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Original Article

Evaluation of the Flexural Strength of Orthodontic Acrylic Resin incorporated with Propolis Nanoparticles: An In Vitro Study

Azam Akhavan[1], Sepideh Arab[2], Negin Eslamiamirabadi[3], Ahmad Sodagar[2], Fatemeh Safari[2]

[1] *Radiation Applications Research School, Nuclear Science and Technology Research Institute, Tehran, Iran*

[2] *Department of Orthodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran*

[3] *Faculty of Dentistry, McGill University, Montréal, Québec, Canada*

Corresponding author: Fatemeh Safari, Department of Orthodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran; Email: safarii_fatemeh@yahoo.com

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Abstract

Aim: Nanopropolis has become the subject of interest in medicine and dentistry as a natural product due to its outstanding properties, particularly antimicrobial activity. This study aimed at investigating the effect of nanopropolis on flexural strength of polymethyl methacrylate (PMMA).

Materials and methods: Three groups of two acrylic resin brands namely Acropars and Triplex containing 0 (control group), 0.5%, and 1% of nanopropolis were prepared in 64×10.0×3.3 mm according to ISO 20795-2 (2013). Fifteen samples were allocated to each concentration. Flexural strength was determined following immersion in water and incubation at 37°C for 50±2 hours using a universal testing machine at a crosshead speed of 5±1 mm/min. Data were analyzed using ANOVA, Tukey HSD, and *t*-test. $P<0.05$ was set as statistical significance.

Results: Control groups of Acropars and Triplex showed the highest mean flexural strength within their own group which both were higher than the recommended 50 MPa. The mean flexural strength of Triplex incorporated with 0.5 and 1% of nanopropolis was higher than that of Acropars with the same percentage.

Conclusions: The mean flexural strength of Triplex remained above the recommended value of 50 MPa after incorporation of both 0.5 and 1% nanopropolis. However, that of Acropars dropped below it.

Keywords

acrylic resin, flexural strength, nanopropolis

INTRODUCTION

In fact, high prevalence of malocclusion amongst growing children entails interceptive orthodontic treatments[1-4] that are commonly performed using removable appliances. In addition, removable appliances have been used as retainers since the 1920s and still remain as practical means for retention.[5] These appliances are mostly fabricated from PMMA due to its various advantages including ease of processing, fitting accuracy, convenience, and reasonable cost.[6] Despite all perfect features, it is highly prone to colonization of microorganisms[7-9] which may lead to dental caries, periodontal diseases, and chronic atrophic candidiasis. Therefore, several efforts have been employed to introduce antibacterial efficacy to PMMA. In this sense,

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PMMA has been incorporated with different antimicrobial agents such as silver nanoparticles[10], silicon dioxide and titanium dioxide[11], carbon nanotubes[12], chlorhexidine diacetate[13], 2-methacryloyloxyethyl phosphorylcholine (MPC), and quaternary ammonium dimethylaminohexadecyl methacrylate (DMAHDM)[14], polyethylene oxide (PEO)[15], nanodiamonds[16] and so on . Despite the incorporation of PMMA with a range of antibacterial agents, there is still lack of consensus in terms of their clinical effectiveness.[17] In this regards, the contemporary trend towards natural products has drawn attentions to propolis (bee glue) as a promising ingredient for both medical and dental applications.[18,19] Propolis has a wide range of outstanding biological scopes including antioxidant and anti-ageing[20], anticancer[21], immunomodulatory[22], antidiabetic[23], anti-inflammatory and anti-allergic[24] properties along with wound healing promotion[25]. Moreover, several investigations have confirmed its activity against wide spectrum of bacteria[26], viruses[27] and fungi[28]. Additionally, propolis has been utilized vastly in dentistry to alleviate dentin hypersensitivity and aphthous stomatitis, prevent dental caries, pulp capping, storing the avulsed teeth and to culture the PDL cells[29] and so on. Propolis mouthwash and paste inhibit *Streptococci mutans* and *Lactobacilli*. Also, propolis paste improves the healing of periodontal socket after extraction by 90% in human subjects.[30] The inhibitory effect of 300 μg/mL propolis nanoparticle against *E. faecalis* for the purpose of root canal disinfection is comparable with 6% NaOCL and 2% CHX.[31] Furthermore, PMMA containing 1 or 2% of propolis nanoparticles has presented antibacterial activity against common oral pathogens such *as Streptococcus mutans*, *Streptococcus sanguinis*, *Lactobacillus acidophilus*, and *Candida albicans*.[32] Therefore, propolis can be considered as a promising agent to incorporate with PMMA due to its numerous beneficial attributes. In spite of that, the effect of propolis on the mechanical properties of PMMA should be taken into account as a crucial requirement in order to endure the loads imposed in the oral cavity. To the best of our knowledge, there is lack of evidence in this respect.

