Advances in Dentistry & Oral Health
ISSN: 2472-6389

Juniper Publishers
key to the Researchers

Research Article
Volume 16 Issue 5 - November 2023
DOI: 10.19080/ADOH.2023.16.555948

Adv Dent & Oral Health

Biological Behavior of Different Retraction Materials in Prosthetic Dentistry: In Vitro Study

Stergidou Aggeliki[1], Theocharidou Anna[2]*, Bakopoulou Athina[2] and Koidis Petros[3]

[1]*Postgraduate student, Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

[2]*Assistant Professor Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

[3]*Professor Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Submission: October 22, 2023; **Published:** November 08, 2023

***Corresponding author:** Theocharidou Anna, Assistant Professor Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

Gingival retraction is essential for an accurate impression in prosthetic dentistry. Various techniques have been used for gingival tissue displacement, classified as mechanical, chemo – mechanical. Retraction pastes have been recently developed and combine mechanical hygroscopic expansion of kaolin matrix with hemostatic activity of aluminum chloride. Four materials for retraction were tested. Three concentration 1:1 (original eluate) and 1:2, 1:4 dilutions with culture medium and two times of exposure were evaluated. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]and ICP Inductively Coupled Plasma atomic emission spectroscopy assays were performed. Higher mitochondrial activity were reported in case of paste subgroups after 24 hours and 48hours especially at subgroups of 10 min exposure. After 72hours a statistically significant increase of mitochondrial activity was reported for paste subgroups, while a mitochondrial activity recovery was reported for all tested materials. These groups caused a temporary cell viability reduction after 10 min exposure. A time dependency of exposure time reported, as cell viability was significantly reduced at all tested subgroups after 20 min exposure at tested materials. In the present study, exposure to retraction paste resulted in higher cell viability than impregnated cord, non – impregnated cord or hemostatic solution for the same tissue.

Keywords: Hemostatic Solution; Prosthetic Dentistry; Gingival Retraction; Kaolin Matrix; Hygroscopic Expansion; Gingival Tissue

Abbreviations: MMP: Matrix Metalloproteinases; ICP–AES: Inductively Coupled Plasma Atomic Emission Spectroscopy; FBS: Fetal Bovine Serum

Introduction

Gingival tissue displacement is an essential clinical procedure in prosthetic and restorative dentistry, which aims at moisture / bleeding control, atraumatic tooth preparation and exposure of preparation margin for impression taking procedure [1,2]. According to [3] materials used for gingival retraction should be efficient in both lateral and vertical displacement without causing permanent tissue destruction and undesirable systemic side effects [1-6]. Chemo – mechanical method is the most commonly used technique [7-10] based on both mechanical displacement action of the retraction material and chemical hemostasis achieved by different medicaments, either vasoconstrictors or astringents [11]. Impregnated gingival retraction cord is the most frequently used chemo – mechanical method by dental clinicians [7-10]. However, the technique is considered relatively sensitive and demanding. Thus [12] They are inserted in gingival sulcus in an injectable form, exerting much lower pressure to the tissues [13] Due to their acidic pH (0.8 – 3), most retraction medicaments can undesirable local side effects both to the exposed dentin and periodontal tissues [14,15,16]. Several in vivo animal and human studies have shown tissue damage caused by impregnated retraction cord and more recently, retraction paste materials [17-22]. Toxicity of retraction agents has also been examined in cell cultures [23] showed the cytotoxicity of different retraction solutions and their dilutions on Chinese hamster lung fibroblasts, using Trypan Blue dye exclusion test, colony forming ability test and MTT assay. Yalcin [24] evaluated cytotoxicity on human gingival fibroblasts with a real – time cell analyzer, while other studies examined cytotoxicity of astringent agents using MTT assay [25,26]. In a different retraction cord and their eluates on human gingival fibroblasts and proved the cytotoxicity of both mechanical and chemo – mechanical agents [27]. However, the

effects of retraction paste materials have not been examined on cell cultures. The aim of this study was to evaluate cytotoxic effects of retraction paste compared to other gingival retraction agents on human gingival fibroblasts.

Materials and methods

Four retraction agents were examined:

a) Retraction paste – 15% aluminum chloride (AlCl3) (astrinGIVAL - AHL Brand)

b) Retraction cord impregnated with aluminum chloride (AlCl3) 0.50 ± 0.1 mg / inch (SURE - Cord® - Knitted Retraction Cord 00)

c) Non – impregnated retraction cord (SURE - Cord® - Knitted Retraction Cord 00)

d) Hemostatic solution – 25% aluminum chloride (AlCl3) (Hemostal - Prevest Denpro).

