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POTENTIAL TOXICITY EVALUATION FOR A NANO-MODIFIED VENEERING CERAMIC SYSTEM. EXPERIMENTAL STUDY IN DOGS

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ABSTRACT

Statement of problem: Although feldspathic porcelain is esthetic and biocompatible, it's considered a weak point in zirconia restorations. Researches are necessary to improve its flexural strength without affecting biological properties.

Purpose: to evaluate the possible toxic effects of incorporating ZrO_2 Nanoparticles into conventional veneering ceramic system.

Materials and Methods: Four veneering ceramic systems [Ex0, Ex5, Ex10&Ex20] were formulated by incorporating zirconia NPs into feldspathic powder [0%, 5%,10% & 20% wt.] respectively. Twenty dogs were grouped into 4 groups to receive the 4 experimental ceramic systems. The 3rd mandibular premolar for each dog was prepared for zirconia crowns. Impressions were taken for production of zirconia copings, veneered with experimental ceramics & delivered. After 3& 6 months gingival specimens were collected& subjected to Real-time PCR test to assess genotoxicity of the 4 experimental veneering ceramics.

Results: No genotoxicity was recorded for all groups in both 3& 6 months. However, appoptic genes recorded in Ex0: 0.64±0.01 &0.65±0.02, Ex5: 0.67±0.02& 0.67±0.01, Ex10:0.72±0.01 &0.73±0.02 and in Ex20: 0.88±0.01& 0.89±0.01 compared to control (housekeeping gene) 0.96± 0.01 & 0.99±0.01 for3&6 months respectively. From clinical perspective; No local biological reactions were recorded.

Conclusion: No genotoxic effect was recorded in all groups. whereas, appoptic genes expressed higher level in proportional to NPs ratio. Generally, a satisfactory soft tissue response was observed.

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INTRODUCTION

Zirconia-based ceramics have attracted the attention of researchers because of their excellent esthetics, superior flexural strength and biocompatibility[1]. On contrary, most veneering ceramics have low flexural strength; putting their strengthening as important research point to reduce fracture incidence of zirconia-based restorations [2].

Evolution of Nanotechnology was a promising solution to produce a novel veneering ceramic system [3]. It is generally assumed that flexural strength of Nano-ceramics is higher than conventional ones**.** Moreover, the "in situ Nano-toughening" effect exerted by nano fillers within amorphous ceramic matrix can provide an extra crack bridging and deflection mechanisms[4]. Additionally, ceramics containing fine metastable zirconia grains [ZrO_2], have a superior benefit of transformation toughness[5]. Hence; yttria stabilized Zirconia Nanoparticles was the first candidate as a reinforcing agent for veneering ceramics.

Oral environment demands certain biological standards for dental restorative materials because of its confidentiality; Saliva is loaded with bacteria & possesses corrosive properties. Moreover, immune system behaves differently in oral epithelium &connective tissue rather than in the rest of body and hence, the Nano modified veneering ceramic intended to be used intra-orally should have strict pre-requisites ; First: harmless to local oral tissues and contain no toxic leachable agents which may be absorbed into circulatory system. Second: It should be free of allergic agents. Third: It should neither contain genotoxic nor mutagenic agents that alter the base pair sequence of DNA resulting in apoptosis or transferring genetic damages to subsequent generations. Finally, it shouldn't be carcinogenic as DNA alterations may promote a generation of malignant tumors [6].

Previous studies have been made to evaluate biocompatibility of Zirconia Nanoparticles NP used in dentistry and there was a great debate regarding to what extent reinforcing NP will benefit or pose risks for human teeth [7], some studies reported short- and long-term significant DNA damage in human cells inducing apoptosis associated with ZrO_2 NP [8] , other studies have reported ZrO_2 NP could induce mild or no cytotoxic effects [9, 10] .Hence, experimental animal research was found to be a must to find out whether the addition of Zirconia NP to conventional veneering porcelain can negatively affect its biological properties or not.

MATERIALS AND METHODS

Twenty dogs were housed in the animal house "Faculty of Veterinary Medicine, Cairo University". Approval for this research was obtained from Research Ethics Committee, Faculty of Dentistry, Tanta University. The procedures were designed in accordance with the guidelines for the responsible use of animals in research as a part of scientific research esthetics recommendation of Research Ethics Committee, Faculty of Dentistry, Tanta University.

Dogs were randomly divided into four groups I, II, III and IV (n=5) based on composition of veneering ceramic EX0, EX5, EX10 and EX20 respectively. Each group received general anesthesia, placed on surgical table, dogs' mouths were opened, and their mandibular teeth were cleaned and dried.

Primary impressions were taken for each dog using alginate for further preparation of custom trays. Mandibular 3rd premolar was prepared so that; a 1mm occlusal reduction & 1mm shoulder finish line with a total convergence 6 degrees was made. Custom trays were loaded with silicon-based impression material" *Zhermack Zetaplus Condensation silicon, Badia Polesine, Italy"* to record impressions of prepared teeth, then poured using type IV extra-hard dental stone into master casts **[Fig 1a&1b]**.