AIM

Thus, this study aimed to investigate the effect of incorporating nanopropolis (NPS) on flexural strength of PMMA in order to induce antimicrobial features in PMMA without compromising its mechanical properties.

MATERIALS AND METHODS

Nanopropolis preparation

Pure propolis was purchased from Gold Zagros (Lorestan, IRAN). Twenty grams of pure propolis was dissolved in 100 ml of ethanol for 7 days at room temperature and then filtered through filter papers (Wattman-40Ashless-Germany) to remove rough particles. Afterwards, pure propolis particles were isolated by adding the solution to distilled water at 1:10 ratio. The suspension was placed in an ultrasonic bath for 20-30 minutes to obtain propolis nanoparticles. The achieved colloidal NPS was centrifuged at 9000 rpm for 20 minutes using a centrifuge machine (HeroLab-22000 rpm, Germany) and then filtered by filter papers. Nanoparticles were verified under scanning electron microscope (SEM; Zeiss, Oberkochen, Germany) at ×65000 magnification **(Fig. 1)**.

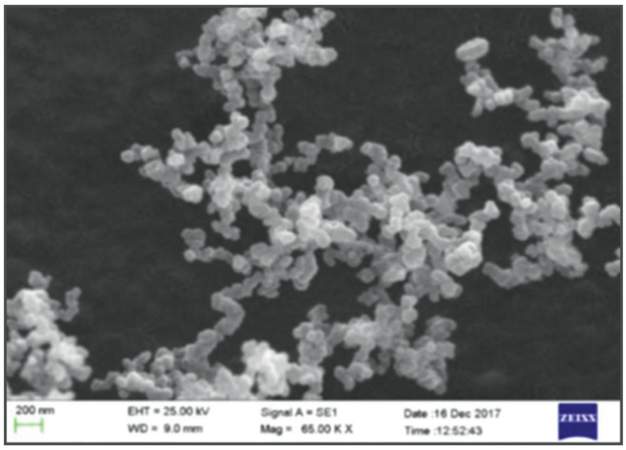

Figure 1. SEM image of NPS at ×65000 magnification.

The colloid was poured to plates and placed in a freezer at −80°C for 20 minutes followed by freeze drying (Freeze dryer, LYOTRAP, LTE scientific, UK) at −70°C for 24 hours to obtain powder form of NPS particles.

Sample preparation

Mold preparation

Stainless still molds were machined in 65×12×4 mm considering the shrinkage of polymer. Impressions were taken of molds using the putty-wash technique (Silicone impression material, Hydro, Detax, Germany).

Sample groups

Two commercial acrylic resin groups including Acropars (Marlic Medical Industries Co, Tehran, Iran) and Triplex (Ivoclar Vivadent AG, Schaan, Liechtenstein) were selected. Three subgroups, each including 15 samples, were prepared for the examination: one control group comprising acrylics without NPS and two experimental groups consisting of acrylic resins incorporated with 0.5% and 1% NPS. Acrylic preparation was preformed according to manufacturer's instruction and the doughy acrylic resins were inserted into the impression mold and pressed using glass slide until the completion of self curing at 26°C. Cured polymeric samples were removed from molds after 1 hour and grinded to 64×10.0×3.3 mm using 60 and 80 grit sandpapers to meet the ISO 20795-2 (2013) standard of the polymeric base of orthodontic appliance.[33] Each sample was meaured three times using a digital caliper (Insize, USA) with a precision of 0.1 mm. Each sample was allocated a number from 1 to 90 **(Fig. 2)**.

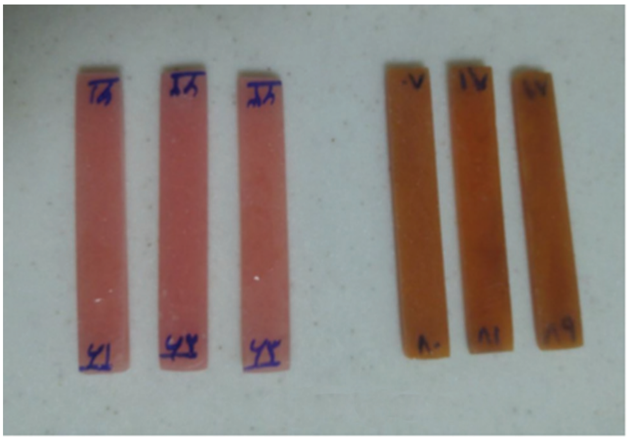

Figure 2. Control samples of Triplex acrylic resin on the left, and Triplex acrylic resin incorporated with 0.5% NPS on the right.