Eluate preparation - dilutions

Paste and cord materials were extracted in culture medium (Dulbecco's Modified Eagle Medium - Biosera) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25μg/ml amphotericin B at 1cm2 / ml surface / volume ratio (ISO 10994 – 12). Hemostatic solution was diluted as 0,6 mg / ml in correspondence with aluminum chloride concentration within the impregnated retraction cord. Materials were incubated for 24h at 37°C, in 100% humidated atmosphere of 5% CO_2. After elution period, eluates were sterilized with syringe filter (Corning®, 0.2 μm pore PES membrane) and diluted again in culture medium. Finally, 3 concentrations were used per material: 1:1 (original eluate) and 1:2, 1:4 dilutions with culture medium.

Cell culture

Human gingival fibroblast cultures were obtained from explants of gingival tissue, after surgical extraction of third molars from healthy donor. Epithelial layer of the gingival tissue was discarded, and the connective tissue was sliced into small pieces (1 mm^3) and placed into 25 cm^2 culture flasks with culture medium (Low Glucose w/ Stable Glutamine w/ Sodium Pyruvate – Biosera) supplemented with 10% fetal bovine serum (FBS, GibcoBRL) and 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25μg/ml amphotericin B. The culture flask was incubated at 37°C, in 100% humidated atmosphere of 5% CO_2. At 80% confluency, cells were subcultured by 0.25% trypsin/ 1mM EDTA solution (Gibco, Invitrogen)) into larger flask 75cm^2 (passage 1). Cell culture of passage 5 was used for the experimental part of the present study.

MTT colorimetric assay

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay28 was used to examine cytotoxicity of the retraction agents. Cells were seeded into 96 - well plates at a concentration of 5 x 103 cells/well, with 200μl culture medium and incubated for 24h at 37°C, in 100% humidated atmosphere of 5% CO_2. Subsequently, after removal of culture medium cells were treated with different retraction agents for 10 or 20 min. Cells left untreated, and cells exposed at dilution medium served as control 1 and control 2 respectively. After incubation, eluates were removed, wells were washed with PBS and 200μl of culture medium was added per well. Viability of cells was measured by MTT assay immediately (0h) and 24, 48 and 72h later.

Inductively Coupled Plasma atomic emission spectroscopy (ICP – AES)

Inductively Coupled Plasma atomic emission spectroscopy (ICP – AES)29 was used to quantify aluminium concentration in eluates of retraction paste, impregnated cord and hemostatic solution both before and after 20 min of cell exposure. Aluminum measurement was conducted using ICP-AES (Perkin Elmer, model 3100XL) at 308.215 nm wavelength with argon plasma at 1350W and detection at ultraviolet light.

Results

MTT colorimetric assay

Results from MTT assay are presented in (Figures 1-4) as a ratio between treated cells and controls, depending on time points. At time point 0h, oxidative mitochondrial activity was decreased significantly only in case of impregnated cord subgroups after 10min exposure time and 20min (1/ 2 concentration)($p<0.05$). Cell metabolic activity of the rest of groups was not reduced significantly ($p>0.05$). (Figure 1). At 24h, paste subgroups (1:2 / 10 min και 1:2 / 20min) presented significantly higher oxidative mitochondrial activity and thus cell viability compared to control group($p<0.05$). In contrast, reduction of cell viability at solution subgroups was observed, in subgroups 1:2 / 10 min ($p<0.05$), 1:4 / 10 min ($p<0.01$), 1:1 / 20 min ($p<0.001$) 1:2 / 20 min ($p<0.01$). Mitochondrial activity reduction was reported at non impregnated cord for exposure time 10min, in subgroups 1:1 / 10min ($p<0.0001$), 1:2 / 10min ($p<0.001$) and 1:4 / 10 min ($p<0.001$). So, after 24h only at paste groups a mitochondrial activity recovery was reported. (Figure 2). At 48h, mitochondrial activity presented a further significant increase at paste subgroups with exposure time 10 min ($p<0.01$), while all the rest of the groups were found to present more reduced values, indicating that the type of retraction (chemical/ paste) and the minimum time of exposure (10min) were more beneficial for mitochondrial activity (Figure 3). After 72h, mitochondrial activity at paste subgroups were increased significantly both after 10 and 20min exposure ($p<0.001$). Cell viability of all cord impregnated subgroups and solution and non-impregnated cord subgroups did not differ from control group ($p>0.05$) after 10min exposure, while subgroups of solution 1:2 / 20min, 1:4 / 20min and non - impregnated cord 1:1 / 20min

