Use of cellulose nanofibers as a denture immersing solution

Wei ZOU¹, Guang HONG^{2,3}, Yukiko YAMAZAKI¹, Kazuma TAKASE⁴, Toru OGAWA¹, Jumpei WASHIO⁵, Nobuhiro TAKAHASHI⁴ and Keiichi SASAKI¹

¹Division of Advanced Prosthetic Dentistry, Graduate School of Dentistry, Tohoku University, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

²Liaison Center for Innovative Dentistry, Graduate School of Dentistry, Tohoku University, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan

³Faculty of Dental Medicine, Airlangga University, Jalan Prof. Dr. Moestopo 47 Surabaya, Indonesia

⁴Department of Prosthetic Dentistry, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan

⁵Division of Oral Ecology and Biochemistry, Graduate School of Dentistry, Tohoku University, 4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan
Corresponding author, Guang HONG; E-mail: hong.guang.d6@tohoku.ac.jp

The purpose of this study was to investigate the influence of cellulose nanofibers (CNF) solution on the mechanical and biological properties of denture base resins (DBR). Two types of CNFs obtained from bamboo (BB) and needle-leaved (NB) trees were used in this study. We prepared 18 different CNF solutions based on their fibrillation (A-low, B-middle, and C-high) and concentration (0.05, 0.10, and 0.20 wt%). A heat-polymerized acrylic resin was used as DBR. The contact angles for each specimen were measured after immersion. The flexural properties of the immersed specimens, and the biological properties of the CNF solutions were examined. Specimens immersed in CNF-NB-C-0.05 wt% solution presented with the lowest contact angles. Specimens in CNF-NB-C and CNF-BB-A groups showed higher flexural modulus values. No cytotoxic or antimicrobial effects were observed for the CNF solutions. This study suggests that CNF solution may improve the surface wettability of the DBR without affecting its flexural property.

Keywords: Cellulose nanofiber, Denture base resin, Surface wettability, Flexural property, Biological property

INTRODUCTION

The number of elderly persons in the population is increasing, especially in developed countries¹. In 2015, the proportion of individuals aged 65 and above was 8.5% of the global population; however, this percentage is expected to increase to 17% by 2050. Increase in the number of elderly persons will lead to increased demands of dental prosthetics with regard to tooth loss, even supposing that intensive oral care may protect them from tooth loss. Removable appliances are most commonly used for oral rehabilitation in edentulous patients. The chewing force is equally distributed to the alveolar membrane and bone by the base of the dental prosthesis. Hence, it is vital that the base material should possess high fracture resistance, be non-toxic, and not impair the oral physiology or cause infections in the oral cavity. Improvements in the properties of denture base materials can contribute to the restoration of oral functions, which in turn may upgrade the quality of life (QOL) in aged denture wearers. Several denture base materials such as wood, silver, porcelain, ceramic, alloys, and polymers have been used. Polymethyl methacrylate (PMMA), a polymer-based material introduced in the 1930s, has been most commonly used for the fabrication of dentures. From a clinical point of view, PMMA has acceptable mechanical and biological properties, satisfying aesthetic performance, and a clearly-defined processing method².

For the removable denture to function well in the oral

cavity, retention and stability are of utmost importance. The retention of removable dentures is defined as the resistance of a denture to vertical movement away from the issues; in addition, it has been described as the inherent quality of the prosthesis to resist the forces of dislodgement along the path of insertion³. The retention of a denture may be influenced by physical, physiological, psychological, mechanical, and surgical factors⁴. Among them, surface wettability of the denture base material is the most important factor for denture retention⁵. However, among the polymeric materials, PMMA is known for its hydrophobic nature⁶. According to most clinicians, the most important factor for achieving retention in complete dentures is the accurate fit of the denture in the oral cavity resulting in a perfect border seal. Several researchers have reported that factors such as atmospheric pressure, vacuum, adhesion, cohesion, wettability, surface roughness, surface tension, viscosity, and capillary attraction affect denture retention^{4,7}.

