the addition of FCN at different concentrations except Group V2; however, the difference was not significant ($p=0.276$). Group V0 (control) (14.20±5.30) had the highest shore A hardness value, while Group V4 (10.70±0.40) had the lowest.

In Group U, the shore A hardness decreased with the addition of FCN when compared to Group U0 (control), but the difference was not significant ($p=0.027$). Group

U0 (control) (26.40±1.00) had the highest shore A hardness value while Group U2 (20.50±0.90) had the lowest.

SEM analysis

Figures 4 and 5 show the morphological analysis of the test groups by SEM imaging. In both denture liner types, higher concentrations of FCNs could be identified by their clear appearance. Particle sizes measured from the images were similar sizes (~15–20 nm) as measured by DLS. The particles were spreaded homogenously in both denture liner specimens, and the test groups exhibited no differences (except Group U4) (Fig. 5E). Fractures were not observed in any tested materials, even at the highest concentration of FCNs (Figs. 5 and 6) .

DISCUSSION

The biocompatibility, antibiofilm and antimicrobial properties of denture liners are important for their clinical usage[3]. Some studies have aimed to increase liners' antibacterial properties without undermining their mechanical properties[4,10,33]. However, it is quite difficult to optimize all their clinical parameters (such as biocompatibility, mechanical properties and physical properties). Most papers reported denture liners were used with several metallic nanoparticles such as silver nanoparticles, to prevent microbial accumulation[4,7]. The search is still on, however, for materials with high biocompatibility and simple production methods *via* advancements in nanotechnology. A variety of carbon sources have been used to prepare FCNs[26]. Different plant extracts and natural sources such as carob molasses, astragalus extract, licorice, and honey, can be used to produce them[24,26]. An important advange of licorice root-derived FCNs used in our study, however, is their natural origin, since FCN synthesis from any carbon source without needing complex instruments is their greatest advantage over other carbon-based nanomaterials[24]. The thermal production method used in our study was also quite simple and economical and eliminates multistep synthesis[26].

FCNs are known for their antioxidant properties[22,26,34]. The new challenge is balancing gains in their antibiofilm and antimicrobial properties while preserving the compliance of the mechanical properties of liners under clinical usage conditions with ISO standards. To achieve these gains, new modified structures by the incorporation of antioxidant agents should be tested that do not present detrimental effects on the structural, physical, and mechanical properties of tissue conditioners and soft denture liners. For this reason, our study evaluated the effects of incorporating licorice root–derived FCNs, not metal elements or pure plants, on the hardness, tear, and tensile bond strengths of an acrylic-based tissue conditioner and a silicone-based soft denture liner.

During clinical usage, tissue conditioners and soft denture liners are exposed to forces that initiate tear and/or debonding. The debonding of these materials results in non-hygienic and non-functional surfaces. Therefore, satisfactory adhesion to the denture base of acrylic resin is critical importance to prevent failures at the bonding interfaces[5,35,36]. The hardness properties of these materials could also change during clinical use, which might render them more susceptible to absorbing the occlusal forces[2]. In the case of cohesive debonding, the tear strengths of tissue conditioners and soft denture liners stands out as a critical and controlling factors[37]. Tear resistance also affects the clinical performance of tissue conditioners and soft denture liners. Chemical and mechanical cleaning procedures could initiate tearing[3,38]. Dootz *et al.*[39] showed that the tear strength of acrylic-based tissue conditioner was higher than silicone-based soft denture liner. In contrast, the tear strength results of the present study were similar with a previous study[38], that is, that silicone-based soft denture liner had higher tear resistance than acrylic-based tissue conditioner. Group U indicated statistically significantly higher tear strength results than Group V ($p<0.001$). Similar to the tensile bond strength results, the tear strength of Group U was significantly weaker with added FCNs than the control group ($p<0.025$). Indeed, the control group indicated the highest value (4.01±1.32 N/mm), while there were no statistically significant differences among the subgroups of Group V ($p=0.028$). These results might be related to the structural-deterioration or porosity of the FCN-embedded tested materials, as was the case in the tensile bond strength tests[2].