How to cite this article: Stergidou A, Theocharidou A, Bakopoulou A, Koidis P. Biological Behavior of Different Retraction Materials in Prosthetic Dentistry: In Vitro Study. Adv Dent & Oral Health. 2023; 16(5): 555948. DOI:10.19080/ADOH.2023.16.555948

1:4 / 20min presented statistically significant reduction ($p<0.05$) (Figure 4). Comparing paste with the rest groups, higher oxidative mitochondrial activity was observed at 1:2 and 1:4 dilutions at all time points (Figure 5). A recovery of mitochondrial activity was observed in all tested groups of 10min exposure, after 72hours (Figure 6). A significant reduction of cell viability was reported at all subgroups at 20 min exposure, except the subgroups of paste retraction material (Figure 7).

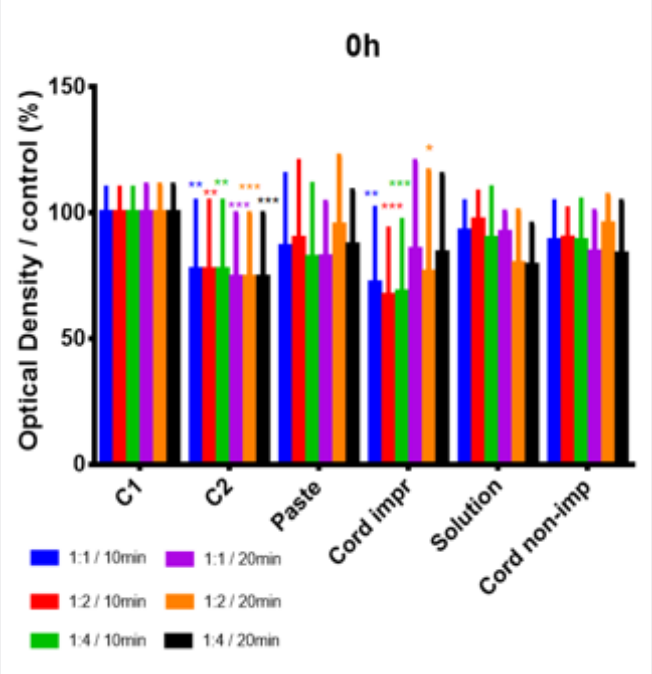

Figure 1: MTT results at 0 hours.

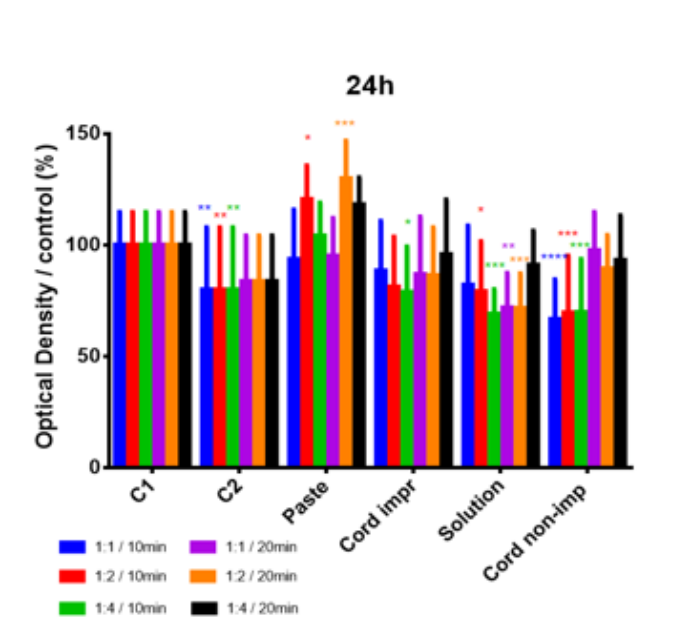

Figure 2: MTT results 24h after exposure.