A major factor involved in the retention of a well-adapted denture is the force related to the wetting of the denture and the mucosal surfaces. Some researchers suggested that increasing the wettability of the mucosal surface might improve denture retention^{8,9}. Monsenego *et al.* pointed out that wettability could reflect the degree of denture retention in the oral cavity⁹. Measurements of contact angles have been utilized to estimate the wettability of different materials⁹. The measuring of contact angle reflects the surface tension of the liquid and the surface energy of the solid. The higher the contact angle, the poorer the wettability of the material. The wettability of dental materials is vital because

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it indicates the ease by which saliva spreads over the surfaces. It is reported that a denture base material with higher wettability should be the primary choice for clinical application¹⁰.

Cellulose from plants and microorganisms is non-toxic, plentiful, renewable and sustainable. It is estimated that the production of cellulose by photosynthesis can range from 10^{11} to 10^{12} tons every year. Natural cellulose-based materials have been used effectively for engineering purposes for thousands of years. The highly crystalline structures formed by natural cellulose with the hierarchical assembly of extended chains in parallel alignment construct nanofibers (width, 3–50 nm), which widely exist in natural plant bodies¹¹. Owing to superior physical properties such as high mechanical strength, low thermal expansion, high aspect ratios, and transparency, cellulose nanofibers (CNF) can be used as an innovative biomass material in the fields of medicine, cosmetics, and pharmaceuticals. Consequently, CNF has been extensively used in academic and industry circles^{12–15}.

Recently, patents and commercial products concerning the industrialization of CNF have been proposed^{14,16}. Moreover, CNF possess superior hydrophilic properties^{16,17}. Therefore, the coating of the surface of a PMMA denture base with CNF might improve its surface wettability and enhance the denture retention in the oral cavity.

The aim of this study was to investigate the influence of CNF solution on the mechanical and biological properties, especially the surface wettability, of denture base resin materials. Our hypothesis is that CNF can be utilized as a solution in which, the denture can be immersed in order to improve its surface wettability.

MATERIALS AND METHODS

Preparation of CNF solutions

The two types of CNF (nanoforest, 10–20 nm, Chuetsu Pulp & Paper, Takaoka, Japan) used in this study were derived from bamboo (CNF-BB) and needle-leaved trees (CNF-NB). Based on the degree of fibrillation (A-low,

Table 1 The immersion solutions used in this study

Group (wt%)	Source of materials	Fibrillation degree	Concentration of CNF (wt%)
CNF-BB-A-0.05	Bamboo tree	Low	0.05
CNF-BB-A-0.10	Bamboo tree	Low	0.10
CNF-BB-A-0.20	Bamboo tree	Low	0.20
CNF-BB-B-0.05	Bamboo tree	Middle	0.05
CNF-BB-B-0.10	Bamboo tree	Middle	0.10
CNF-BB-B-0.20	Bamboo tree	Middle	0.20
CNF-BB-C-0.05	Bamboo tree	High	0.05
CNF-BB-C-0.10	Bamboo tree	High	0.10
CNF-BB-C-0.20	Bamboo tree	High	0.20
CNF-NB-A-0.05	Needle-leaved tree	Low	0.05
CNF-NB-A-0.10	Needle-leaved tree	Low	0.10
CNF-NB-A-0.20	Needle-leaved tree	Low	0.20
CNF-NB-B-0.05	Needle-leaved tree	Middle	0.05
CNF-NB-B-0.10	Needle-leaved tree	Middle	0.10
CNF-NB-B-0.20	Needle-leaved tree	Middle	0.20
CNF-NB-C-0.05	Needle-leaved tree	High	0.05
CNF-NB-C-0.10	Needle-leaved tree	High	0.10
CNF-NB-C-0.20	Needle-leaved tree	High	0.20
Control	Distilled Water	—	—
Blank*	—	—	—

* The specimen was immersed in distilled water for 24 h at room temperature for the three-point bending test only. CNF: cellulose nanofibers.

