Color Changes and Stainability of Complete Dentures Manufactured Using PMMA-TiO_2 Nanocomposite and 3D Printing Technology - one Year Evaluation

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*Color stability through wear and time, one of the most important esthetic factor, influencing patient's acceptance of an acrylic removable prosthesis, was investigated in vivo in a limited number of studies and solely on prefabricated denture teeth.The aims of the present paper were to assess the color changes occurred with the commercially available PMMA for 3D printing technique, improved by doping with titania nanoparticles, and to evaluate the color stability and stainability of the PMMA with 0.4%TiO_2 denture teeth, on denture wearers, over a period of one year. Ten patients rehabilitated with removable complete dentures or implant retained overdentures manufactured using a new nanopolymer, PMMA doped with 0.4% by weight of TiO_2 as denture base and artificial teeth material and Digital Light Projection (3D printing) technology, were evaluated in the present study. The color of each denture was assessed with a spectrophotometer, before and after use, in CIE L*a*b* system. The before measurement was made on the positive control - PMMA with nanoTiO_2 (PC), while the after was measured the color of the tooth after one year complete denture use. Negative control - PMMA without nanoTiO_2 (NC) was also compared to PC and the following parameters were registered: Value (L), Chroma (C), Hue (H), redness/greenness (a) and yellowness/blueness (b). A color difference (ΔE) between 1.63 and 5.24 was measured for all patients, while for the NC ΔE was triple (17.65). The highest ΔE value for all denture wearers with TiO_2 nanofiler inclusions was below the maximum acceptability threshold, in accordance to the patient's subjective evaluation, who were unable to identify the color change.*

Keywords: nanocomposite PMMA - TiO_2, complete denture, color change, spectrophotometry, acrylic bondings

Color, one of the most important esthetic parameter in restorative dentistry influences patient's acceptance of treatment. The restorative materials should reproduce, as accurate as possible, the shades of natural teeth and possess color stability in the oral cavity, over a long period of time [1].

Polymer poly(methyl methacrylate) (PMMA), the most popular material for partial and complete denture manufacturing in daily practice, has several drawbacks mainly due to a great color instability caused by both intrinsic and extrinsic factors [2]. The intrinsic factors are mostly related to the physical and chemical changes in the matrix of the material itself [3,4], the type of polymerization procedures as well as the contained inorganic materials, such as amorphous silica and glass filler [1] and are due to the oxidation of unreacted pendant methacrylate or chemical bonding breakdown. The extrinsic factors are related to a process of absorption and adsorption of liquids dependent upon oral environmental conditions. The absorption is a slowly process over a period of time and is due primarily to the polar properties of the resin molecules, leading to a staining of the material.

Changes in color can be measured using clinical methods but instruments such as spectrophotometers, colorimeters and spectroradiometers, can help overcoming the shortcomings of visual method by bringing accuracy and facilitating color matching, communication and reproduction. Instrumental devices reduces errors and are more accurate when compared to measurement by eye, being a valuable tool in shade verification (quality control) [5].

Despite of a lower accuracy, the most commonly used shade-matching method is the visual method. Vitapan Classical (Vita Zahnfabrik, Bad Sackingen, Germany), with distinctive shades empiric-based organized, is probably the most popular shade guide in dental practice. The same manufacturer has also an evidence-based shade guide, Vitapan 3D-Master, described according to the Munsell color space in terms of Hue, Value, and Chroma [6].

The perception of tooth color is a complex phenomenon and can be influenced by a number of factors, including the type of incident light, the reflection and absorption of light by the tooth, the adaptation state of the observer and the context in which the tooth is viewed, leading to inconsistencies and bias [7].

Spectrophotometers, measuring the amount of light energy reflected from an object at 1-25 nm intervals along the visible spectrum, are amongst the most accurate, useful and flexible instruments for color matching in dentistry [8].

A spectrophotometer contains a source of optical radiation, means of dispersing light, an optical system for measuring, a detector and means of converting light obtained to a signal that can be analyzed and transformed into a form equivalent to a dental shade guide.