How to cite this article: Stergidou A, Theocharidou A, Bakopoulou A, Koidis P. Biological Behavior of Different Retraction Materials in Prosthetic Dentistry: In Vitro Study. Adv Dent & Oral Health. 2023; 16(5): 555948. DOI:10.19080/ADOH.2023.16.555948

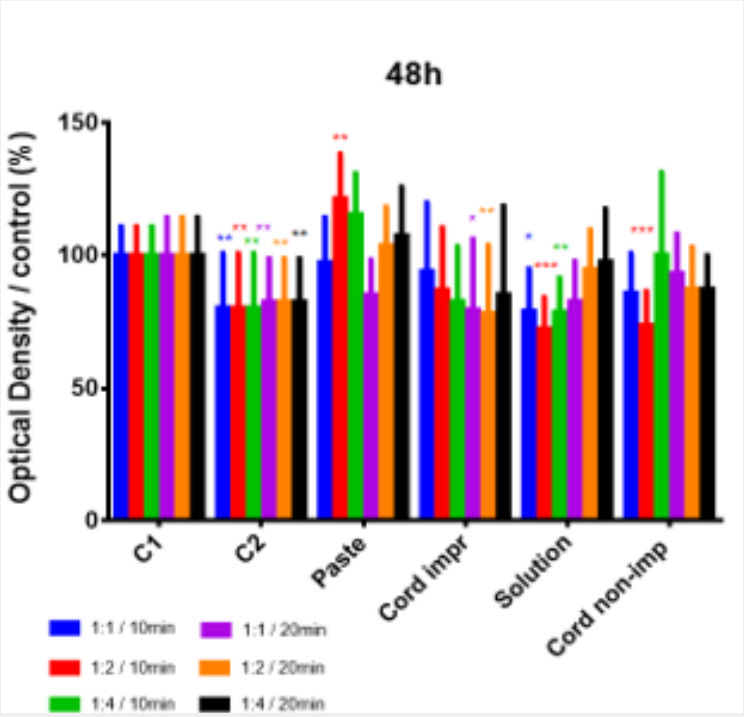

Figure 3: MTT results 48h after exposure to retraction materials subgroup.

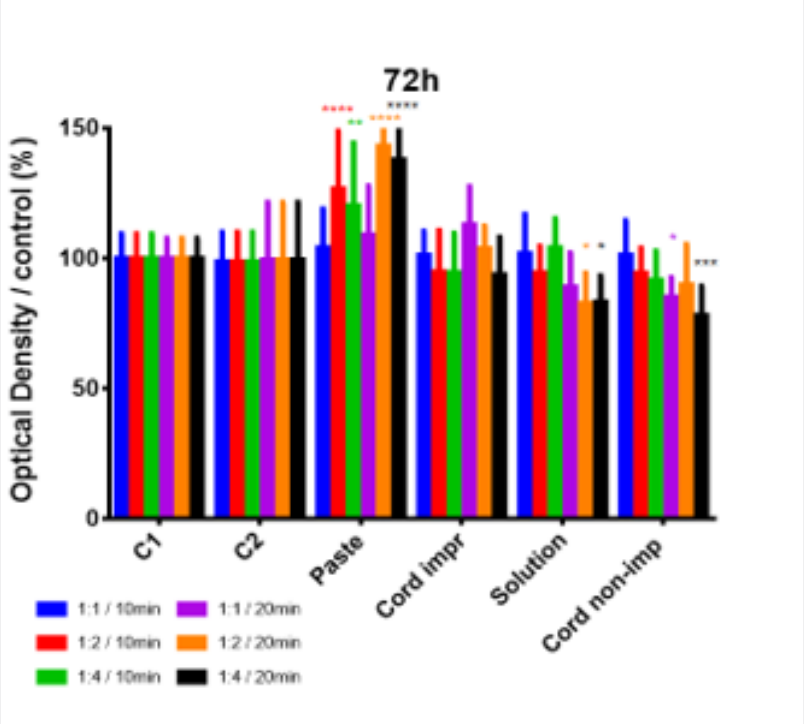

Figure 4: MTT results 72h after exposure to retraction materials subgroup.

