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Modulus of Elasticity, Flexural Strength and Biocompatibility of Poly(methyl methacrylate) Resins With Low Addition of Nanosilica

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Research Article

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ABSTRACT

Background: Reinforcement of PMMA has been an issue since it has been first introduced in the dental practice. Various improvements have been suggested, out of which addition of nano particles presents most recent method. Effect of nano particle addition on mechanical and biological properties off PMMA materials remains to be determined.

Materials and methods: The materials used were mixed with SiO_2 nano particles ranging from 0.05% to 2 wt.%. Determination of flexural strength and modulus of elasticity was done by three-point bending test on Universal testing machine. Most successful nano-modified materials were chosen for cytotoxicity assessment using L929 and MRC 5 cell lines.

Results: The highest values for flexural strength and modulus of elasticity were obtained with 0.05% or 0.1% nanosilica addition, depending on the composition of the material. The secondary peek was observed at 2%. Addition of silica nano particles affected cytotoxicity of the material that was concentration depended.

Conclusion: Modulus of elasticity and flexural strength are strongly dependent on material nano-particle content. All of the materials tested proven satisfactory regarding their cytotoxic potential.

INTRODUCTION

Fracture of dentures presents an issue in clinical practice, where prevalence occurs up to 63% in the first three years of their use [1,2]. Repairing of dentures is a demanding procedure, involving additional time and money. It should be emphasized that denture wearers are usually older patients with limited mobility, and transport to dental office could also present a serious problem. This is why improvement of mechanical properties of dentures, while maintaining their biocompatibility, remains a necessity. Most profound fracture effect of upper dentures is located in the midline of the palate, where cyclic shifting of flexural forces occurs, and as such was addressed in this study. Silica nanoparticle addition was introduced as a method for reinforcement. While the benefits related to the mechanical properties have been proven, the influence of nano-modified materials on living tissues, especially the correlation between quantity of nano addition and its effect on biocompatibility of PMMA, has not been addressed [3-6]. The incorporation of nanoparticles is a method for enhancing material's mechanical properties, with the secondary goal of maintaining it's toxicity level. This affects polymerization process and forming of polymer chains with different internal and external

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structure, which is especially important when contacting under laying tissues of dentures. Alteration of material's structure can shift the balance between occurence and releasing of potentially harmful components in oral cavity, thus adding another factor in the complex chain of mutual interactions between material's components, degradation products and living organism [7]. It is expected that this could altogether affect materials toxicity, and appropriate testing should be conducted.

The aim of this study was to investigate the mechanical properties-elastic modulus, flexural strength and cytotoxicity of nano-modified materials on two different cell cultures. The null hypothesis was that no difference exists between common and nano-modified materials depending on strength and cytotoxicity.

MATERIALS AND METHODS

Materials

Materials used in this study are listed in **(Table 1)**. Preparation of samples was done by mixing the AEROSIL R812 (Evonik Degussa, Essen, Germany) silica nano particles in as-received condition with the liquid component of the PMMA base materials Materials were modified with dispersions of SiO_2 nanoparticles, containing 0.05%, 0.1%, 0.2%, 0.5%, 1% and 2% wt., with hexamethyldisilazane (HDMS) hydrophobic surface layer. The control group consisted of unmodified materials. Nanoparticles were weighed using a laboratory balance (Adventurer Pro; Ohaus, Parsippany, New Jersey, accuracy: 0.0001 g).

Table 1. Materials as specified by the manufacturers.