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Paul and co-workers [9], found that spectrophotometers offered a 33% increase in accuracy and a more objective match in 93.3% of cases when compared with observations by the human eye or conventional techniques.

Spectrophotometers precisely measures color from reflectance or transmittance data [5] and have been extensively used in dental research and applications [10], including the quantification of color change caused by processing dental materials [1,11] descriptions of coverage error of dental shade guides [12], color accuracy and precision [13], color perceptibility and acceptability [14] and translucency parameter [15].

To objectively calculate color changes, the CIE-L*a*b* system, established by the Commission Internationale de l'Eclairage (CIE), can be use [16]. The CIE L*a*b* system specifies color perceptions in terms of a 3-dimensional space by comparing the color of the tooth surface with the color of the corresponding control groups through wavelength vs reflection. The *L* axis represents luminosity and extends from 0 (black) to 100 (perfect white). The *a* coordinate represents the amount of red (positive values) and green (negative values), while the *b* coordinate represents the amount of yellow (positive values) and blue (negative values) [17]. The coordinates *a* and *b* coexist in the same plane of this 3-dimensional space.

To improve the characteristics of the acrylic composite for complete denture manufacturing, functionalized TiO_2 nanoparticles have been added, demonstrating better mechanical, antibacterial characteristics and also high physicochemical stability [18–20]. In order to increase the accuracy of the final restoration and also to reduce the unreacted methacrylate, 3D printing technology with an improved nanocomposite was used lately in complete denture manufacturing [21,22].

The gloss and color changes after adding 0.4% by weight TiO_2 nanoparticles and the stainability of the newly obtained nanocomposite have not been yet investigated.

Therefore, the aims of the present study were:

-to assess the color changes occurred after adding 0.4% TiO_2 nanoparticles in the commercially available PMMA for stereolithographic technique;

-to evaluate the color stability and stainability of the PMMA with 0.4%TiO_2 denture teeth on denture wearers over a period of one year.

Experimental part

Ten patients out of the thirty-five enrolled in the cohort study registered with ClinicalTrials.gov Identifier: NCT02911038 and approved by the Romanian Research Bioethical Committee (# 98/2016) have been selected for denture color stability evaluation.

All the patients were rehabilitated with removable complete dentures or implant retained overdentures manufactured using 3D printing technology. An improved nano-polymer, PMMA doped with 0.4% by weight of TiO_2 was used as denture base and artificial teeth material and each complete denture/overdenture was fabricated by Digital Light Projection using the EnvisonTEC Perfactory 3D printer (Gladbeck, Germany) [18]. Dentures were individually designed in 3Shape software (Copenhagen, Denmark) and processed by applying specific manufacturing stages detailed described elsewhere [18]. The materials used for preparing the nanocomposite have been: poly (methyl methacrylate) polymer mixture used for 3D printing procedure (eDent 100, Envision TEC GmbH, Germany) which contains: poly (methyl methacrylate), methyl methacrylate, benzoyl peroxide, silicium and aluminium oxides; TiO_2 (anatase, Aldrich, Germany) which was functionalized according to a previous presented procedure [19]. The surface morphology of the nanocomposites obtained have been investigated with scanning electron microscopy (SEM) technique, using an Oxford Instruments equipment.

The following criteria have been applied for the inclusion in the present study:

-color A2, according to Vitapan Classical shade guide, was choose as teeth shade for denture manufacturing;

-patient should be at least rehabilitate with maxillary denture/overdenture;

-complete maxillary dentures were required to be in good condition, with intact dental anatomy of the maxillary central incisors and with no cracks or fractures in the denture base;

-at least one year of uninterrupted wearing of 3D printed complete dentures.

All the patients signed a consent form and data were assessed during the one year follow-up scheduled visit.

A portable spectrophotometer (Easyshade VITA Zahnfabrik, H. Rauter GmbH, Germany) was used to evaluate the denture teeth color.