How to cite this article: Stergidou A, Theocharidou A, Bakopoulou A, Koidis P. Biological Behavior of Different Retraction Materials in Prosthetic Dentistry: In Vitro Study. Adv Dent & Oral Health. 2023; 16(5): 555948. DOI:10.19080/ADOH.2023.16.555948

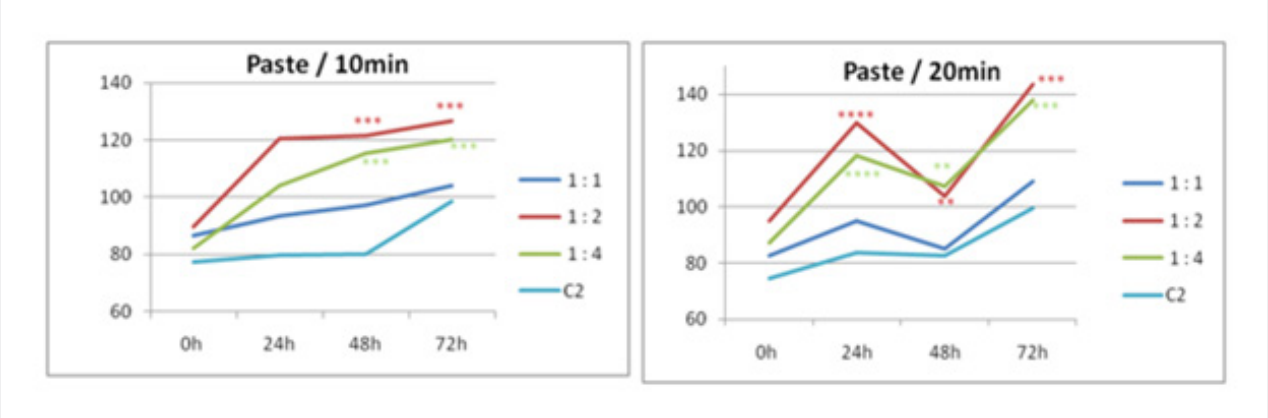

Figure 5: Mitochondrial activity of h-GFs, after 10- and 20-min exposure at paste retraction material, at several dilutions (1:1, 1:2, 1:4).

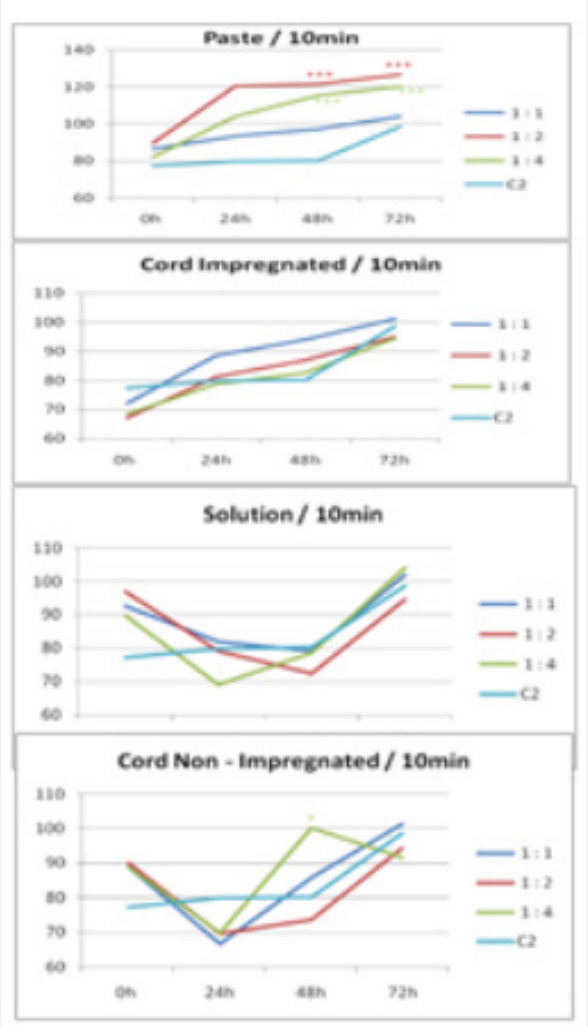

Figure 6: MTT assay revealed that after 72h mitochondrial activity recovered in all cases of 10 min exposure.

How to cite this article: Stergidou A, Theocharidou A, Bakopoulou A, Koidis P. Biological Behavior of Different Retraction Materials in Prosthetic Dentistry: In Vitro Study. Adv Dent & Oral Health. 2023; 16(5): 555948. DOI:10.19080/ADOH.2023.16.555948