PMMA materials and manufacturers	Powder/Liquid ratio	Composition		Lot Numbers	
		Powder	Liquid	Powder	Liquid
Triplex Hot; Ivoclar Vivadent, Shaan, Liechstenstein	23.4 g/10 ml	PMMA, pigments, catalysts (0.5-1.5% benzoil peroxide)	MMA, EDMA (2.5-10%)	N17392	N47618
Polihot; Polident, Volcja Draga, Slovenia	22 g/10 g (30 ml/11 ml)	PMMA, pigments, catalists	MMA, other unspecified additions	18	86
Biocryl-RN; Galenika, Belgrade, Serbia	20 g/10 g	PMMA, pigments and catalists (0.31%)	MMA, hydrohynon	1306	1524
Nano particle material and manufacturer	Nano particle size and type	Surface layer	Surface area	Lot number	
AEROSIL R812; Evonik Degussa, Essen, Germany	7 nm SiO_2	HDMS hydrophobic	220 ± 25 m^2/g	3158032735	

The mixing of nanoparticles and the liquid component (MMA - based) was done using a magnetic stirrer (MM-530; Tehtnica, Zelezniki, Slovenia), with the speed of 500 rpm. The size of the particles in the liquid component was determined by Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) analyzer. The size distribution of particles in the liquid component of Biocryl material is shown in **(Figure 1)**, with similar results obtained for Triplex and Polyhot. It can be seen that regardless of the SiO_2 nanoparticle content, particle size in the liquid component is similar. Afterwards, the powder and dispersion (liquid in control group) components were thoroughly mixed with a spatula and left to rest for 10 min, at room temperature in a closed mixing cup. Upon reaching a dough like state, sufficient quantity of resin was placed in a 40°C isolated flask halves, pre-filled with hardened plaster. The mold was made by boiling out the wax patterns, dimensions of 50×50×4 mm. The flasks were closed, the whole set was pressed at 80 bar pressure and clamped. Polymerization was conducted in water environment, by subsequent heating to 100°C, and maintaining that temperature for 45 min. Afterwards, the samples were cooled at room temperature for 30 min, followed by cooling in cold water. Cutting of the material was done using a standard metallographic abrasive cutting machine with water specimen cooling (Discotom; Struers, Copenhagen, Denmark). Overall 10 samples were made for each experimental group. Finishing of the samples was done with 1500 grit SiC paper (P1500; Struers, Copenhagen, Denmark) and subsequently checked with a micrometer (Altraco; Hyundai Measurement, Seoul, Korea, accuracy: 0.01 mm).

Mechanical property testing

Determination of flexural strength and modulus of elasticity was done by three-point bending test, on samples with dimensions: 50×6.25×2.5 mm, according to the ISO178 standard [8]. The testing was conducted on Universal testing machine (AT-L-118B, Toyoseiki, Tokyo, Japan) with a crosshead speed of 1 mm/min. Flexural strength was calculated using Equation (1)

$$\sigma = \frac{3F_{max}L}{2BH^2}$$

Where σ is flexural strength [MP_a], F_{max} is maximum load [N], L is the distance between supports [mm], B is the width of the samples [mm] and H is the height of the samples [mm].

Modulus of elasticity was calculated using Equation (2)

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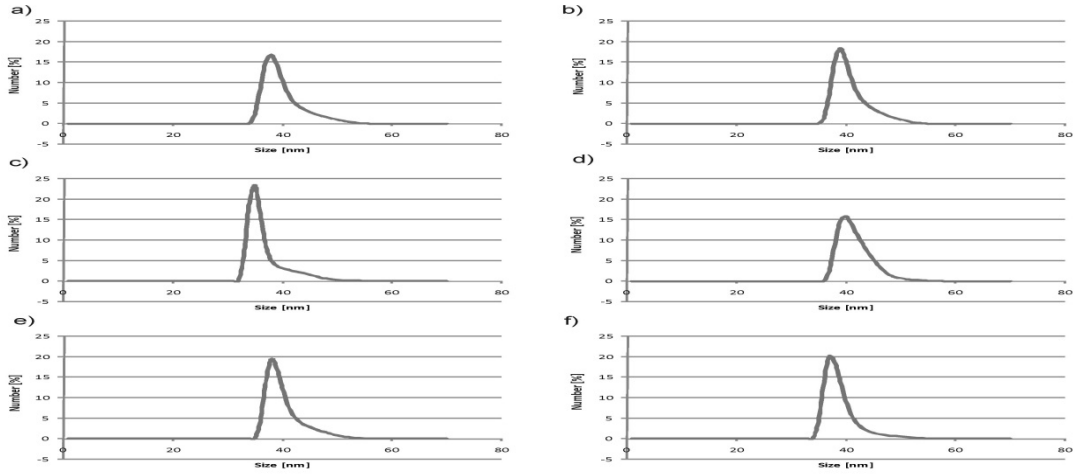