B-middle, and C-high), the CNF was divided into 6 different materials (Lot Number: CNF-BB-A: 22B17-S-1BBA; CNF-BB-B: 22C17-S-1BBC; CNF-BB-C: 22C17-S-1BBC; CNF-NB-A: 20G17-S-1NBA; CNF-NB-B: 18A17-S-1NBB; CNF-NB-C: 30C17-S-1NBC). These 6 kinds of CNF were diluted with distilled water to three concentration (0.05, 0.10, and 0.20 wt%), and to prepare a total of 18 different CNF immersion solutions (Table 1). The denture in the control group was immersed in distilled water. Additionally, the blank group was also immersed in the distilled water but only for 24 h and used in the three-point bending test. Each solution was stored in a plastic container at $23\pm 2^\circ\text{C}$. One week after immersion, the specimens were removed from the solution and washed using flowing distilled water. The specimens were then immersed in fresh volumes of solution. This procedure was repeated weekly for 24 weeks.

Preparation of acrylic resin specimens

The heat-polymerized acrylic resin denture base specimens (Acron, Lot No. Powder-1606092, Liquid-1704071, GC, Tokyo, Japan) were polymerized to form rectangular blocks ($64.0\times 10.0\times 3.3$ mm) according to the manufacturer's instructions and ISO20795-1 (ISO20795-1: 2013 Dentistry-Base Polymers-Part 1, Denture base polymers)¹⁸. Both surfaces of the test specimens were abraded with #600, #800, #1000, and #1200 grit waterproof emery papers. After washing the specimens with water for 15 s, they were immersed in distilled water at room temperature ($23\pm 2^\circ\text{C}$) for 24 h, and evenly divided into 20 groups containing 7 specimens each.

Contact angle measurement

After 0, 7, 14, 28, 56, 70, 84, 98, 112, 126, 140, 154, and 168 days of immersion, the surface was gently air-dried. The contact angle was measured using a portable contact angle meter (PCA-1, Kyowa Interface Science, Niiza, Japan). An auto pipette and a goniometer were employed to ensure the uniformity of the distilled water droplet volume (2 μL). The FAMAS software (Kyowa Interface Science) was adopted to analyze the images.

All measurements were performed at room temperature at 50% humidity. Two separate points were chosen to measure the contact angles in each of the seven specimens in the different groups (Fig. 1).

Three-point bending test

After immersion for 0 and 168 days, the flexural properties of the specimens were determined by three-point bending test with a crosshead speed of 5.0 mm/min with a 2kN load cell (Serial No.: UK268) according to ISO20795-1 using a material testing machine (Model 5565, Instron, Canton, MA, USA) at room temperature¹⁹.

The flexural modulus (E) was calculated as follows:

$$E = F_1 l^3 / 4bh^3 d$$

where F_1 is the load at a point in the straight-line portion (with the maximum slope) of the load/deflection curve, l is the span distance between the supports (50.0 mm), b represents the width of the specimen (10.0 mm), h represents the height of the specimen (3.0 mm), and d is the deflection at the load F_1 .

The flexural strength (δ) was calculated using the following equation:

$$\delta = 3Fl / 2bh^2$$

where F is the maximum load during the flexural test.

Cytotoxicity evaluation

Following culture with a human gingival fibroblast cell line (p10–13) in a 24-well plate (1×10^4 /well) for 24 h, the sample was placed in cell culture inserts so that it would account for 10% of the entire culture medium (1,500 μL /well). The sample was cultured at 37°C under 5% CO_2 for 24 h. Next, the optical density (OD) measured at 450 nm ($n=5$) using a WST-8 cell counting kit (Sigma-Aldrich Japan, Tokyo, Japan)²⁰. For apoptosis measurements, the cells (1×10^4 /well) were exposed to the specimens in 24-well plates for 24 h. Following exposure, the cells were trypsinized, centrifuged and resuspended in 50 μL fetal bovine serum (FBS) containing 10 $\mu\text{g/mL}$ Hoechst 33342 (Thermo Fisher Scientific, Tokyo, Japan) and 10 $\mu\text{g/mL}$ propidium iodide (Sigma, St. Louis, MO, USA). After incubation for 30 min in dark at room temperature, the cells were spread onto microscope slides. A minimum of 300 cells were counted using a fluorescence microscope.