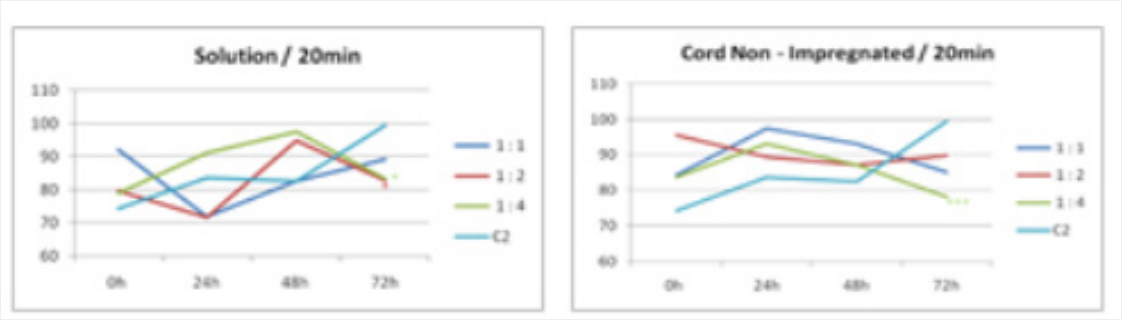

Figure 7: Cell viability reduction reported at solution and cod impregnated Subgroups after 20 min of exposure.

Inductively Coupled Plasma atomic emission spectroscopy (ICP – AES)

Results from ICP – AES evaluation are presented in Table 1. Aluminium concentration in 1:1 paste eluate before cell exposure was 188,3 mg/L , whereas after cell exposure 109,7 mg/L. In contrast, aluminum concentration in 1:1 impregnated cord eluate and 1:1 hemostatic solution was 1,11 mg/L and 0,35 mg/L before, and 0,87 mg/L and 0,15 mg/L after cell exposure, respectively (Table 1).

Table 1: ICP results.

Test group	Eluates	Dillution	Result/ mg/L
1	Paste Astringival		188.3
2	Cord ipmregnated		1.11
3	AlCl3		0.35
4	DMEM		0
5	Paste Astringival	10-Jan	13.61
6	Cord ipmregnated	1/10	0.16
7	ACl3	10-Jan	0.12
	Eluates After 20 min exposure		
1	Paste Astringival		109.7
2	Cord ipmregnated		0.87
3	AlCl3		0.15
4	DMEM		
5	Paste Astringival	10-Jan	4.71
6	Cord ipmregnated	1/10	0.33
7	ACl3	10-Jan	0.08

Discussion

Retraction pastes were introduced as both materials and methods for chemo – mechanical way of gingival retraction in prosthetic dentistry. They are viscous retraction materials containing 15% aluminium chloride, 50% fillers (commonly kaolin matrix), 15% H2O and some modifiers30. It has been documented that paste materials exert much less pressure to gingival tissue than retraction cords (up to 37 times)13, better patient tolerance without requiring local anesthesia and significant less trauma on sulcular and junctional epithelium [22]. Regarding cytotoxicity of retraction agents, several studies examined vasoconstrictor and astringent solutions and gels [23-26] in vitro, but there is little literature concerning the comparative evaluation of mechanical and chemo – mechanical retraction agents, both by direct contact and eluates on human gingival fibroblasts [27]. Although clinical and histopathological effects of paste materials have been examined [22,30,31] there is lack of evidence concerning cytotoxicity and ion release.

How to cite this article: Stergidou A, Theocharidou A, Bakopoulou A, Koidis P. Biological Behavior of Different Retraction Materials in Prosthetic Dentistry: In Vitro Study. Adv Dent & Oral Health. 2023; 16(5): 555948. DOI:10.19080/ADOH.2023.16.555948

In the present study, eluates of impregnated cord caused a significant decrease in oxidative mitochondrial function at both the 10 and 20 min exposure times, as showed by MTT assay. This finding is in agreement with Liu et al., who examined gingival retraction cords impregnated with aluminium sulphate, DL-adrenaline HCl and non-impregnated cord eluates [27]. Although mitochondrial function reached control levels for most groups at 72h (except hemostatic solution subgroups 1:2 και 1:4 and non impregnated cord 1:1 and 1:4 at exposure time 20min), indicating the reversibility of cytotoxic effects, pattern of mitochondrial response varies depending on material, concentration and exposure time. Kopac et al.23 found greater reduction of mitochondrial function after 48h, with no intermediate time point.