Flexural strength test

Samples were immersed in water and incubated at 37°C for 50±2 hours (Incubator, PECO-Iran). Then flexural strength test was carried out using a universal testing machine (Zwick Z250, Germany). Specimens were undertaken an increasing load at a crosshead speed of 5±1 mm/min to the failure point. Flexural strength was calculated according to the following equation:

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

where F delegates the force in Newton at failure point, l stands for the distance between supports in millimeters with an accuracy of ±0.01 mm, b and h are the width and height in millimeters, respectively, at the center of the sample.

Statistical analysis

Statistical analysis was performed by IBM SPSS version 25 using one-way ANOVA, two-way ANOVA, Tukey HSD, and T-test. *P* less than 0.05 was considered statistically significant.

RESULTS

Triplex and Acropars incorporated with 0, 0.5%, and 1% NPS were prepared for flexural strength test. The experiment was conducted on 90 samples of six groups. Results of the flexural strength showed a descending trend in flexural strength with increasing the concentration of NPS **(Fig. 3)**.

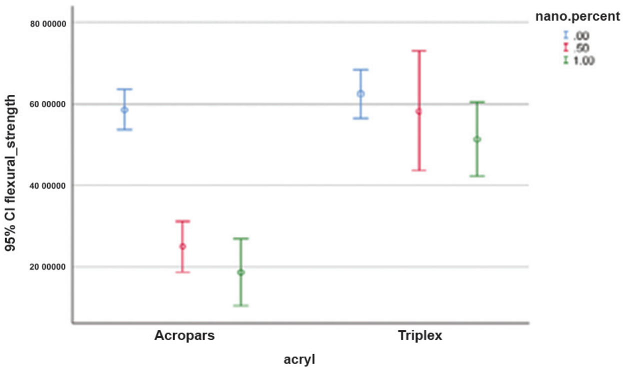

Figure 3. Mean flexural strength (MPa) of two acrylic resins incorporated with 0, 0.5, and 1% of NPS.

So that the mean flexural strength of Acropars incorporated with 1% of NPS dropped to 18.60±14.81 MPa and that of Triplex came to 51/33±16/47 MPa.

The highest mean value was recorded in acrylic resins without NPS in both Acropars and Triplex groups **(Table 1)**. The two way ANOVA test indicated a different effect of NPS percentage on flexural strength according to the type of resin, denoting a significant interaction between acrylic resin type and NPS percent.

The mean flexural strength of control group of Acropars (58/58±8/87 MPa) and Triplex (62/43±10/61 MPa) showed no significant difference (p=0.29, 95% CI). On the other hand, the mean flexural strength of Triplex+0.5% NPS (58/25±26/49 MPa) was significantly higher than that of Acropars with the same percentage of NPS (24/91±11/22 MPa) (p<0.001, 95% CI). Likewise, the mean flexural strength of Triplex+1% NPS (51/33±16/47 MPa) was higher than Acropars+1% NPS (18/60±14/81 MPa) significantly (p<0.0001, 95% CI). Incorporation of both 0.5 and 1% NPS had no adverse effect on the flexural strength of Triplex although decreased that of Acropars. Among Acropars samples, the mean flexural strength was significantly different between the subgroups using one-way ANOVA test (p<0.0001). Acropars without NPS represented significantly higher mean flexural strength than either 0.5% or 1% NPS incorporated samples (p<0.0001, 95% CI)

Table 1. Mean flexural strength of Acropars and Triplex incorporated with 0, 0.5, and 1% of NPS.

Acrylic resin group	NPS%	Number	Flexural strength value MPa	
			Mean	SD
Acropars	0	15	58/58	8/87
	0.5	15	24/91	11/22
	1	15	18/60	14/81
Triplex	0	15	62/42	10/61
	0.5	15	58/25	26/49
	1	15	51/33	16/47

Table 2. Comparison between the mean flexural strength of Acropars and Triplex incorporated with 0%, 0.5%, and 1% of NPS.