Prior to the evaluation, biofilm removal and disinfection of the dentures was performed applying the following protocol:

-immersion for 15 min in a container containing an effervescent tablet, Corega (Glaxo SmithKline, Romania) dissolved in 250 mL of warm water and rinsing afterward in running water;

-washing with neutral soap, thoroughly scrubbing with it by hand friction for 30 s and rinsing in running water [23];

-disinfecting with chlorhexidine 2% (Gluco-Chex, Cerkamed, Poland) by immersing in the solution for 10 min and rinsing in running water.

This procedure is described to be effective only on the biofilm and do not remove the stains that occurred on the acrylic teeth [24].

Measurement protocol

Calibration of the measuring device:

To evaluate the repeatability and the reliability of the measuring instrument, 12 set of measurement of the same tooth have been performed. After each set of measurements the device was turn off, restarted and recalibrated. A good agreement among all color components *L*, *a*, and *b* (repeatability >.91 and reliability >.89) have been obtained, in agreement to other studies [1,25,26].

The Vita Easyshade spectrophotometer has an embedded fiber optic light and can record the tooth shade under any light condition. However, to avoid bias, color was evaluated extra orally with a black background, standard ambient lighting and temperature on the middle third of the buccal surface of the maxillary right central incisor for all dentures. All measurements were made by a single trained operator. Before any measurement, the device was calibrated on its own white ceramic block, according to the manufacturer instructions. The single tooth option was selected from the menu and probe tip was secured perpendicular to tooth surface flushing the whole surface. After the initiation of the measurement procedure, tip was kept stable until the long beep and the tooth shade for each complete denture was recorded with *l, a, b*, values.

To evaluate the color stability, e-DENT 100 sample, with no addition of TiO_2 and polymerized with EnvisonTEC Perfactory 3D printer, using the protocol described by the manufacturer, was considered as negative control (NC). The positive control (PC) was a complete maxillary denture obtained from e-DENT 100 with 0.4% nanoTiO_2 inclusions. Both positive and negative control have been processed 12 month ahead of the measuring date and stored under ideal conditions (in a dark, dry place, at room temperature).

The positive control denture teeth as well as the negative control and all the ten assessed dentures were A2, according to the manufacturer described parameters [27].

Differences between colors were calculated in the CIE L*a*b* system using the color distance between the coordinates of two stimuli with the following Euclidean formula [17]:

Fig. 1. Vita Easyshade spectrophotometer was used to perform measurements: a – A2 shade tab from the Vitapan Classical shade guide was verified prior to any measurement. b-Positive control (PC) was compared to A2 shade using the option Restoration. The differences of Value, Chroma and Hue are represented and *Fair* is the overall color accuracy. c- The screen shows the positioning of the tooth's color measured with respect to the color space coordinates for the A2 shade. ΔE is the color difference calculated using CIE L*a*b* system and ΔE_{LC} is calculated excluding H. d -Color coordinates in CIE L*a*b* system (L, a, b, C, H) for the measured specimen using the Tooth single option are displayed on the screen

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (1)$$

Sequence of measurements:

-Positive and negative control were compared to A2 predefined color Vitapan Classical and the threshold value ÄE was registered (fig. 1 a, b and c).

-The color of each denture was evaluated before and after use. The *before* measurement was made on the positive control (PC), while the *after*" measurement was made on the tooth (central incisor) after one year complete denture use. Negative control (NC) was also compared to positive control (PC). Three consecutive measurements were performed for each specimen and the following parameters were registered: Value (L), Chroma (C), Hue (H), redness/greenness (a) and yellowness/blueness (b) (fig. 1 d).

A questionnaire regarding dentures color assessment was used to subjectively evaluate color change in the artificial teeth. The subjective data collected were compared to the objective data obtained from the spectrophotometric analysis.

Results and discussions

The color assessment of the negative control (e-DENT 100 3D printed as indicated by the manufacturer) and the positive control (e-DENT 100 with nano-TiO_2 unused denture) evaluated with the option *Restoration* of the spectrophotometer are indicated in table 1.