Figure 1. Particle size in Biocryl liquid with different contents: a) 0.05 %; b) 0.1 %; c) 0.2 %; d) 0.5 %; e) 1 %; f) 2 %.

$$E = \frac{FL^3}{4BH^3d}$$

Where E is the modulus of elasticity [GP_a], d is the deflection [mm] that corresponds to the load F, while L, B and H are as in the previous equation.

SEM, EDX and DSC analyses

Fractured samples were examined by a scanning electron microscope (SEM) (JSM-6460LV; JEOL, Tokyo, Japan) operating at 25 kV. The samples were previously coated with gold (SCD-005; Bal-tec/Leica, Wetzlar, Germany). Furthermore, examination of agglomerated nanoparticles was done by energy dispersive X-ray analysis (EDX), using an INCA Microanalysis system (Oxford Instruments, Abington, UK).

Differential Scanning Calorimetry (DSC) analysis was performed on a DSC device (Q20;TA Instruments, New Castle, USA). The analysis was conducted within the 40-160 °C temperature range.

Biocompatibility testing

The cell lines used in the study included L929 (mouse fibroblasts, American Type Culture Collection CCL1) and MRC-5 (human fibroblasts, American Type Culture Collection CCL 171). The cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose, supplemented with 10% of fetal calf serum (FCS; Sigma) and antibiotics and antimycotics solution (Sigma). The cells were sub-cultured twice a week and a single cell suspension was obtained using 0.1% trypsin in EDTA (Serva, Heidelberg, Germany). All cell lines were cultured in flasks (25 cm^2, Costar, Corning, NY, USA) at 37 °C in the 100% humidity atmosphere and 5% of CO_2. Exponentially growing cells were used throughout the assays. The cell number and percentage of viable cells were performed by dye exclusion test (DET) with trypan blue [9]. Viability of cells used in the assay was over 90%.

Material samples were extracted four times consecutively in 4 mL of Dulbecco's modified eagle medium ([DMEM] Sigma) without serum for 3, 5, 7, and 21 days. After each elution period, the extracts were removed and the vials were filled again with fresh medium. The incubation of cells with eluate lasted for 48 hours.

Growth inhibition was evaluated by tetrazolium colorimetric MTT assay [10]. Viable cells were seeded into 96-well micro-titer plates (Costar, Corning, NY, USA) at optimal seeding density of $5 \cdot 10^3$ cells per well, to assure logarithmic growth rate throughout the assay period. Serial dilutions of the various eluates in 100 µL volumes were added, and cells were treated for 48 h at 37 °C/5% CO_2. After treatment, 10 µl of MTT solution (5mg/ml, sterilized by filtration) was added to all of the wells. Plates with cells and MTT were incubated for 3 hours at 37 °C, after which the medium and MTT were removed by suction. The formazan product was solubilized in 100 µl 0.04 M HCl in isopropanol. After a few minutes, the plates were read on a spectrophotometer plate reader (Multiscan MCC340, Lab systems, Finland) at 540/690 nm. The wells containing only complete medium and MTT acted as blank. The experiment was repeated twice.

Inhibition of growth was expressed as a percent of a control according to the Equation (3):

$$\%K = \frac{A_{test}}{A_{control}} \times 100$$

where A is absorbance of test and control sample respectively.