According to ICP – IES results, retraction paste eluates had much higher aluminium concentration than both cord impregnated and hemostatic solution eluates. Also, aluminum concentration after cell exposure decreased, indicating possible cell intake. Jones [31] investigated the effect of ions on cell proliferation and supported that aluminium ions stimulate cell proliferation, inducing both S-phase DNA synthesis and cells' mitosis. This result could explain the better biological behavior of paste in the present study. More specifically, aluminium concentration at paste subgroups 1:2 and 1:4 that stimulated mitochondrial function was 3500 μmol/L and 1750 μmol/L. respectively. In agreement with the results of this investigation, Wataha [32] examined cytotoxicity of metal ions and using MTT assay observed that unlike other ions (Ag+1, Co+2, Cu+3, Zn+3 etc) aluminium ions could not affect negatively cell metabolic reactions even at high concentrations, approximately 3000 μmol/L. Stimulation of mitosis induced by aluminium ions at certain concentrations can be an explanation of higher cell mitochondrial function rates at certain paste subgroups (1:2 and 1:4).

Also, activation of oxidoreductive mitochondrial potential as a reactive defensive action of cells to the impact of astringents has been proposed as a possible explanation of cell metabolic stimulation [26]. However documentation of molecular pathway remains unclear. Due to heterogeneity among studies that examined in vitro cytotoxicity of retraction agents concerning many parameters (exposure time, examination time points, clinical form and chemical composition of retraction agents, dilution media, cytotoxic assays), results are not comparable with the present study. Definitely, gingival epithelial cells are mainly in contact with gingival retraction agents. However, difficulty of isolation and development of primary epithelial cell cultures lead to the gingival fibroblast use in the present study. Human gingival fibroblasts are frequently in contact with retraction agents due to trauma and ulceration during retraction techniques, so their use has certain clinical relevance. It cannot be assumed from the present in vitro study that results will have the same effect in clinical conditions. Protective epithelial barrier in comparison with host defence mechanisms (gingival crevicular fluid, inflammatory mediators, cytokines, matrix metalloproteinases - MMPs) may alter the overall cytotoxic effects clinically. Also, different pressure exerted during retraction procedure and possible consequent trauma is not considered.

Conclusion

Within the limitations of this in vitro study, it can be concluded that:

i. Impregnated retraction cord eluates caused the highest initial cytotoxic effect on human gingival fibroblasts.

ii. A time dependence was reported concerning the exposure at non impregnated cord eluates. Longer exposure (20min) resulted in irreversible reduction of oxidative mitochondrial function.

iii. At the rest of experimental groups the initial biological effect on mitochondrial activity was reversible. That is why proliferation rate of tested groups was comparable with that of the control group at the last time point.

iv. Retraction paste eluates presented the least aggressive cytotoxic effects.

In case of paste eluates cell exposure at certain concentrations (1:2 and 1:4) resulted in faster metabolic recovery and further stimulation of mitochondrial activity, indicating a dose – dependent effect.

References

1. Benson BW, Bomberg TJ, Hatch RA, Hoffman W (1986) Tissue displacement methods in fixed prosthodontics. J Prosthet Dent 55(2): 175-181.
2. Rosenstiel S, Land M, Fujimoto J (2006) Contemporary fixed prosthodontics. 4th ed. St Louis, MO: Mosby - Yearbook Inc p. 434 -440.
3. Donovan TE, Gandara BK, Nemetz H (1985) Review and survey of medicaments used with gingival retraction cords. J Prosthet Dent 53(4): 525-531.
4. La Forgia A (1964) Mechanical-chemical and electrosurgical tissue retraction for fixed prosthesis. J Prosthet Dent 14(6): 1107-1114.
5. Nemetz H (1974) Tissue management in fixed prosthodontics. J Prosthet Dent 31(6): 628-636.
6. Shillingburg HT, Hobo S, Whitsett L (1997) Fundamentals of Fixed Prosthodontics. 3rd ed. Chicago, IL: Quintessence Publishing pp. 257-279.
7. Shillingburg HT, Hatch RA, Keenan MP, Hemphill MW (1980) Impression materials and techniques used for cast restorations in eight states. J Am Dent Assoc 100(5): 696-699.
8. Shaw, DH, Krejci RF (1986) Gingival retraction preference of dentists in general practice. Quintessence International 17(5): 277-280.
9. Hansen PA, Tira DE, Barlow J (1999) Current methods of finish-line exposure by practicing prosthodontists. J Prosthodont 8(3): 163-70.
10. Ahmed SN, Donovan TE (2015) Gingival displacement: Survey results of dentists' practice procedures. J Prosthet Dent 114(1): 81-5. e1-2.