As could be observed in figure 1 b and table 1, the modified material PMMA - nano TiO_2 (PC) had a better

Table 1

COMPARISON OF POSITIVE AND NEGATIVE CONTROL TO SHADE A2

Specimen	ΔL	ΔC	ΔH	ΔE	ΔE_{LC}
PC	3.4	1.5	5.0	4.1	3.7
NC	-4.6	0.5	10.8	5.7	4.5

PC=positive control, NC=negative control, ΔL=differences in Value between specimen and A2, ÄC=differences in Chroma between specimen and A2, ΔH=differences in Hue between specimen and A2, ΔE= color difference between specimen and A2 calculated as Euclidean distance between the two points (colors) in the three-dimensional space, ΔE_{LC}= ΔE calculated excluding H.

color match (A2), when compared to the unmodified PMMA (NC). On the screen of the spectrophotometer (fig. 1b), the difference between the color space positioning of the PC tooth tristimulus values and the corresponding shade tab, could be seen. The bar graphs show that the tooth is lighter, slightly higher chromatic, and yellowish than A2. The overall evaluation was *Fair*, meaning that an expert at shade matching may see a noticeable but acceptable difference between the restoration and the target shade to which it has been verified. NC's overall evaluation was Adjust, meaning that a noticeable difference could be observed between the restoration and the target shade to which it has been verified, an important *mismatch* appears in hue.

The mean values of the parameters measured (Value, Chroma, Hue, redness/greenness and yellowness/blueness) and also the overall estimated color according to Vitapan Classical and Vitapan 3D-Master shade guides, for all the analyzed samples, are displayed in table 2.

As could be observed in table 2, none of the analyzed samples had the original color (A2). The grater color difference appears at the negative control (overall color

Table 2

MEAN VALUES OF THE MEASURED PARAMETERS

Specimen	Vitapan Classical	Vitapan 3D-Master	Value (L)	Chroma (C)	Hue (H)	Redness(a>0) / greenness(a<0)	Yellowness(b>0) / blueness(b<0)
1	A3.5	4M3	82.6	44.7	84.8	4	44.5
2	A3.5	4.5M3	79.5	45.8	84.4	4.5	46.8
3	A3.5	4M3	82.9	43.7	88.1	1.4	43.7
4	A3.5	4M3	83.2	47.8	88.0	1.6	47.8
5	A3.5	4M3	82.2	43.8	88.1	1.4	43.8
6	A3.5	3.5M3	80.9	39.9	85.7	2.8	43.5
7	A3.5	4.5M3	79.9	48.0	85.2	4.0	47.9
8	A3.5	4.5M3	79.8	45.6	84.2	4.6	45.4
9	A3.5	3M3	81.2	39.4	85.6	2.7	43.3
10	A3.5	4.5M3	80.0	48.5	84.5	4.6	48.3
NC	B3	2.5M3	80.8	29.9	91.6	-0.8	29.9
PC	A3.5	4M3	84.3	47.0	87.1	2.4	46.9

PC=positive control, NC=negative control

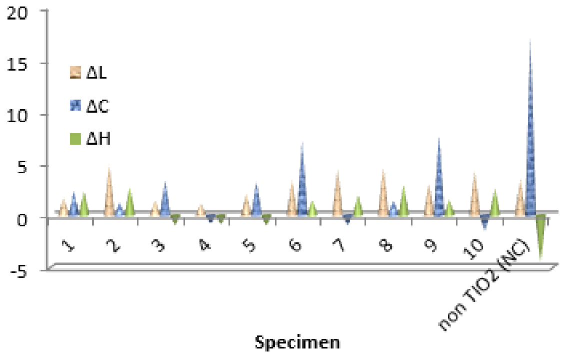


Fig. 2 Differences in Value (ΔL), Chroma (ΔC) and Hue (ΔH) between the specimen analyzed, negative control (NC) and positive control (PC)