Statistical analysis

Statistical analysis was performed in Minitab 16 (Minitab Inc, State College, PA, USA) software. The data was analyzed using a one-way analysis of variance (ANOVA) with Tukey's post-hoc test. The significance level was set at α=0.05

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RESULTS

Mechanical properties

The modulus of elasticity, flexural strength and results of statistical analysis are shown in **(Figures 2 and 3)** as well as **(Tables 2 and 3).** The highest values for modulus of elasticity were obtained with 0.05% nanosilica addition in Triplex and Polyhot, or with 0.1% in Biocryl **(Figure 2).** Higher nanosilica additions caused a drop in modulus of elasticity. The lowest values were recorded at 0.2%, that gradually rose until 2% of addition was reached. Standard deviations showed a notable rise when nanosilica was added. The statistical analysis showed that the modulus of elasticity of unmodified and modified Triplex samples are significantly different. In Polyhot, only the samples modified with 0.05% nanosilica are different in relation to unmodified ones, while in Biocryl, nanosilica modified samples are different only in samples modified with 0.2% nanosilica.

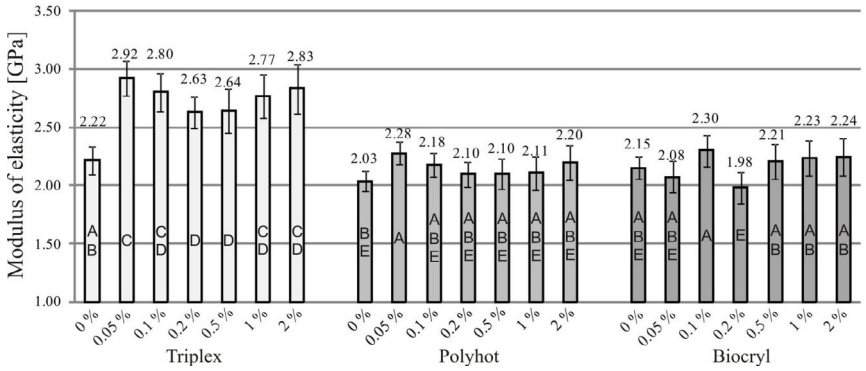

Figure 2. Modulus of elasticity of tested materials with standard deviations and the results of statistical analysis (means that do not share a letter are significantly different).

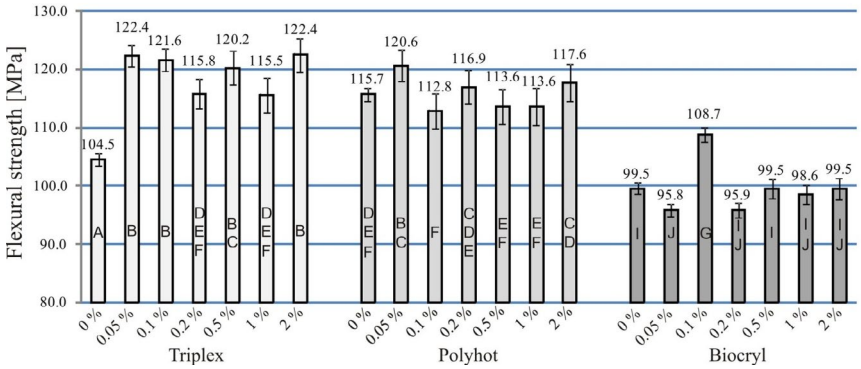

Figure 3. Flexural strength of tested materials with standard deviations and the results of statistical analysis (means that do not share a letter are significantly different).

Table 2. Analysis of variance for modulus of elasticity.

Source	df	Sum of Squares	Mean Square	F Ratio	P
Model	20	17.5184	0.8759	44.42	0.000
Residual	189	3.7271	0.0197		
Corrected total	209	21.2455			

Table 3. Analysis of variance for flexural strength.

Source	df	Sum of Squares	Mean Square	F Ratio	P
Model	20	17707.01	885.35	170.05	0.000
Residual	189	983.99	5.21		
Corrected total	209	18691.00			

The flexural strength of unmodified and modified samples showed similar behavior as the modulus of elasticity. The highest values were obtained with 0.05% nanosilica addition in Triplex and Polyhot, or with 0.1% in Biocryl. Further addition of nano particles caused dissipation of the results, that varied among the materials. The secondary peak of flexural strength was recorded at 2% addition (Tryplex, Polyhot), while it missed out in Biocryl. As in modulus of elasticity, standard deviations of the the modified samples were higher than those of the control. At highest flexural strengths, statistical analysis of unmodified and modified samples showed a significant difference in all three tested materials. Samples modified with 2% nanosilica, were statistically

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different only in Triplex material, values of which are similar to 0.05% nanosilica. In Polyhot and Biocryl, the samples modified with 2% nanosilica did not statistically differ from the samples with 0.05% nanosilica, or from the control samples.