Fig. 1 The different contact angles with various concentrations in comparison with the control group over the 168 days of immersion. θ is the contact angle.
(a) CNF-NB-C-0.05 wt%; (b) control group; (c) CNF-BB-B-0.2 wt%.

(BZ-9000, Keyence, Osaka, Japan) with an excitation filter of 340–380 nm, and the cells were classified as apoptotic, necrotic, or viable.

Antimicrobial property evaluation

The disk-diffusion method²¹⁾ was used to determine the antimicrobial property of the CNF solution. The cell suspensions of *Streptococcus mutans* (*Sm*) and *Porphyromonas gingivalis* (*Pg*) were spread on blood agar plates. Sterile filter-paper disks (8 mm in diameter) were placed on the surface of the plates, and 75 μ L of various concentrations (0.05, 0.10, and 0.20 wt%) of CNF solutions were impregnated onto the disks. The plates were incubated anaerobically at 37°C for 24 (*Sm*) or 48 h (*Pg*)²²⁾. Subsequently, the presence of a bacterial growth inhibition circle around the disc was visually inspected.

Statistical analysis

The contact angles, flexural properties, and cytotoxicity measurements were analyzed by one-way analysis of variance (ANOVA), combined with Student-Newman-Keuls multiple comparisons tests (SNK test, $\alpha=0.05$). Three-way ANOVA was used to determine the effects of the type and content of CNF solutions used as well as the immersion time on the contact angle and flexural properties. All analyses were computed with SPSS for McIntosh operating system (SPSS 22, SPSS Japan, Tokyo, Japan).

RESULTS

Contact angle

The variations in contact angles at different time points in each group are shown in Figs. 2 and 3. The contact angles demonstrated significant decrease from day 0 to day 56 of immersion in CNF solution. The lowest values in the majority of the groups were observed after 56 days after immersion.

After 56 days of immersion, specimens immersed in CNF-BB-A-0.10, CNF-BB-A-0.20 and CNF-NB-C-0.05 wt% solutions showed significantly lower contact angles when compared with those in the control group ($p<0.05$, SNK test, Fig. 4).

After 168 days immersion, specimens immersed in CNF-NB-C-0.05 wt% solution presented with the lowest contact angles, whereas those immersed in CNF-BB-B-0.20 wt% solution presented with the highest contact angles among all the groups ($p<0.05$, SNK test, Fig. 5).

Flexural properties

The specimens immersed in CNF-BB-A (0.05 and 0.10 wt%) and CNF-NB-C showed significantly higher values of flexural modulus when compared with those in the blank and control groups ($p<0.05$, SNK test, Fig. 6).

Figure 7 shows the flexural strength in specimens in each group. The specimens immersed in CNF-NB-C-0.05 and CNF-NB-C-0.20 wt% solutions were demonstrated

Fig. 2 Variations in contact angles $\pm$ standard deviation (SD) in the CNF-BB group over the 168 days of immersion. (a) CNF-BB-A; (b) CNF-BB-B; (c) CNF-BB-C. The contact angles showed a decreasing trend from day 0 to day 56 and remained stable thereafter until day 168.

Fig. 3 Variations in the contact angles in the CNF-NB group over the 168 days of immersion. (a) CNF-NB-A; (b) CNF-NB-B; (c) CNF-NB-C. A decreasing trend was noted from day 0 to day 56, after which, the values remained stable until day 168.

Fig. 4 The values of the contact angles after 56 days of immersion. (a) CNF-BB groups; (b) CNF-NB groups. The contact angles of specimens immersed in CNF-BB-A-0.1 wt% and CNF-BB-A-0.2 wt% were significantly lower when compared to those in the control group ($p < 0.05$). Specimens that immersed in CNF-NB-C-0.05 wt% presented with the lowest contact angles when compared with the rest of the specimens ($p < 0.05$).