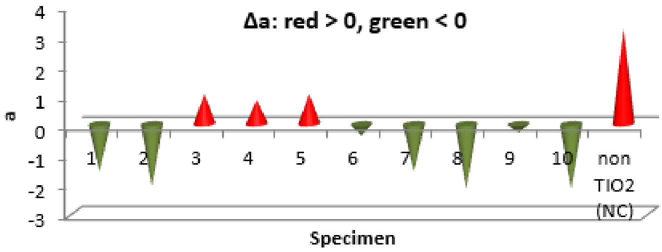


Fig 3 Differences in color on *a* axis (red-green) between the specimen analyzed, negative control (NC) and positive control (PC)

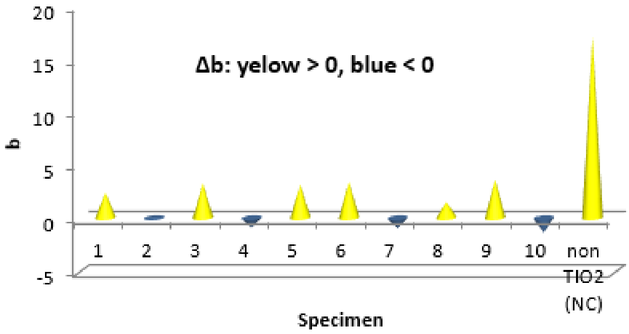


Fig. 4 Differences in color on *b* axis (yellow-blue) between the specimen analyzed, negative control (NC) and positive control (PC)

turns to B3), mainly due to a lower chromatic and a higher color tendency to greenish. However, after one year, all of the samples with nanofillers inclusions registered as main color A3.5. The reason of this color mismatch could be explained by the optical tooth properties, different of those of acrylic resin. Light scattering and absorption within enamel and dentin give rise to the intrinsic color of the tooth, enamel being relatively translucent confers dentin a major role in the overall color [17,28].

One of the aims of the present study was to evaluate the color stability and stainability of the PMMA with 0.4%TiO_2 denture teeth on denture wearers over a period of one year, therefore, each specimen, including negative control (PMMA without nanofiller) was compared to positive control (un-wear denture teeth). The study variables related to the color change for all enrolled participants and also for the negative control, compared to the positive control are listed in table 3 and represented in figure 2-4.

Acrylic resins undergo color changes in the oral cavity. Alteration in the color of acrylic resin denture teeth were described in several in vitro and in vivo studies [1,16,29].

The highest ΔE values observed in these studies varied from 1.82 to7.94 for the in vitro experiments [16,29,30] and up to 11.03 over a period between 6 months and 4 years, in clinical environment [1].

Table 3
STUDY VARIABLE RELATED TO COLOR CHANGE

Specimen	ΔL	ΔC	ΔH	Δa	Δb	ΔE
1	1.7	2.3	2.3	-1.6	2.4	3.35
2	4.8	1.2	2.7	-2.1	0.1	5.24
3	1.4	3.3	-1.0	1.0	3.2	3.63
4	1.1	-0.8	-0.9	0.8	-0.9	1.63
5	2.1	3.2	-1.0	1.0	3.1	3.88
6	3.4	7.1	1.4	-0.4	3.4	4.82
7	4.4	-1.0	1.9	-1.6	-1.0	4.79
8	4.5	1.4	2.9	-2.2	1.5	5.23
9	3.1	7.6	1.5	-0.3	3.6	4.76
10	4.3	-1.5	2.6	-2.2	-1.4	5.03
NC	3.5	17.1	-4.5	3.2	17.0	17.65

NC=negative control, ΔL=differences in Value between specimen and PC, ΔC=differences in Chroma between specimen and PC, ΔH=differences in Hue between specimen and PC, ΔE= color difference between specimen and PC calculated with the Euclidean formula (1)

All performed studies evaluated denture prefabricated teeth and to our knowledge, this is the first study evaluating 3D printed denture teeth color changes.

However, in all cases evaluated for a one year period, except for the negative control, without nanofiller inclusions, the highest ΔE value was 5.24 (table 3), significant lower than the maximum reported by Barão and co-workers [1] on dentures manufactured with commercially available prefabricated teeth.