Overall, the highest modulus of elasticity and flexural strength was obtained in nanosilica modified Triplex material. The highest modulus of elasticity in unmodified materials was obtained in Triplex, while the highest flexural strength was obtained in Polyhot.

The comparison to other commercial materials reveal that the materials tested have an increased mechanical properties versus the unmodified and microwave irradiated specimens. Such behavior is notices not only by the nanoparticle modified, but the unmodified specimens as well. Namely, in accordance to the results obtained in [11], the flexural strength of Simgal R (Galenika, Belgrade, Serbia) is between 59 MP_a for the unmodified and 77 MP_a of the microwave irradiated specimen (650 W for 5 min), while for Akrilat-R (ADA Dental Products, Belgrade, Serbia in collaboration with Dentaurum, Ispringen, Germany) is between 66 MP_a for the unmodified and 83 MP_a for the microwave irradiated specimen (650 W, 4 min).

SEM, EDX and DSC analyses

The results of SEM and EDX analyses are shown in **(Figures 4 and 5)**. Both figures depict agglomerates detected in 2% nanosilica loading. Presence of nanosilica agglomerate with dimensions of 272 nm can be seen by secondary electron and back scattering electron mode in Biocryl material **(Figure 4).** The difference between this agglomerate and the particle size measured in the liquid component indicated that the agglomeration continues during polymerization process that is, synthesis of the specimens after liquid and powder components are mixed. Crack in the form of a void is present around the agglomerate, probably formed as a result of fracture during bending. The results of EDX test, which includes Polyhot agglomerate at fracture surface, are presented in **(Figure 5).** It can be seen that in EDX test, irradiation overheats the materials surface, which bulges and degrades, except in the nanosilica agglomerate. Also, EDX spectrum shows the presence of Si **(Figure 6).**

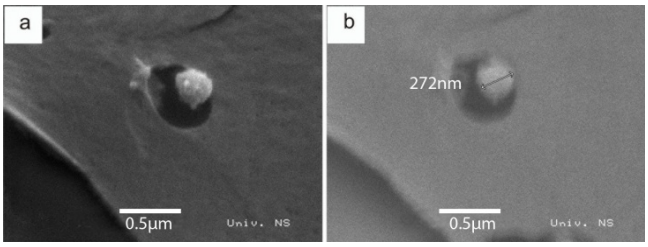

Figure 4. Agglomerate, having a size of 272 nm in Biocryl modified with 1 % of nanosilica: a) secondary electron; b) back scattering mode.