Fig. 5 The contact angles of the specimens after 168 days of immersion. (a) CNF-BB groups, (b) CNF-NB groups. Specimens immersed in CNF-BB-B-0.2 wt% presented with the highest contact angles when compared to those in the other groups ($p < 0.05$), whereas specimens immersed in CNF-NB-C-0.05 wt% had the lowest contact angles ($p < 0.05$).

Fig. 6 The flexural modulus (GPa) of specimens after immersion in CNF solutions. (a) CNF-BB groups, (b) CNF-NB groups, (c) control and blank groups. The specimens immersed in CNF-BB-A-0.05 wt% and CNF-BB-A-0.1 wt% solutions showed significantly higher values of flexural modulus when compared with those in the blank and control groups ($p < 0.05$). The CNF-NB-C groups showed significantly higher values of flexural modulus than the blank and control groups ($p < 0.05$).

higher values when compared with those in the other groups. However, no significant difference was noted among the CNF groups and the controls.

Cytotoxicity of CNF solutions

No cytotoxic effects of the CNF solutions on human gingival fibroblasts were observed (Fig. 8).

Antimicrobial property of CNF solutions

After incubation, no bacterial growth inhibition circle was observed in any of the disks indicating that CNF had no inhibitory effect on *Sm* and *Pg* growth (Figs. 9 and 10).

Fig. 7 Flexural strengths (GPa) of specimens after immersion in the CNF solutions. (a) CNF-BB groups, (b) CNF-NB groups, (c) control and blank groups. The flexural strengths of specimens immersed in CNF-NB-C-0.05 wt% and CNF-NB-C-0.2 wt% were higher than those in the other groups. However, no significant difference was noted when compared with the blank and control groups.

Fig. 8 Qualitative analysis of cytotoxicity for the CNF solutions. (a) CNF-BB solution, (b) CNF-NB solution.

DISCUSSION

Our hypothesis that CNF could be utilized as a denture immersing solution to improve the surface wettability of the denture base resin has partially been accepted. Although surface wettability was promoted by the CNF solutions, the effect was limited. Moreover, the concentration, degree of fibrillation, and origin of CNF must be suitable in order to obtain the desired effect.

The surface wettability of PMMA, the most widely used denture base material, is influenced by its low surface energy²³. Several attempts have been made to improve the wettability and retention of denture bases by surface modification via sandblasting, silicon tetrachloride treatment, H_2O_2 -Ti solution treatment, vacuum discharge, and titanium trichloride with hydrogen peroxide treatment⁷⁻⁹. In 2015, it was reported that air abrasion of the basal surface could significantly improve the retention of the denture base by making the surface hydrophilic in nature²⁴.

The isolation of nanocellulose from bioresources using various methods is an actively researched area of interdisciplinary nanotechnology. Cellulose is a renewable and multifunctional material, which serves as a perspective substitution of non-renewable materials²⁵. CNF have high surface energy and hydrophilic properties. Since the hydroxyl groups located on the surface of these fibers contribute to their hydrophilic

behavior²⁶. Because of these hydroxyl groups (Fig. 11), the hydrophilicity can be improved, which results in the enhancement of the surface wettability of the immersed denture base resin. It may suggest that CNF might be used as a new immersing solution to improve the surface wettability of denture base materials.

Furthermore, considering the difficulties associated with various methods of surface treatment, the use of a new immersing solution to enhance the wettability of dental bases might prove beneficial in the clinical setting. Therefore, the current study aimed to evaluate the effects of CNF immersing solutions on denture wettability. The method used to measure the contact angle in the present study has been proven to be effective due to its ability to minimize the susceptibility to errors⁶. The present *in-vitro* study used 18 different CNF immersing solutions, which were grouped according to the type of material, degree of fibrillation, and concentration. Moreover, the contact angles of the denture base resin that immersed in the 18 CNF solutions were measured, respectively. In this study, the CNF-NB-C-0.05 wt% solution significantly improved the wettability of the denture base resin.

The contact angles of all specimens immersed in all CNF solutions were lowest after 56 days if immersion, after which, the values remained stable without any significant changes until 168 days of immersion. Comparisons of the contact angles between the two time points (56 and 168 days of immersion) in all groups

Fig. 9 The inhibition circle by *S. mutans* after incubation for 24 h. The inhibitory effect was not seen in all the groups.