	P value					
	Acropars 0%	**Acropars 0.5%**	**Acropars 1%**	**Triplex 0%**	**Triplex 0.5%**	**Triplex 1%**
Acropars, 0%	-	<0.001*	<0.001*	0.291	-	-
Acropars, 0.5%	<0.001*	-	0.323	-	<0.01*	-
Acropars, 1%	<0.001*	0.323	-	-	-	<0.001*

* significant difference according to *P*

in pairwise comparisons using Tukey HSD test. There was no significant difference between the flexural strength of 0.5% and 1% nanoprolis incorporated Acropars (p=0.323, 95% CI). Among Triplex samples, mean flexural strength showed no significant difference between the subgroups (p=0.283, 95% CI).

DISCUSSION

Several attempts have been made to incorporate antimicrobial agents into PMMA to address the microbial colonization.[17] In this regard, NPS might serve as a promising alternative to metal antimicrobial agents due to its remarkable antibacterial, antiviral, and antifungal effectiveness along with biological safety and natural source.[34] Indisputably, PMMA incorporated with NPS should also meet the recommended flexural strength for clinical practice as well. Nanoparticles may act as small-sized fillers and therefore enhance or reduce mechanical properties.[35-37] Various nanoparticles have been introduced to PMMA in several previous investigations. Nanodiamonds at a concentration of 0.5% improved flexural strength of PMMA due to the crystalline structure of diamond, intense chemical bonds, and suitable diffusion of nanoparticles in the resin matrix. However, higher concentration of nanoparticle resulted in lower flexural strength due to the inadequate ratio of polymer.[38] Addition of zinc oxide nanoparticles up to 1.4% increased the flexural strength of PMMA.[39] Incorporation of 2.5% and 5% zirconium oxide nanoparticles boosted flexural strength of PMMA regardless of resin thickness.[40] Silicon dioxide nanoparticles revealed a dose-dependent reducing effect on flexural strength, higher concentration leading to lower value due to the presence of voids and agglomeration of nanoparticles.[41]

Since the antibacterial activity of NPS modified-PMMA against *S. mutans*, *S. sanguinis*, *L. acidophilus*, and *C. albicans* has been proven by the authors lately[32], this study was designed to evaluate the effect of NPS incorporation on flexural strength of two acrylic resins namely Triplex and Acropars. In control groups, the mean flexural strength of Triplex was slightly higher than that of Acropars although the difference was insignificant and both were more than 50 MPa which is the minimum flexural strength base on ISO 20795-2:2013.[33] Current results indicated that the mean flexural strengths of Triplex+0.5% and 1% NPS were lower than that of control Triplex although the differences were insignificant (p=0.821 and p=0.258, respectively) and remained above the recommended value.[33] On the other hand, incorporation of both 0.5% and 1% NPS resulted in significant reduction of the mean flexural strength of Acropars (p<0.0001) to less than the accepted value. This may be explained by the interference of nanoparticles as impurities with resin polymerization. Moreover, nanoparticles may act as plasticizers and result in higher amount of unreacted monomers.

The differences between various acrylic resins have been considered in previous studies.[42] Triplex and Acropars self-curing acrylic resins opted in this study are different in terms of composition and concentration of oligomers, plasticizers, crosslinkers, initiators, and accelerators which may have to diverse effect of NPS on flexural strength of each. The present results for Acropars groups containing NPS are consistent with the previous reports with some other particles. Incorporation of higher percentage of Al_2O_3, TiO_2, and SiO_2 (3-5%)[43], 10% ratio of TiO_2, ZrO_2, Sic-nano, Si_3N_4 and HA-nano[44], 0.5 and 1% of either TiO_2 or SiO_2[45] decreased flexural strength of PMMA significantly. Kul et al. revealed no significant differences in flexural strength of PMMA (Heraus Kulzer) following the addition of SiC, Al_2O_3 or Ag by 10 wt%[44] which is in line with the current values achieved in 0.5 and 1% NPS enriched-Triplex. Nevertheless, there are controversies on how the incorporation of various antibacterial agents would affect the flexural strength of PMMA. For instance, in the study conducted by Yadav et al., incorporation of 10% mass of silver zinc zeolite, chlorhexidine (CHX), and fluconazole decreased the flexural strength of PMMA (Trevalon) significantly which was in accordance with our results in the Acropars groups. Their observations may be due to an increase in residual monomer in the zeolite porosities or disruption of the physical structure of the polymer by either CHX or large particles of fluconazole. It might also be attributed to the high percentage of the nanoparticles they used.[46] Contrariwise, in the research by Ratanajanchai et al., addition of 1.0% w/w of potassium sorbate, 0.5% w/w of sodium metabisulfite and 0.25% w/w of zinc oxide particles as antimicrobial agents increased the flexural strength and decreased the flexural modulus of PMMA but within the acceptable range.[47] Similarly, in a study by Lee et al., incorporation of 0.5%, 1%, and 2% graphene-oxide nanosheets into PMMA introduced antimicrobial activity without any adverse effect on flexural strength. Besides, addition of 0.5% graphene-oxide improved flexural strength which may be due to the ability of graphene-oxide to deflect cracks.[48] Incorporation of 0.4%, 0.8%, and 1.6% of Galla Chinese extract into Acropars conferred antibacterial property along with improvement in the flexural strength of PMMA which the latter attributed to the covalent bonds creation between Galla extract and Acropars.[49] To our knowledge, despite promising addition of propolis to other substrates such as glass ionomer or resin composite, there is lack of evidence on mechanical properties of PMMA incorporated with NPS. Regarding the addition of propolis to other substrates, incorporation of 25% and 50% ethanolic extracts of propolis into glass ionomer inhibited *S. mutans* with no adverse effect on shear bond strength.[50] Similarly, 2% and 5% NPS incorporated into composite resin exhibited antibacterial effect against *S. mutans* and *S. sanguinis* beside acceptable shear bond strength.[51] Several factors including but not limited to nanoparticle's type, formulation, structure, and concentration along with the rate of dispersion in resin matrix and probability of interfering with the polymerization might affect the results. In addition, the properties of the matrix, which the nanoparticles introduced to such as formulation or mode of polymerization are other key factors in achieved data. Thus, further studies considering the aforementioned variables along with subsequent evaluation of antimicrobial efficiency, cytotoxic effect of nanoparticles, roughness of fractured surface and other major mechanical properties such as modulus of elasticity are recommended. In vitro experiments considering the effect of time, aging and so on to simulate clinical conditions are required. Ultimately, clinical investigations are essential to generalize the results to the practice.