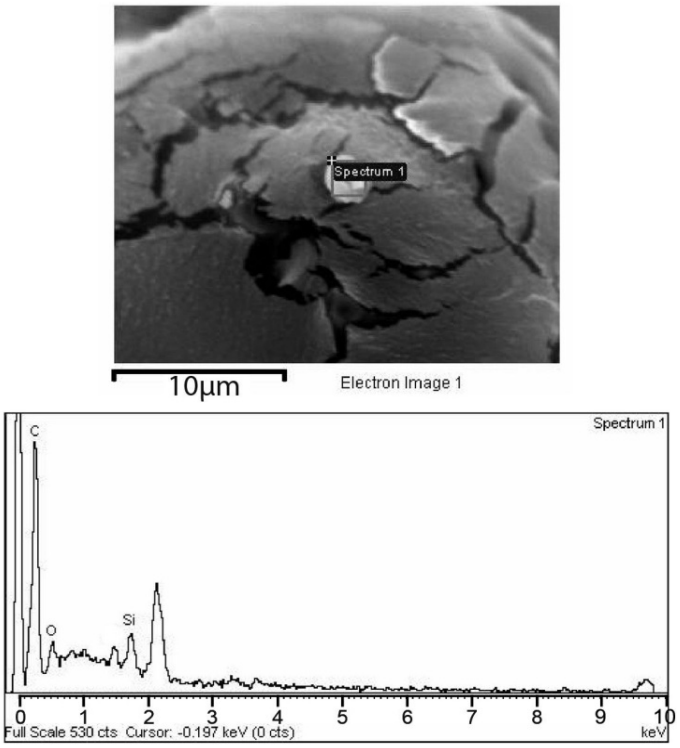

Figure 5. EDX analysis showing the tested agglomerate and the spectrum revealing an elevated Si content in Polyhot modified with 2 % nanosilica.

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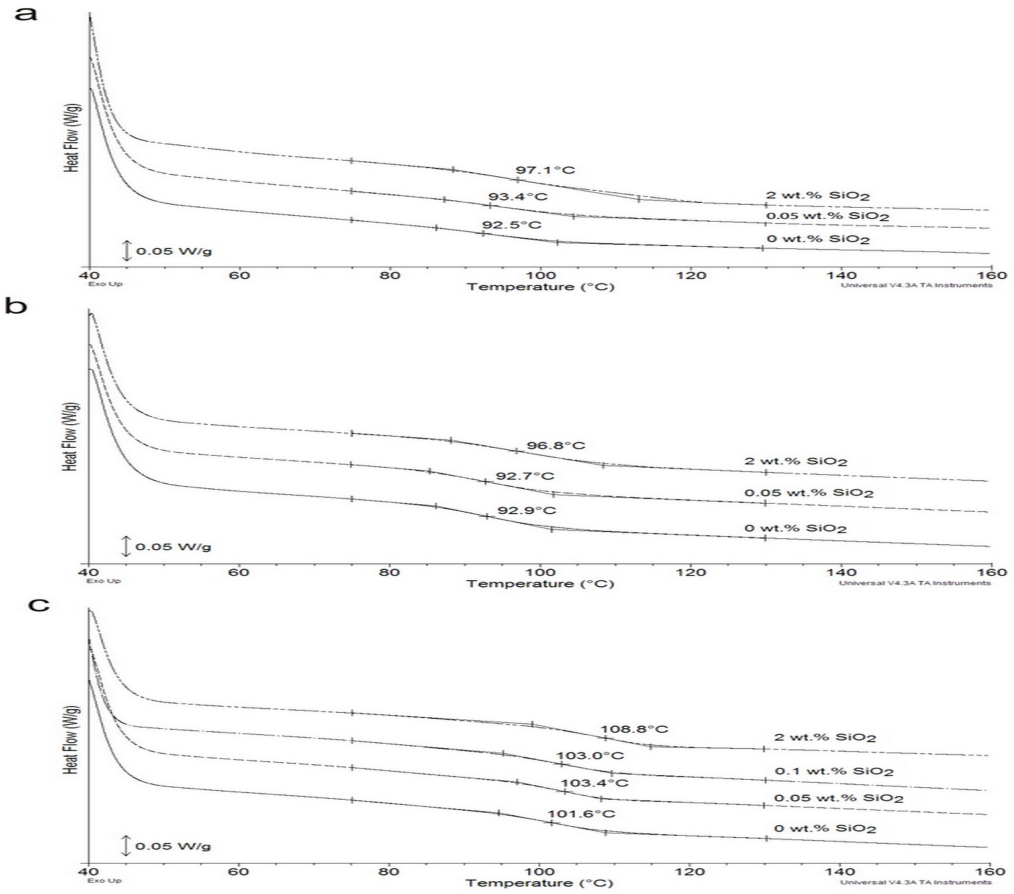

Figure 6. DSC thermograms of: a) Triplex Hot, b) Polyhot, c) Biocryl.