(a) 1=CNF-BB-A-0.05 wt%; 2=CNF-BB-B-0.05 wt%; 3=CNF-NB-A-0.05 wt%; 4=CNF-NB-B-0.05 wt%; 5=CNF-NB-C-0.05 wt%; Co.=Control (only paper disk). (b) 1=CNF-BB-A-0.10 wt%; 2=CNF-BB-B-0.10 wt%; 3=CNF-NB-A-0.10 wt%; 4=CNF-NB-B-0.10 wt%; 5=CNF-NB-C-0.10 wt%. (c) 1=CNF-BB-A-0.20 wt%; 2=CNF-BB-B-0.20 wt%; 3=CNF-NB-A-0.20 wt%; 4=CNF-NB-B-0.20 wt%; 5=CNF-NB-C-0.20 wt%. (d) 1=CNF-BB-C-0.20 wt%; 2=CNF-BB-C-0.10 wt%; 3=CNF-BB-C-0.05 wt%; Co.=Control (only paper disk).

Fig. 10 The inhibition circle by *P. gingivalis* after 24 h of incubation. The inhibition effect was not observed in all groups.

(a) 1=CNF-BB-A-0.05 wt%; 2=CNF-BB-B-0.05 wt%; 3=CNF-NB-A-0.05 wt%; 4=CNF-NB-B-0.05 wt%; 5=CNF-NB-C-0.05 wt%; Co.=Control (only paper disk). (b) 1=CNF-BB-A-0.10 wt%; 2=CNF-BB-B-0.10 wt%; 3=CNF-NB-A-0.10 wt%; 4=CNF-NB-B-0.10 wt%; 5=CNF-NB-C-0.10 wt%. (c) 1=CNF-BB-A-0.20 wt%; 2=CNF-BB-B-0.20 wt%; 3=CNF-NB-A-0.20 wt%; 4=CNF-NB-B-0.20 wt%; 5=CNF-NB-C-0.20 wt%. (d) 1=CNF-BB-C-0.20 wt%; 2=CNF-BB-C-0.10 wt%; 3=CNF-BB-C-0.05 wt%; Co.=Control (only paper disk).

Fig. 11 The chemical structure of CNF.

showed that, the specimens immersed in CNF-NB-C-0.05 wt% solution had the lowest contact angles at both time points (Figs. 2 and 3), which indicated superior hydrophilicity among specimens in all the groups. The result of the multivariate linear regression analysis demonstrated that the type of material, concentration, time, and degree of fibrillation affected the hydrophilicity of denture base materials; moreover, the impacts of these factors decreased in turn. We inferred that CNF

from needle-leaved trees show a stronger hydrophilicity than that from bamboo trees. One of the non-desirable characteristics of acrylic denture base resins is porosity. The porous surface may promote the adsorption of CNF on to the surface of the acrylic denture base resin²⁷. Based on the results of the contact angle measurement, most of the samples' contact angles declined with time. However, when the contact angles reached a certain value, the angles did not decrease anymore and remained stable. We inferred that one reason of this phenomenon was that the number of pores was unchangeable with the limited Permeated OH groups. These hydrophilic CNF may permeate into the pores of the acrylic resin surface and expose the hydrophilic hydroxyl (OH) groups, which might accelerate the absorption of moisture, thereby improving the surface wettability of the denture base resin. However, extensive studies are necessary in order to understand the interactions between the CNF and the acrylic resin interface.

The mechanical evaluation of denture base materials frequently involves the determination of

flexural properties²⁸); numerous studies have examined the flexural properties of denture base resins^{29,30}. Water molecules can penetrate a polymer structure and act as a plasticizer, leading to a reduction in the mechanical properties of the structure³¹. In the current study, the flexural strengths of specimens in the blank group (no treatment) were high (0.090 ± 0.0122 GPa), but not significantly different from those in other groups. Specimens in the CNF-NB-A (0.05, 0.10, and 0.20 wt%) and CNF-NB-B (0.05 and 0.10 wt%) groups showed a significantly negative effect on flexural strength after immersion when compared with those in the other groups. This finding is consistent with the results of previous studies by Lee *et al.*, where water or humid environment was reported to reduce the physical properties of some polymer resins^{28,32}. The observation may be partially explained by immersion liquid absorption, which may influence the mechanical properties of denture base acrylic resins. Moreover, this may also account for the reason why water acts as a plasticizer after being absorbed onto the surface of the resin *via* diffusion.