The important consideration is the range of color changes and if the eye perceives these changes. In the CIE L*a*b*color space, this range of color change is shown by ΔE, which is the algebraic difference between the two colors and almost all the studies have used this system after its introduction [31], leading to measurements in an objective way.

Several evaluation of visual judgments of dental restorations reported a 50:50% CIE L*a*b* perceptibility threshold to human eye (ΔE) of 1.0 [32], 1.8 [10] or 2.6 [33], different than corresponding 50:50% acceptability threshold (ΔE) of 3.5 [10] or 5.5 [33]. However, the highest ΔE value (5.24) for the specimens with TiO_2 nano-filler inclusions is below the maximum acceptability threshold, in accordance to the patient's subjective evaluation. The subjective data obtained from all the participants asked whether they had noticed any changes in the coloring of the artificial teeth in their dentures were negative (none of the subjects noticed color changes) and, therefore, could not be correlated to the objective data obtained from the spectrophotometric analysis.

For the NC (PMMA without nano-inclusions), ΔE was 17.65 (table 3), when compared to PC (PMMA with nano-inclusions) and 5.7 when compared to A2 color (table 1), greater than the human acceptability threshold.

The ability of denture teeth to remain color stable through wear and time is of critical importance and most of the time measures the longevity of patient acceptance of a removable prosthesis.

The etiology of artificial tooth discoloration is multifactorial. Some of the most important factors that contribute to intrinsic and extrinsic staining are: denture wear, lack of patient maintenance, the effect of composite structure and chemical characteristics, exposure to stains, and many others [30,34].

Poly (methyl methacrylate) slowly absorb water in time, which is a property attributable to the polar nature of the resin molecules. Color changes of materials might be mediated by an accelerated oxidation or by penetration of colored solutions.

The sorption of various water soluble metabolites with ionizable groups onto the polymeric dental device surface could be a reason of color change.

The poly (methyl methacrylate) used as polymeric matrix for the dental devices under study present a certain degree of hydrophilicity due to solutions diffusion through intermolecular space. It is known that residual monomer present in the polymerized compound might increase the water sorption [35]. However, due to the technology applied for the denture manufacturing, namely 3D printing, the amount of residual monomer is reduced, therefore its influence is not significant. On the other hand, the polymeric web chains are characteristic for syndiotactic PMMA, therefore it is expected that the pendent groups of PMMA (fig.5) would not allow the molecular chains to get

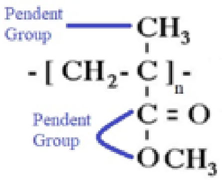


Fig. 5. Poly (methyl methacrylate) -pendent groups

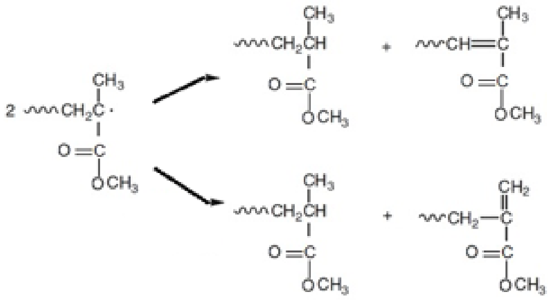
close each other and in consequence would hinder the adjacent chemical groups.

As consequence, within amorphous PMMA the polymeric chains almost do not slip between them. Being rigid, the inner PMMA structure also embraces void areas where adsorbed solutions/soluble metabolites could be accommodated. These diffusing metabolites could cause the color change of the PMMA made dental devices. In the meantime, it is important to underline that through the 3D printing technology the PMMA crosslinking is almost complete and thus, a lower level of fluids sorption would be expected. In such case, one should consider other possible reasons for color change, as well.

The used initial polymeric matrix contains partially polymerized methyl methacrylate (under 25% conversion) and poly (methyl methacrylate) and a peroxide (benzoyl peroxide, fig. 6) as photoinducer of the complete polymerization according to the scheme 1 where a general scheme for PMMA photo-polymerization is introduced.