Biocompatibility testing

Most successful nano-modified materials (0.05%, 2%) were chosen for cytotoxicity assessment. Control was set at 0% addition. The results are presented in **(Tables 4 and 5)**, which represent the two different cell lines, meaning L929 mouse fibroblasts and MRC 5 human lung fibroblasts.

In **Table 4**, it can be seen that most profound cytotoxic effect of unmodified materials was caused by Triplex Hot, in relation to the other two materials. In most cases, addition of nano particles diminished the viability of cells. The highest cytotoxicity was observed for 2% nano particle addition for all of the materials, Triplex Hot being the most cytotoxic. Viability of cells decreased gradually over the course of elution period (up to 21 days), except for Polyhot at 2% concentration. Nano addition of 0.05% improved viability of cells in eluates obtained from Triplex Hot, starting from day 5 to day 21.

Table 4. Viability of L929 mouse fibroblasts.

%	0				0.05				2			
Days*	**3**	**5**	**7**	**21**	**3**	**5**	**7**	**21**	**3**	**5**	**7**	**21**
Bio	96.6 ± 4.5	93.8 ± 0.4	100.2 ± 0.75	94.8 ± 1.2	96.5 ± 0.5	95.1 ± 0.7	91.4 ± 2.9	93.2 ± 4.0	96.4 ± 1.8	87.5 ± 1.6	84.2 ± 0.6	75.5 ± 3.7
Trip	98.2 ± 0.2	87.7 ± 0.8	84.6 ± 3.0	85.7 ± 4.5	95.7 ± 1.4	91.8 ± 0.5	85.6 ± 2.2	93.5 ± 2.2	89.3 ± 1.5	84.1 ± 1.6	70.8 ± 1.7	73.7 ± 1.7
Poly	99.0 ± 1.2	97.1 ± 0.5	92.5 ± 1.4	92.1 ± 1.4	95.2 ± 0.3	93.9 ± 0	93.7 ± 0.6	91.5 ± 0	86 ± 0.9	85.6 ± 0.5	85.5 ± 2	88.2 ± 0.9

*Days of elution period

Table 5. Viability of MRC 5 human lung fibroblasts.

%	0				0.05				2			
Days*	**3**	**5**	**7**	**21**	**3**	**5**	**7**	**21**	**3**	**5**	**7**	**21**
Bio	93.8 ± 2.4	98.7 ± 1.8	96 ± 0.8	97.5 ± 0.5	96 ± 0.5	92.4 ± 1.9	92.3 ± 0.9	85.9 ± 4.8	93.7 ± 2.8	88.2 ± 1	75.8 ± 3.3	74.6 ± 2.9
Trip	97.9 ± 1.4	96.1 ± 1.8	91.1 ± 0	83.5 ± 1.5	92.7 ± 2	84.6 ± 3.7	79.9 ± 0.9	77.6 ± 0.7	88.2 ± 0.9	73.3 ± 2.9	71.2 ± 1.09	69.5 ± 2.5
Poly	94.5 ± 2.6	90.7 ± 0	87.6 ± 0.5	85.8 ± 1.4	94.6 ± 0.5	90.6 ± 0.2	92.2 ± 1.4	95.8 ± 4.9	88.2 ± 0.9	73.3 ± 2.9	71.9 ± 0.9	70.3 ± 0.1

*Days of elution period

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In **Table 5**, the highest cytotoxicity of unmodified materials was recorded for Polyhot. Modified materials showed lowest viability in 2% groups, were Triplex Hot and Polyhot yield similar results, while Biocryl proved slightly better. The viability of all cells decreased with eluates obtained over the course of 21 days, with the exception of unmodified Biocryl, were viability improved. Polyhot with 0.05% shoved better results in elution periods of 7 and 21 days, in relation to the control.

Comparing the two different cell lines used in this study, it can be concluded that the results are similar, with MRC 5 cell line being slightly more sensitive to the tested materials. In both cell lines, nano addition of 2% lowered the viability of cells up to 30% (most pronounced in Triplex), which means that the material is considered to be slightly cytotoxic, according to the ISO 10993-5 [12].