The specimens immersed in CNF-NB-C (0.05, 0.10 and 0.20 wt%) and CNF-BB-A (0.05 and 0.10 wt%) solutions showed significantly higher values of flexural modulus when compared to those in the other groups, especially the blank and control groups. Some researchers reported that the immersing solutions (especially for disinfection) exerted no significant effects on flexural/elastic modulus after immersion when compared to distilled water^{33,34}. A previous study suggested that the denture base material has a lower flexural/elastic modulus, and can release high forces on to the underlying mucosa thereby irritating the gingival tissues and promoting the resorption of the residual alveolar ridge³⁵. In this study, we suggested that there may be a cross-linking of CNF with the microstructure of the resin, of which the major driving forces may be the weak key of hydrogen bond or van der Waals force. This cross-linking of CNF with the microstructure of the resin may lead to an increase in flexural modulus. Thus, an increase in flexural modulus by immersion in CNF-NB-C (0.05, 0.10 and 0.20 wt%) or CNF-BB-A (0.05 and 0.10 wt%) solutions might prove beneficial for the denture bases materials. Extensive studies are needed to explore the cross-linking between CNF and the acrylic resin.

The surface wettability of denture base acrylic resins affects not only the retention force of the denture but also the rate of deposition of microorganisms on to its surface. The two phases for the adhesion of *Candida albicans* on to the denture base are as follows: an initial phase based on electrostatic interactions, Van der Waals forces, and hydrogen interactions, and a second phase comprising specific interactions between the cell walls of the microorganisms and the particles on the acrylic resin plate³⁶. It has been shown that the hydrophilic surface prevents the first stage of microbial adhesion. *Candida* species exhibit a strong affinity for the acrylic base resin because of its hydrophobic properties³⁷. The findings of

the present study indicated that immersion in CNF-NB-C-0.05 wt% solution increases the hydrophilicity of the denture base resin.

Alternatively, biosafety is a very important factor to be considered while developing a novel denture immersion solution. The CNF solutions used in the current study did not demonstrate any cytotoxic effects on the cells in the cytotoxic analysis. CNF has been widely used for medical, cosmetic, and pharmaceutical applications owing to their superior biocompatibility and biosafety. Thus, a denture immersion solution that has the potential to improve the surface wettability and antimicrobial activity of the denture base material will prove beneficial for elderly individuals using removable dentures, by reducing the incidence of aspiration pneumonia. However, CNF did not appear to have any inhibitory effects on *Sm* or *Pg* growth in the present study.

In summary, the denture base resin specimens immersed in CNF-NB-C (0.05 wt%) showed the highest hydrophilicity when compared with the other specimens; however, the flexural properties of these specimens appeared to be unaffected. In addition, the CNF-NB-C (0.05 wt%) solution exhibited good biocompatibility; thus, further studies investigating the application of this solution in clinical settings are warranted.

The current study has a few limitations. First, it is *in vitro* study, and did not simulate the actual oral environment. Second, the flexural properties of the materials were measured in the dry state. Third, a drop of distilled water was used to measure the contact angle. To overcome the limitations of this study, the flexural properties should be measured in the wet state, and artificial saliva may be used to measure the contact angle. We believe that it may be interesting to add a cleansing agent to the CNF solution and explore their effects on the denture base materials for commercial use.

CONCLUSION

Within the limitations of this study, our results suggest that low concentrations of the CNF-NB-C solution can improve the surface wettability of the denture base resin, without affecting its mechanical properties. In conclusion, CNF may be safely used as a denture immersion solution to improve the retention of complete dentures.

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