Master casts were scanned "*Smart optic",* data was digitized where copings were designed with

exocad software so that; axial walls thickness was 0.6 mm and occlusal thickness was 0.6mm. Zirconia blocks "*katana™* " were milled and post sintered at1500□C to produce zirconia copings. Experimental veneering ceramics (EX0 ,EX5, EX10 and EX20) were then manually layered over their corresponding zirconia cores, and underwent firing cycle at 930 °C for 45min according to manufacturer's recommendations . Finished crowns were cemented to their corresponding prepared teeth using *Panavia F 2.0* **[Fig 1c]**.

After three months, Animals were again anesthetized and fixed on surgical and Two vertical incisions were made with a horizontal releasing incision extending from mesiobuccal to distobuccal line angles of 3rd premolar to take gingival samples from all dogs and same procedures were repeated after 6 months to collect gingival samples. Gingival samples of all dogs at 3months and 6months underwent *Real-time Polymerase chain reaction* [***PCR***] to determine ***caspase-3 gene*** expression level (appoptic genes) compared to ***GAPDH*** the housekeeping gene (control genes) for assessment of possible genotoxicity of experimental .

Gene expression level values of both Caspase-3 and GAPDH were collected for all experimental groups (I, II, III and IV), tabulated. And underwent statistical analysis.

RESULTS

Evaluation of Genotoxicity:

Numerical casepase-3 gene expression level values for experimental groups I,II,III & IV and for **GAPDH** (control group) after three and six months. One-way ANOVA and Tukey-test were used to compare difference between groups within the same period. Paired t- test was then used to compare gene expression levels at 3 and 6months.

After 3months of crowns insertion in experimental animal mouths, GAPDH expression level recorded a mean value 0.96± 0.01 and reached **0**.99±0.01 after 6months. A highly significant reduction in Caspase-3 gene expression level values was observed in all investigated groups compared to control GAPDH gene. On the other hand, Caspase 3 gene expression level showed highly significant increase in all experimental groups compared to each other as; group I was (0.64±0.01)&(0.648±0.016) after 3 and 6months respectively, Group II recorded (0.67±0.02) & (0.669±0.014), Group III recorded (0.72±0.01) & (0.733±0.015) and (0.88±0.01) & (0.891±0.010) in Group IV [Fig.2].

Correlation analyses were performed to analyze the relation between Caspase-3 gene expression level at 3 and 6 months to identify the effect of time factor and the result was that there's no correlation exists.

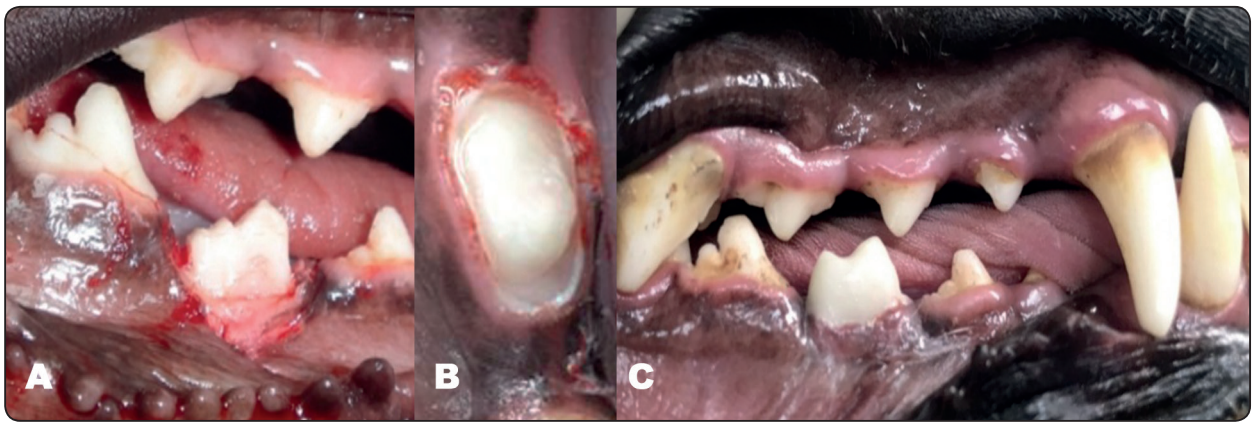

Fig 1. (a) 1.5mm occlusal & 1mm axial reduction for mandibular 3rd premolar, (b) circumferential shoulder finish line and (c) cemented crown in place

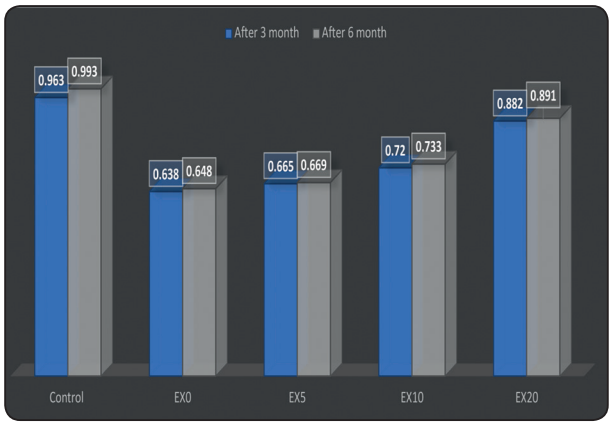

Fig. (2) Caspase-3 gene expression level values in experimental groups compared to control gene after 3 and 6 months.