No significant differences were observed between the tested and control groups in the shore A hardness parameter and among all the Group V subgroups in the tear and tensile bond strength parameters. Therefore, the null hypothesis that the incorporation of FCNs affects the mechanical and physical properties of tissue conditioners and soft denture liners depending on concentration was partially accepted. On the other hand, the addition of FCNs resulted in a statistically significant decrease in the tear and tensile bond strength values of Group U.

Previous studies have also demonstrated the mechanical weakening effect of antimicrobial additives were incorporated into the tissue conditioners or soft denture liners[4,13]. The methodological steps of *in vitro* studies may have a critical effects on these results, however. FCNs have a very strong aggregating tendency[40]. Increasing the number and size of nanoparticles leads to aggregation, without which is difficult to obtain a homogenous dispersions of filler, as it reduces the surface areas and thus the effectiveness of the nanoparticles[10,41]. Water-soluble FCNs also have minimum cytotoxicity[40]. Finally, FCN agglomerates easily form solids, but it remains isolated in water. In all of the test groups, FCNs were dissolved in water. In the present study, however, SEM images revealed the homogeneous dispersion of FCNs in the matrices of the tissue conditioner and soft denture liner (Figs. 4 and 5). Demonstrating that water was an appropriate solution for dispersing the FCNs, and did not affect the unity of the resulting denture liners. The presence of FCNs in the

test specimens did not affect or change any of the surface functional groups (Fig. 7). Further, the interaction between the FCNs added at changing concentrations and the tissue conditioner and soft denture liner specimens prepared was evaluated with FT-IR spectroscopy. As depicted in Fig. 7, FT-IR spectra of both specimens did not exhibit shifting in peak positions and revealed a small change in the intensity of peaks at increased FCN concentrations revealing an increased number of functional groups, but the change observed was not significant. These results showed that FCN integration to the tissue conditioner and soft dentures prepared did not reveal any change in the hybridization state or electron distribution of the materials, which attributed to a poor chemical interaction between the FCNs and the dental materials prepared[42,43].

Weak bonds are problems in tissue conditioners and soft denture liners, as they enable bacterial growth at interfaces with the resin bases and hinder prosthesis cleaning and thus contributing to the patient discomfort[2,35]. The tensile bond strengths of Group U2, U3 and U4 in this study were weaker than the control group's. The mean bond strength observed in the Group U subgroups was similar to the values reported by Mutluay and Ruyter[44] and Lassila *et al.*[45]. A significant decrease was observed in the tensile bond strength as the percentage of FCNs increased. The failure type become adhesive in Group U and cohesive in Group V (Fig. 8). This result can be explained by the differences in chemical composition of the tested denture liners. The failure type of Group V was generally cohesive because of the chemical similarity between tissue conditioner that contain acrylic polymers and acrylic based denture base material. In contrast, the failure type of Group U was generally adhesive. Similarly, this result can also be explained with different chemical composition between silicone based denture liner and acrylic based denture base material. This causes that the weakest area of bonding surface was the interface of bonding area and results in adhesive failure.

Additionally, failure types transitioned from adhesive to mixed as FCNs were added (Fig. 8). These results suggest that the FCN addition could lead to structural deterioration. Decreased values after FCN addition may also be related to the particle size, resulting in matrix fragility and porosity of the modified material[2]. However, the tensile bond strength results for the tissue conditioner and soft denture liner should be higher than 1 MPa according to the ISO standard for clinically acceptable usage[32], and in the present study, although decreased tensile bond strength values were obtained after FCN addition, they were still higher than 1 MPa. Therefore, all the FCN concentrations fulfilled the ISO standard requirement, presenting values within the clinically acceptable limits. The bonding between denture base materials and the tissue conditioner or soft denture liner depends on the diffusion or penetration of the monomers into the denture base material[35]. Thus, when the FCN addition ratio increased, the surface area contact of the tissue conditioner or soft denture liner contacted with the acrylic denture base material was reduced. This may also result in decreased tensile bond strength values.