DISCUSSION

Based on the results presented it can be seen that the addition of nano particles to PMMA materials can go both ways, regarding mechanical and biological properties. While in the case of Biocryl, addition of 0.05% silica diminished mechanical properties, in other two materials (Triplex Hot, Polyhot) it improved flexural strength and modulus of elasticity. The strengthening effect for Biocryl was achieved with 0.1% nano addition, which is double the amount of the other two materials. The higher amount of addition needed is probably related to the lower powder/liquid ratio and further need for saturation of the material. Decreasing the powder/liquid ratio increases the amount of residual monomer which can be shown by the lower flexural strength of unmodified Biocryl material. Higher amounts of residual monomer present a weak spot and predilection for denture fracture. Pretreatment of powder and addition of nanoparticles, should thus enable construction of more resilient dentures in relation to unmodified ones, or a thinner construction with same properties [4,5,6,13]. This is considered beneficial regarding the comfort of wear and thermal isolation of underlying tissues. For two of the materials tested, secondary peak is observed for 2% nano particle addition. From mechanical point of view this is also considered to be beneficial, but requires additional quantity of the material and increases the difficulty of handling. Limiting the addition to 0.05% prevents the formation of agglomerates while forming more uniform crystal lattices, and as such should be recommended [3]. Well distributed nano particles reinforce the material by restraining the movement of polymer chains, while agglomerates act as a weak spot due to absence of inter covalent bonding. Agglomeration of nano particles is considered to be an issue when handling these type of materials, because Van der Waals forces found in the agglomerates are inadequate to prevent crack formation [14].

Addition of silica nanoparticles up to 2%, did not cause more profound increase in toxicity of the materials. Smaller quantities of silica (0.05%) enhanced mechanical properties of PMMA while not significantly affecting materials biocompatibility. This differed among the cell lines and materials being used, which is understandable when considering their different starting components. The lowest cytotoxicity among modified materials was observed for addition of 0.05%, which increased with the time of elution period. Similar results were obtained from eluates of unmodified specimens, which concurs with the results of previous research [15,16]. Concentration dependent toxicity is expected in prolonged exposition to the material.

In some materials, addition of 0.05% silica improved cell viability **(Tables 4 and 5).** Reinforcing material with silica nano particles creates a more rigid structure with uniform particle distribution, that act as a web when in contact with or in other medium. Residual monomer and other toxic leachable substances are thus incorporated inside of the material which makes it harder to diffuse into surrounding. The issue of remaining monomer while remaining inevitable could thus be minimized [17]. This ability should also prove beneficial in regarding to uptake of soluble agents dispersed in the saliva. This reverse effect should decrease the colonization of microorganisms and retention of unwanted particles, thus improving cleanliness of dentures. Materials modified with 2% nanosilica has proven to be slightly cytotoxic, especially in longer extraction periods. This varied among the materials being used. The polymerization process was identical for all of the materials tested, so the difference is most probably related to their starting components and their mutual interactions with silica nanoparticles. Cytotoxicity of silica nanoparticles has been previously documented [18].

Limitations of this study are related to the number of materials tested. Previously there has been a lot of discussion related to various polymerization cycles, which demands a wider survey of materials which are currently on the market. Additionally, our future research will be based on other biological effects of silica nano addition, meaning adhesion, bactericidal and fungicidal properties, having in mind plausible results obtained from nano addition of TiO_2 and Pt by the other researchers [5,19].

CONCLUSIONS

In accordance with the results presented, the following conclusions can be drawn:

- Modulus of elasticity and flexural strength are strongly dependent on material nano-particle content. The highest modulus of elasticity and flexural strength in all tested materials were obtained with the lowest 0.05 % nano silica particle addition. Higher additions had either a lower reinforcing effect, or even a negative effect, due to agglomeration.
- Addition of silica nano particles to PMMA resin affects cytotoxicity of the material that is concentration depended. Longer extraction periods affects viability of cells depending on the starting composition of the material.
- All of the materials tested were none to slightly cytotoxic.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest relevant to this article.

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