Evaluation of Local Biological Reactions

Clinical evaluation of all experimental restorations showed absence of any signs of gingival inflammation around restorations during three and six months of the experiment except for some plaque retention. No signs of pulpal injury (hypersalivation, loss of appetite and bad breath) was detected. Surgical sites healed well. No allergic reaction provoked neither in the form of Lichenoid nor erosive lesions in oral mucosa or skin in experimental animals.

DISCUSSION

Experimental work was necessary in the current study to investigate biological effect of incorporating ZrO_2 NPs into conventional veneering ceramic on local tissues [Gingival, pulpal & mucosal] and on DNAs [Genotoxicity] of dogs [11]. Dog" was selected as an animal model because its teeth size is comparable to those of humans indicating an occlusal force near that of humans particularly; housed dogs due to their starch-rich diet. The selected tooth to be prepared was mandibular 3rd premolar not only due to its accessibility but also because of absence occlusal contacts at dog's premolars [12].

After three and six months from restorations delivery, samples of gingival tissue close to experimental restorations were cut to undergo Real-time Polymerase Chain Reaction (PCR) test for assessment of genotoxicity. Real-time PCR was selected rather than conventional PCR as it's less time consuming, easier lab work& more sensitive and precise [13].

In this current study, the piece of tested DNA was Caspase3- Gene which plays vital role in regulating inflammatory responses and apoptosis when cells are threatened by noxious agents and therefore, it was selected to be screened in the present study[14]. On the other hand, Housekeeping genes e.g. GAPDH were used as control group as they're stable, normally expressed in cells and tissues and do not show changes under experimental or disease conditions [15].

Results of the present study showed that after 3months and 6 months, there was significant reduction in the expression level of caspase enzyme in all investigated groups compared to control gene. Decreased expression of apoptotic genes [Caspase-3 gene] constitute the potential mechanism of prolonged longevity [non-appoptic activity] an indicator for being non-genotoxic [16] in accordance with **Noushad et al., (2009)** [11] and **Covacci et al.,(1999)**[17]. on the contrary, the current study disagrees to **Atalaya et al., (2018)** [18] and **Brunner et al., (2006)** [19] who concluded that ZrO_2 NPs can induce DNA damage and apoptosis at all studied concentrations. This different finding may due to the smaller particle size in **Atalaya's**[18] and different shape and composition of NPs in **Brunner's**[19] besides, the different assessment method.

Caspase 3 gene expression level showed highly significant increase in all experimental groups compared to Ex0 after 3months explained by presence of noxious agent in experimental samples, hence the initiator caspases were activated to stimulate Executioner Caspases [including Caspase 3; the gene under investigation] to start apoptosis as a defense response. On the other hand, after 6months, an increase in the level of Caspase 3 gene

expression was observed in all groups. The increased expression of appoptic genes proportionally with ZrO_2 NP concentration comes in accordance with **Brunner et al., (2006)** [19], **Atalaya et al., (2018)**[18] and **Dhanalekshmi and Meena (2016)** [20] who accounted for DNA damage associated with ZrO_2 nanoparticle due to their tiny size, greater surface area and high surface energy [21] that enable them to intercalate within DNA strands resulting in DNA damage. **Di Virgilio et al.** [22] added that the higher concentration of NPs, the more micronucleus frequency and DNA damage.

As for the ***Time Factor***, the current study showed that gene expression level at the initial 3months was higher than that of 6months recall. This finding can be explained by the fact of ceramic ion leaching in saliva is always of higher rates, initially whereas, it reaches a constant rate after < 100 days of exposure to the oral aqueous medium in accordance with **Wataha and Lockwood (1998)** [23] and **Elshahawy et al., (2013)** [24]. Therefore, there's no correlation between 3- and 6-months gene expression level.

Regarding ***Clinical Findings***, satisfactory soft tissue response was observed except for plaque retention on experimental crowns surface this may be due to lack of glaze firing cycle and resultant surface roughness.

CONCLUSION

- Experimental ceramics had no genotoxic effect.
- Appoptic gene expression recorded the highest level in Ex20, followed by Ex10, then Ex5 and finally Ex0.
- Satisfactory soft tissue response was observed for all experimental groups

RECOMMENDATIONS

Controlled clinical study should be carried out by applying experimental ceramics into a human volunteer over short period of time.

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