Among various chemical compositions of tissue conditioner and soft denture liners, silicone-based soft denture liners have been widely used because of their high clinical performance. They remain more stabler over long periods of time, while acrylic-based tissue conditioner lose cushioning effect over time[36], so the mechanical and physical comparison of these materials results, in different values depending on the chemical structure (Tables 3, 4, and 5). The acrylic-based tissue conditioner (Group V) displayed nonsignificantly lower surface hardness than the silicone-based soft denture liner (Group U) (p=0.100). Although the differences were nonsignificant, this result can be explained by the differences in chemical composition of the tested materials. The acrylic-based tissue conditioner (Group V) contained acrylic polymers, plasticizers and a methyl methacrylate-based monomer. These components directly affected the softness of the material. The loss of plasticizers (an aromatic ester) and alcohol from tissue conditioner can decrease its viscoelasticity and capacity to absorb impact[46]. The silicone-based soft denture liner (Group U) contained dimethylsiloxane and polydimethylsiloxane, so plasticizers are not necessary to provide the desired softness[28].

A major complication associated with the use of tissue conditioner and soft denture liner is the change in hardness over time. As the hardness of denture liners increases, their ability to absorb the chewing forces decreases[41,47]. Shore A hardness, a metric for the softness of the lining materials. The shore A hardness of long term soft denture liner (silicone-based soft denture liner; Group U) after 24 h of aging in distilled water at 37°C for soft and extra-soft materials should range from 25–50 units according to the ISO standards[32]. Despite a slight decrease, no statistically significant difference was obtained with different FCN additional rates in tissue conditioner and soft denture liner (Group V, p=0.276; Group U, p=0.027; respectively). The hardness values of all subgroups in Group U presented lower hardness values than ISO standards (25–50 units). In Group U, the hardness decreased with the increasing concentration of FCN. In the concentrations from 0.5% to 10%, the hardness values were lower than 25 units Shore A; therefore, these materials did not conform to the requirements of the ISO standard for soft denture liners. Additionally, this findings could be associated with the period from the end of curing to measurement[41]. It would be more accurate to evaluate the change in hardness values with different addition rates instead of numerical hardness values. Chladek *et al.*[41] reported that it is difficult to assess the effects of the material composition on the hardness changes. Furthermore, Mancuso *et al.*[28] reported that aging resulted in a significant differences in the hardness values of silicone-based soft denture liner and acrylic-based tissue conditioner. However, in our study, test parameters were not evaluated after aging procedure. The limitation of this study was that

the effects of thermocycling were not tested. For this reason, this *in vitro* study's results for tear strength, tensile bond strength, and hardness test results might be a guide for future studies.

From a clinical standpoint, the denture liners with added FCNs could be alternatives to the tested soft denture liner and tissue conditioner because of their clinically acceptable mechanical properties, except the tear and tensile bond strengths of Group U. Nonetheless, to ensure clinical success, their other mechanical and physical properties, such as surface roughness, color change, and water absorption/solubility, should be investigated. Additionally, the use of new forms of FCNs on antibiofilm efficacy and the mechanical and physical properties of denture liners might be a promising research topic.

CONCLUSION

This *in vitro* study revealed that incorporating licorice root extract-based FCNs, which are known for their antioxidant and antibacterial properties, into acrylic-based tissue conditioner had no adverse effects on their mechanical properties. A silicone-based soft denture liner, however, was susceptible to FCN addition, which led to decreased tear strength and tensile bond strength values —however, these lower values were still within clinically acceptable limits. The fact that FCNs, which have antioxidant and antibacterial effects, had no adverse effects on the mechanical properties of denture liners gives rise to hopes about the usage of FCN-added denture liners againts bacterial accumulation in oral environments.

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