CONCLUSIONS

The effect of NPS on flexural strength of acrylic resin is dependent on the commercial type and concentration of the nanoparticle. Addition of 0.5% and 1% propolis nanoparticles to Triplex does not have any adverse effect on its flexural strength. However, it drops that of Acropars below the acceptable value.

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Competing Interests

The authors have declared that no competing interests exist.

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Оценка прочности на изгиб ортодонтической акриловой смолы с наночастицами прополиса: исследование in vitro

Азам Акаван[1], Сепиде Араб[2], Негин Есламиамирабади[3], Ахмад Содагар[2], Фатеме Сафари[2]

[1] Научно-исследовательский отдел радиационных технологий, Научно-исследовательский институт ядерной науки и радиационных технологий, Тегеран, Иран

[2] Кафедра ортодонтии, Факультет дентальной медицины, Тегеранский университет медицинских наук, Тегеран, Иран

[3] Факультет дентальной медицины, Университет „Макгил", Монреаль, Квебек, Канада

Адрес для корреспонденции: Фатеме Сафари, Кафедра ортодонтии, Факультет дентальной медицины, Тегеранский университет медицинских наук, Тегеран, Иран; E-mail: safarii_fatemeh@yahoo.com

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Резюме

Цель: Нанопрополис стал предметом интереса в медицине и стоматологии как натуральный продукт благодаря своим выдающимся свойствам, в частности антимикробной активности. Целью данного исследования было изучение влияния нанопрополиса на прочность на изгиб полиметилметакрилата (PMMA).

Материалы и методы: Три группы акриловых смол двух марок, а именно Acropars и Triplex, содержащие 0 (контрольная группа), 0.5% и 1% нанопрополиса, были приготовлены в размерах 64×10.0×3.3 mm в соответствии с ISO 20795-2 (2013). Для каждой концентрации было выделено пятнадцать образцов. Прочность на изгиб определяли после погружения в воду и инкубации при 37°C в течение 50±2 часов с использованием универсальной испытательной машины при скорости траверсы 5±1 mm/min. Данные были проанализированы с использованием ANOVA, Tukey HSD и t-критерия. $P<0.05$ принимали за статистическую значимость.

Результаты: Контрольные группы Acropars и Triplex показали самую высокую среднюю прочность на изгиб в своей группе, которая в обеих группах превышала рекомендуемые 50 MPa. Средняя прочность на изгиб Триплекса с добавлением 0.5 и 1% нанопрополиса была выше, чем у Акропарса с тем же процентом.

Заключение: Средняя прочность на изгиб Триплекса оставалась выше рекомендуемого значения в 50 MPa после добавления как 0.5, так и 1 % нанопрополиса. Однако показатель Acropars опустился ниже этого уровня.

Ключевые слова

акриловая смола, прочность на изгиб, нанопрополис