It is known that peroxides are photoinitiators for different radicalic reactions in organic compounds or composites. For instance, the benzoyl peroxide absorbs light of 378 nm which causes their excitation and further cleavage into radicals as it splits at O-O bond generating two radicals.

Thus, it might initiate in the system under discussion, a free radical polymerization up to a complete reticulated polymer following the known stages of initiation, propagation and termination, when the poly(methyl


(2)

methacrylate) radicals undergo mainly disproportionation, see equation 2 [36].

Regarding the initiation of the free radical polymerization mechanism, it is very likely that no matter the initial radical formation, it is possible to be produced hydroperoxydes following a reaction with oxygen [37]. Such hydro-peroxides are photolabile and are decomposing generating radicals which could subtract hydrogen atoms from poly (methyl methacrylate) and in this way to lead to photooxidation of the polymer. It is then possible a chemical change of color of the polymeric matrix due to its oxidation. Much more, if there is any unpolymerized methyl methacrylate than oxidation at the pendant methacrylate groups level will occur. The consequence would be a change in color.

The material used for polymeric matrix, e-Dent 100 also contains SiO_2 filler. Such filler could adsorb liquid metabolites onto the surface. An important amount of liquids adsorbed hydrolyzes the silica filler. This might cause the appearance of micro crevices and cracks into the polymeric matrix (fig. 7.a).

The existence of developed defects as presented could be the reason for stain penetration and significant color change of the dental devices studied. Another reason for color change of the nanocomposite after 3D printing procedure could be assigned to the possible porosities of the surface [38,39]. However, as our study proved, the 3D printed dentures using the PMMA - 0.4%TiO_2 nano-composite was less affected by color change compared with the 3D printed dentures manufactured from PMMA. This improved behavior could be due to a more compact

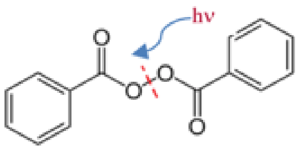


Fig. 6. Benzoyl peroxide cleavage under UV radiation (λ = 378nm)

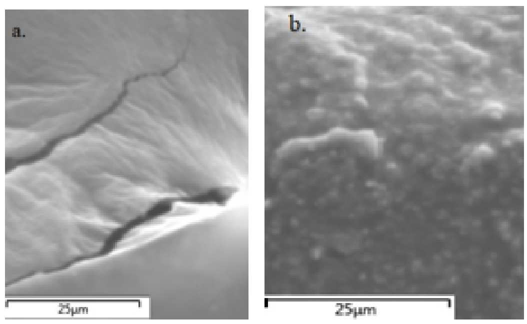


Fig. 7 SEM images of the 3D printed dental devices using different polymeric matrix. a. PMMA; b. nanocomposite PMMA- TiO_2

BPO + hν → BPO*	(excited state) (1)
BPO* + MMA → BPO + MMA* → MMA·	(initiation) (2)
MMA· + x MMA → $(MMA)_x$ MMA·	(propagation) (3)
$(MMA)_x$ MMA· + ·R → $(MMA)_x$ MMA R	(chain-transfer and termination) (4)

Scheme 1. Photopolymerization mechanism. BPO (benzoyl peroxide) photosensitizer; MMA (methylmethacrylate) monomer; R = free radical or polymer fragment

structure of the printing material when no crevices are present (fig. 7b).

Conclusions

To our knowlege, this is the first study assesssing the color changes and stainability of the 3D printed denture teeth in vivo.

Despite the color instability (ΔE) between 1.63 and 5.24, measured in all denture wearers with TiO_2 nanofiler inclusions, the color change over one year period was below the maximum acceptability threshold, in accordance to the patient's subjective evaluation, who were unable to identify the color change. However, for the NC, without nano-titania inclusions, ÄE was triple (17.65), leading to the conclusion that color stability increases with doping the polymer for dental usage with 0.4% by weight TiO_2 nanoparticles.

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