How to cite this article: Stergidou A, Theocharidou A, Bakopoulou A, Koidis P. Biological Behavior of Different Retraction Materials in Prosthetic Dentistry: In Vitro Study. Adv Dent & Oral Health. 2023; 16(5): 555948. DOI:10.19080/ADOH.2023.16.555948

11. Mohan M, Gupta A, Shenoy V, Parolia A (2011) Pharmacological Agents in Dentistry: A Review. Br J Pharm Res 1(3): 66-87.
12. Poss S (2002) An innovative tissue-retraction material. Compend Contin Educ Dent 23(1 Suppl): 13-17.
13. Bennani V, Aarts JM, Schumayer D (2016) Correlation of pressure and displacement during gingival displacement: An in vitro study. J Prosthet Dent 115(3): 296-300.
14. Land M, Rosenstiel S, Sandrik JL (1994) Disturbance of the dentinal smear layer by acidic hemostatic agents. J Prosthet Dent 72(1): 4-7.
15. Land M, Couri CC, Johnston WM (1996) Smear layer instability caused by hemostatic agents. J Prosthet Dent 76(5): 477-482.
16. Ayo-Yusuf OA, Driessen CH, Botha AJ (2005) SEM-EDX study of prepared human dentine surfaces exposed to gingival retraction fluids. J Dent 33(9): 731-739.
17. Harrison J (1961) Effect of retraction materials on the gingival sulcus epithelium. J Prosthet Dent 11(3): 514-520.
18. de Gennaro GG, Landesman HM, Calhoun JE, Martinoff JT (1982) A comparison of gingival inflammation related to retraction cords. J Prosthet Dent 47(4): 384-386.
19. Shaw DH, Krejci RF (1986) Gingival retraction preference of dentists in general practice. Quintessence International 17(5): 277-280.
20. Kopac I, Cvetko E, Pavlica Z, Marion L (2002) Gingival inflammatory response induced by chemical retraction agents in beagle dogs. Int J Prosthodont 15(1): 14-19.
21. Akca EA, Yildirim E, Dalkiz M, Yavuzyilmaz H, Beydemir B (2006) Effects of different retraction medicaments on gingival tissue. Quintessence Int 37(1): 53-59.
22. Phatale S, Marawar PP, Byakod G, Lagdive SB, Kalburge JV (2010) Effect of retraction materials on gingival health: A histopathological study. J Indian Soc Periodontol 14(1): 35-39.
23. Kopac I, Batista U, Cvetko E, Marion L (2002) Viability of fibroblasts in cell culture after treatment with different chemical retraction agents. J Oral Rehabil 29(1): 98-104.
24. Yalçin M, Barutcigil C, Umar I, Bozkurt BS, Hakki SS (2013) Cytotoxicity of hemostatic agents on the human gingival fibroblast. Eur Rev Med Pharmakoi Sci 17(7): 984-988.
25. Farzin M, Barfi A, Hashemi A, Pardis S, Amir Ali RKA (2016) Cytotoxic Effect of Astringent Agents on Human Gingival Fibroblasts. J Oral Health and Dental Manag 15(3): 179-182.
26. Nowakowska D, Saczko J, Kulbacka J, Choromanska A (2010) Dynamic oxidoreductive potential of astringent retraction agents. Folia Biol (Praha) 56(6): 263-268.
27. Liu CM, Huang FM, Yang LC, Chou LS, Chou MY, et al. (2004) Cytotoxic effects of gingival retraction cords on human gingival fibroblasts in vitro. J Oral Rehabil 31(4): 368-372.
28. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1-2): 55-63.
29. Velez G (2009) Inductively coupled plasma: The future of heavy metals testing. Life Science, Technical Buttelin 7: 1-2.
30. Poss S (2002) An innovative tissue-retraction material. Compend Contin Educ Dent 23(1 Suppl): 13-17.
31. Wataha JC, Hanks CT, Craig RG (1993) The effect of cell monolayer density on the cytotoxicity metal ions which are released from dental alloys. Dent Mater 9(3): 172-176.
32. Jones TR, Antonetti DL, Reid TW (1986) Aluminum ions stimulate mitosis in murine cells in tissue culture. J Cell Biochem 30(1): 31-39.

DOI: 10.19080/ADOH.2023.16.555948

How to cite this article: Stergidou A, Theocharidou A, Bakopoulou A, Koidis P. Biological Behavior of Different Retraction Materials in Prosthetic Dentistry: In Vitro Study. Adv Dent & Oral Health. 2023; 16(5): 555948. DOI:10.19080/ADOH.2023.16.555948